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IUFRO Poplar and Willow Genetics 2.08.04

8th INTERNATIONAL POPLAR SYMPOSIUM (IPS-VIII):

Poplars and Willows in the Era of Global Change: Agroforestry, Environmental Improvement, and Ecosystem Services to Enhance Livelihoods

BOOK OF ABSTRACTS

4th October-6th October 2022, Novi Sad, Serbia

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Session 1:
Genetics, Genomics, Breeding, and Conservation

Oral Presentations

CELL WALL COMPOSITIONAL ASSESSMENT OF GREY POPLAR HYBRIDS

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Abstract

Interspecific hybrid poplars, such as grey poplar (*Populus* × *canescens*), are rated among the most promising tree species for biofuel and bioenergy production due to a minimal requirement for fertilizer and an ability to grow on marginal infertile lands. In this study, we focused on the assessment of the chemical profiles for the artificial interspecific hybrids of grey poplar in terms of the content of main woody cell wall components and extractives, lignin monomer composition and neutral saccharide composition. The clone T-9, (*P.* × *canescens*) × *P. grandidentata*, showed the reduced lignin content (down to 17.6%) with the abundance of syringyl units (syringyl-to-guaiacyl ratio in lignin equals 2.3), and the increased proportion of exctractives (4.9%). From the saccharides, the proportion of D-glucose achieved 51.3%, whereas for D-xylose and D-galactose it was 18.1 and 4.1%, respectively. The proportions of L-arabinose and D-mannose were not higher than 2.2% for each. The micropropagated plants of the T-14 clone, *P. tremula* × (*P.* × *canescens*), also showed the lignin content lower than 20% (i.e., 18.2%), almost identical syringyl-to-guaiacyl ratio in lignin (2.3), but the proportion of exctractives was lowered (down to 3.3%). From the saccharides, the proportion of D-glucose achieved up to 69.8% that was the most distinctive difference between the two examined clones. Also, the proportions of D-xylose and D-mannose were increased (22.4 and 4.2%, respectively), whereas for D-galactose and L-arabinose it was less than 2.0% for each. The cell wall compositional characteristics of the T-14 micropropagated plants were then compared to those of the T-14 plants propagated from root cuttings. These plants were used as the counterparts to the micropropagated plants because root cuttings are the preferred option of source material over the poorly rooting stem cuttings for conventional vegetative propagation of grey poplar and its artificial interspecific hybrids. The performance of the T-14 micropropagated plants was higher for the content of cellulose, D-glucose and D-mannose. On the other hand, the performance of the T-14 plants propagated from root cuttings was superior for the content of hemicelluloses, D-xylose and L-arabinose. The T-14 micropropagated plants were found to be a promising renewable resource of fermentable sugars for the biofuel industry.

Keywords: Biofuels, Cellulose, D-Glucose, Extractives, Lignin
Acknowledgments: This work was supported by the Slovak scientific grant agency VEGA (1/0450/19).

INITIAL WORKS FOR THE PRODUCTION OF GENERATIVE SEEDLINGS AND SELECTION OF EUROPEAN BLACK POPLAR (*POPULUS NIGRA* L.) CLONES

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Abstract

European black poplar, as one of the most significant tree species in riparian forests of Croatia, is also one of the more endangered species in the whole area of its distribution range. In Croatian forestry, there are few records on the cultivation of black poplar plants by a generative way and their further use in breeding and production in nurseries.

The catkins were gathered in the Forest Administration Osijek, Forest Office Valpovo and were stored in the greenhouse of the Croatian Forest Research Institute. When the catkins were opened, the seeds were separated from the cotton layer by a sieve and sowed. For sowing were used two types of substrates, were the first type was fine-grained sand, and the second was a mixture of sand and neutral peat. Sowing in the different substrate was used to compare i.e. test their effectiveness. In first year plants were transplanted two times into larger containers where plant selection was performed during each transplantation. The selection criterion was the height growth and the development of the plant, i.e. the number of leaves.

The results showed differences in height growth between plants sown in different substrates. The average height growth was significantly higher in black poplar plants grown in a mixture of peat substrate and sand, compared to plants grown in pure sand. Also, it was observed that pure sand as a substrate, in conditions of frequent watering and high temperatures, favors the occurrence of fungal diseases because they are only recorded on plants sown in the sand. Obtaining seeds by the applied method of collecting branches and fruit manipulation proved to be a successful method because sufficient quantities of branches were collected with catkins, from which a sufficient amount of seeds was obtained later after opening. The success of seed germination was the same in both substrates, i.e. there was no significant difference in germination between different substrates. Growing black poplar plants from seed is a rarely used method of reproduction and it is necessary to improve it and to find the best protocol for growing quality seedlings. The use of quality plants from seeds will increase genetic diversity, preserve the gene pool, and later selection by genotype and genetic testing expect that it will be possible to identify several clones of black poplar that will be competitive with poplar clones currently in use in Croatian forestry.

Keywords: *Populus* sp., seed, gene pool, substrate, selection

IDENTIFICATION ON THE BASIS OF GENETIC MARKERS OF POPLAR CLONES USED IN WOODLOTS AND PLANTATIONS IN POLAND.

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Abstract

The research topic was carried out in 2018-2020. The main premise for the realization of the topic was the need to develop a universal tool that can be used to identify the clonal affiliation of selected poplar cultivars economically used commercially in Poland.

Poplars are far superior to all other tree species in terms of speed and volume of timber production under natural conditions in Poland. Due to their exceptional growth characteristics and sometimes specific wood properties, poplars are of great economic importance and are grown in almost all countries of the northern temperate zone. However, it should be kept in mind that correct identification is the basic requirement for full exploitation of potential production possibilities and evaluation of selected poplar cultivars in plantation or afforestation management. This is often complicated by the high degree of cultivars as regards many morphological characteristics. Most of the poplars recommended for cultivation are interspecific hybrids. Traditionally, poplar cultivars have been distinguished using a combination of morphological characteristics and phenological traits. These methods have been described and are generally accepted, but one must be aware of their complexity due to the expertise, time, and subjectivity required. The development of molecular biology has made it possible to replace morphological and phenological markers with genetic analyses.

Based on the problems presented, the main objective of the present research was formulated, which was to obtain individual genotypes of cultivars (cultivated clones, i.e. cultivars) in the following poplar sections with the help of microsatellite DNA: Black poplars (*Aigejros*), Balsam poplars (*Tacamahaca*) and their hybrids used in tree stands and plantations in Poland.

Cultivars of 20 poplars, including interspecific hybrids from the Aigeiros and Tacamahaca sections, served as study material. We collected 5 leaves from each of 5 trees that were clones of a cultivar for which an individual genetic profile was established. We obtained positive amplification of a DNA fragment for all selected markers, but the quality was satisfactory for only 19. Of the selected markers, after final review of the obtained genotypes, we recommend 11 for commercial use to verify the clonal affiliation of the cultivars studied in the project. For these markers, a patent application number TM - 08- GP was filed to obtain legal protection for the set.

THE IMPACT OF DIFFERENTIALLY EXPRESSED GENES IN MONOCLONAL AND POLYCLONAL PLANTINGS OF *P. deltoides* FOR AGRICULTURAL NITROGEN MITIGATION

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Abstract

Excessive nitrogen run off can cause the eutrophication of water bodies. The establishment of riparian crops, such as *Populus spp*., can reduce excessive run off from agricultural fields before it enters nearby water bodies. Polyclonal plantings of *Populus spp*. are expected to increase site resource utilization through niche differentiation (e.g., different poplar varieties may have root structures that together occupy greater portions of the soil), which in turn would increase efficacy of *Populus spp*. ability to intercept nitrogen from agricultural fields. However, underlying molecular mechanisms of niche differentiation and changes in site resource utilization are poorly understood. We propose using RNA-Seq to determine if differentially expressed genes (DEGs) are an underlying molecular mechanism of niche differentiation.

RNA sequencing is a high through-put tool that can determine the condition of the genetic transcriptome and provide accurate information on transcription response to the environment. Following RNA-Seq, we will utilize Salmon database for transcript quantification and *edgeR* in R statistical software to import our transcript estimates, correct for changes to the average transcript length across samples, and to provide gene-level estimate counts. Next, we will conduct a gene set enrichment analysis (GSEA) and a Gene Ontology (GO) analysis to identify and interpret relationships between enriched gene sets. We will use the KEGG pathway enrichment database to examine the relationship between the quantity of DEGS and their associated mapped pathways, specifically analyzing pathways involved in nitrogen utilization and tree biomass growth.

We expect that our two varieties of *Populus deltoides* in polyclonal plantings will show greater variation/regulation of gene expression compared to our two varieties planted in monocultures. We expect that our polyclonal plantings will display an increase in site resource utilization through associated physical data such as changes in concentrations of measured inorganic forms of nitrogen from our groundwater samples along with tree height and diameter measurements to support tree biomass growth.

A better understanding of these mechanisms in niche differentiation can aid in the development of tools to predict what combinations of poplar varieties will provide the greatest growth and nitrogen mitigation potential. Our determination of DEGs and their relationships in biological pathways may help us better understand morphological variability in *Populus deltoides* varieties and could provide data for future genetic studies aimed at further improving the nitrogen mitigation potential and growth of this crop.

Keywords: *Populus spp.,* Differentially Expressed Genes (DEGs), Niche Differentiation, RNA-Seq.

GENETIC RESOURCES OF EUROPEAN BLACK POPLAR IN POLAND: THE CURRENT STATE OF KNOWLEDGE AND FUTURE RESEARCH

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Abstract
European Black poplar (*Populus nigra* L.) occurs along major river valleys in Poland with a high frequency, but we observe a progressive reduction in its population size. Furthermore, many trees have reached terminal age. Natural regeneration of this species is possible only in a few areas, where the river dynamics still allow periodic flooding. The gene pool of pure black poplar in Poland is also endangered by introgressive hybridization with Canadian poplars and Lombardy poplar.
Using a set of nuclear microsatellites and species-specific DNA markers we analyzed the genetic variation of the black poplar populations along the Oder (9 stands – 592 adult trees) and Middle Vistula (2 stands – 124 old trees, 53 young trees, and 205 seedlings) river valleys in Poland. The Oder is highly transformed, whereas the Middle Vistula is modified to a far lesser extent.
We did not find any naturally regenerated seedlings resulting from sexual reproduction along the Oder river. All seedlings and saplings neighboring adult trees turned out to be clones of up to a few individuals. Repeated genotypes were unique to a particular population. The degree of clonality was significantly higher in the Oder (13-55%) than in the Middle Vistula (0-11%). The results indicate that the level of genetic variation in the studied black poplar stands is still high and accompanied by a low level of inbreeding, which is comparable to the results of studies conducted along other rivers in Europe. Nevertheless, we identified 34 cryptic hybrids in the Oder populations, mainly in the downstream stands. Introgression was also evident in the Middle Vistula – there were 9 hybrids in the group of old trees and 3 hybrid seedlings. The effective population size was much lower in the Oder than in the Middle Vistula (N_e = 5-134 vs. N_e = 93-435), and the gene flow was limited to neighboring stands. Signatures of past bottlenecks were detected in all populations.
Further regulation of rivers in Poland is planned in the upcoming years by building flood management structures. For this reason, black poplar may soon be left with no habitats to regenerate naturally. Therefore, we will conduct complementary research using microsatellite and SNP markers in order to develop a comprehensive conservation strategy for the genetic resources of black poplar in Poland.
Keywords: European black poplar, Canadian poplar, genetic variation, introgression.

Acknowledgments: The research was funded by the Polish National Science Centre (grants no. 2021/41/B/NZ9/00722 and 2016/21/N/NZ9/01515).

COMPARATIVE INDICATORS OF MORPHOLOGICAL TRAITS OF BLACK POPLAR LEAVES *IN SITU* AND *EX-SITU*

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Abstract
Black poplar (*Populus nigra*) is one of the most important species of forest trees that inhabit alluvial habitats along river banks in Europe. This species is on the verge of extinction due to habitat devastation, river regulation, and over-exploitation. In recent decades it has been exposed to hybridization with non-native black poplar species and Euro-American hybrids. This research aims to determine whether there are correlations in the morphological traits of black poplar leaves from in situ habitats, i.e. from natural populations, and those found in ex-situ conditions, i.e. in the clone archive raised from clone material selected in the studied natural populations.
The first part of the research included the measuring leaf traits in seven natural populations with different ecological conditions (populations Doboj, Čapljina, Konjic, Bugojno, Kakanj, Tegare, and Banja Luka). The authors selected ten trees per population and measured fifty leaves per tree. The following traits were measured: leaf blade length (LBL), leaf blade width (LBW), petiole length (PL), and distance between the leaf widest part and the leaf base (DBW). The second part of the research included measurements of the same traits from leaves collected on clone material originating from these populations. The clones were planted in the clone archive, i.e. in *ex-situ* conditions, where the same ecological conditions prevail. Each of the analysed clones was presented with 50 leaves. After measurement, the data were processed in the statistical program SPSS 26.0. Descriptive statistics, variance analysis and the Pearson correlation coefficient between leaf traits in adult black poplar trees in situ and young trees in the clone archive were calculated.
The results of variance analysis showed statistically significant differences among natural populations and among the clones for all leaf traits. Pearson's correlation coefficient between the values of traits in natural populations and clones derived from these populations had positive values for all traits. The highest value of Pearson coefficient was for leaf blade length, 0.698, and the lowest for the distance of the leaf widest part from the leaf base, 0.224.
These results have their practical application in answering the question of to what extent the morphological traits of leaves are hereditary, i.e. how much habitat conditions affect these traits. Research needs to be continued to determine the correlation between leaf traits and incremental possibilities and biomass production, which will make a significant contribution to the selection of the most productive black poplar clones.

Keywords: Populus nigra, leaf traits, natural populations, clonal archive

THE FIRST STEP IN TESTING NEW POPLAR CLONES FOR BIOMASS PRODUCTION IN BOSNIA AND HERZEGOVINA

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Abstract
The need for wood and wood products is growing in Bosnia and Herzegovina. In the past decade, many cities in the country have switched heating systems to woodchips and biomass. Therefore, the need for establishing plantations that would provide new quantities of wood was recognized.
The experiment with 7 poplar clones obtained for testing thanks to Biopoplar s.r.l. Italy: *Orion*, *Westen*, *Oudenberg*, *H7*, *H11*, *H17* and *H33* was set in 2018. Clone I-214 was used as a control clone. The experiment was set in the Center for Seed and Nursery Production - Doboj (central part of Bosnia and Herzegovina) in the continental climate on pseudogley soil. The experiment was divided into two parts: irrigated and non-irrigated part of the experiment. Each of these parts consists of 3 replicates, in which there are 30 seedlings per clone. Planting distance is 2.5 x 1 m. Survival was recorded and the height were measured at the end of the first and second vegetation period.
The results in the first year of monitoring indicate that irrigation had a positive effect on clones' survival while not having a significant effect on height growth. The highest survival rate at the first year was recorded for clone *H17* for both treatments: 98.89% (irrigated) and 93.33% (non-irrigated). In the second year survival rates were 97.77% for *H17* clone (irrigated) and 91,11% for the same clone (non-irrigated). The highest values of height in the first year were recorded for the *Orion* clone in the non-irrigated part of the experiment (163.81 cm) and in the irrigated part for the *H17* clone (168.58 cm). In the second year clone *H33* was the highest clone non-irrigated and irrigated with heights of 310,12 cm and 311.17 cm respectively. Control clone *I-214* had a significantly lower percentage of survival and reaches significantly smaller dimensions compared to other clones.
Clones, such as *Orion*, *H17* and *H33*, stand out with above-average results in terms of survival and potential productivity, and these clones in the future should be considered when determining the clone for biomass production on the same or similar sites.

Keywords: poplar clones, two years old, biomass production

Acknowledgment: The research was supported by Biopolar s.r.l. company, Cavallermaggiore, Italy, and PFE "Forests of Republic or Srpska" j.s.v. Sokolac, Republic of Srpska, Bosnia and Herzegovina

Session 1:
Genetics, Genomics, Breeding, and Conservation

Posters

GROWTH AND REPRODUCTIVE CHARACTERISTICS IN CROSSINGS BETWEEN RELATIVES IN *Populus nigra* L.

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Abstract
Although black poplar is an autochthonous tree of large area of Europe, it is, besides other things, highly threatened by the loss of native habitats resulting from human activity and thus by the fragmentation of ever-shrinking populations. In such populations, there is a high probability of inbreeding, which causes reducing the fitness of individual genotypes, i.e. inbreeding depression.
In three years (2010, 2011, 2013) controlled pollinations were carried out to obtain both I_1 and I_2 generation. Progeny derived from open pollination of respective mother trees was used as a control. There were assessed germination of obtained seeds and the health state (resistance against leaf rust caused by *Melampsora larici-populina*, mortality) and growth traits (diameter, height, yield) of the offspring were evaluated in a randomized field trials with four repetitions. Inbreeding depression was proved in all traits. Offspring derived from cross-pollination had significantly higher mean values of all observed traits compared to offspring derived from inbreeding; offspring derived from self-pollination were the worst.
The flowering phenology of surviving plants was monitored. Although the black poplar is considered a dioecious species, about 10 % monoecious individuals have been found among flowering trees. These young monoecious *P. nigra* trees originated from different crossings. It has been demonstrated that both male and female flowers of monoecious *P. nigra* trees can be fertile, and furthermore these monoecious trees are self-compatible. However, spontaneous selfing of monoecious tree in nature was not proved by microsatellite markers.
Results of this study could be applicable in conservation genetic and bring us new information that monoecious individuals are more frequent than it is expected at least when the trees are young. It is necessary to continue in flowering observation in following years. Study in small populations will continue to observe mating and associated events and their effect on growth and survival as one part of the project 'Black poplar conservation and its application in water management and forestry'.

Keywords: black poplar, small population, inbreeding depression, fitness, monoecious trees

THE USE OF EUROPEAN BLACK POPLAR AS AN ALTERNATIVE FOREST TREE IN THE CZECH REPUBLIC

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Abstract

The impacts of climate changes on forestry in the Czech Republic have caused a mass dieback of forest stands in recent years. Forest regeneration using pioneer deciduous trees and their amelioration function is a way to improve the nutrition and stability of the stands and their adaptability to the permanently ongoing changes in the environment. Native poplar species can be used as one of the alternatives for reforestation, European black poplar (Populus nigra L.) is a suitable substitute tree for the lower altitudes. This species is native in the Czech Republic, but has been neglected and greatly reduced in the past. Forest inventory of potential reproductive forest genetic resources of black poplar phenotypically quality trees in Moravia and Bohemia and their genetic variability is monitored as a part of our research.

The genetic variability of black poplar trees were investigated with nuclear microsatellites. The selection of microsatellite markers was focused on markers sufficiently polymorphic with more uniform frequency of represented allele sizes for more relevant results of variability evaluation. PCR products were analyzed using the Applied Biosystems 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The genetic diversity parameters were calculated using the statistical program GenAlEx 6.501 (PEAKALL, SMOUSE 2006, 2012).

239 samples of black poplar trees were genotyped with the 11 nuclear polymorphic microsatellite loci (WPMS01, WPMS04, WPMS07, WPMS10, WPMS11, WPMS13, WPMS14, WPMS16, WPMS19, WPMS21, WPMS22) described by Van der Schoot et al. (2000) and Smulders et al. (2001). The number of alleles ranged from 10 (WPMS16) to 40 (WPMS04) and the mean number of effective alleles from 5.43 (WPMS16) to 14.41 (WPMS01). The values of observed heterozygosity ranged from 0.55 to 0.98, and expected heterozygosity from 0.82 to 0.93. The highest value of allele frequency was 27 % (WPMS16). Comparison of analysis results of matching multilocus genotypes showed significant diversity among most of the studied individuals, in which allele sizes differed at nine microsatellite markers. The knowledge from phenotypic evaluation and genetic investigation of black poplar will be used in the field of protection of genetic resources as well as in forestry and agroforestry. The genetically polymorphic and morphologically quality trees are using for *ex situ* conservation strategies in the field gene bank of the FGMRI. Selected genotypes are evaluated on newly established research plots to compare their yield with yield of hybrid poplars and determine the optimal management of reforestation.

Keywords: European black poplar, Genetic variability, Protection of genetic resource

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RELATIONSHIP BETWEEN BASIC DENSITY AND ACOUSTIC WAVE PROPAGATION: PREDICTION OF WOOD QUALITY OF IMPROVED WILLOW CLONES IN ARGENTINA

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Abstract

The objective of this work was to evaluate the relationship between basic density and acoustic wave propagation in standing trees to provide a new rapid and simple phenotyping tool for wood quality selection of structural use in new genotypes of willows (Salix spp.).
The present study was carried out in 82 trees of 12-year-old trials implanted in two differential sites with respect to flood protection (highly protected and unprotected). The willows under study were: three hybrids S. matsudana Koidz. x S. alba L. (Agronales, Los Arroyos and Tehuelche) and a genotype achieved by open pollination of S. alba L. (Yaguareté). Yaguareté and Los Arroyos proved to be clones tolerant to waterlogging, while Agronales and Tehuelche were not.
For each clone acoustic wave propagation (AWP - Microsecond Timer, Fakopp) was measured in standing trees. Subsequently, felling and sawing of each tree was carried out to cut small-sized pieces and semi-structural pieces obtained from the internal and external areas of the log. The small-sized pieces were used for basic density (BD) determination. Also, on each semi-structural piece the AWP was measured.
A statistical correlation analysis between AWP in standing trees and BD was carried out.
Depending on the clone and implantation site the results were variable. Yaguareté and Los Arroyos presented negative correlations between BD and AWP (-0.87 p=0.05 and -0.64 p=0.12, respectively) in unprotected site, turning out to be the best place for both of them. Agronales and Tehuelche presented a significant but contrary correlations between these variables in the highly protected site (-0.71 p=0.002 and 0.65 p=0.009, respectively).
Finally, the correlations between AWP on the standing tree and in semi-structural pieces are under development, as well as the generation of juvenile-adult prediction models.
According to these results, AWP can be used as a rapid non-destructive measure to estimate BD in Yaguareté, Agronales and Tehuelche clones.
Significant correlations, as well as future prediction models to be obtained between growth, technological variables and acoustic measurement (taken as a non-destructive grading technique) will allow us to provide a new rapid and simple phenotyping tool for the breeding program currently underway.

Key words: *Salix spp.*; flood protection; non-destructive technique; breeding program.

IMPROVED WILLOWS FOR "DUAL PURPOSE" TECHNOLOGICAL USES

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Abstract
The INTA's willow (*Salix spp.*) breeding program, based on interspecific hybridization followed by clone selection, is focused to increase the genetic diversity of willows and to improve volumetric yield values, and wood quality for a "dual purpose" standard, including attitudes for the paper industry (focusing on newsprint and packaging), sawmilling and other applications. As a result of the program activities, between 2013 and 2021, eight improved willows have been released. Growth variables (total height and DBH) were recently assessed in three trials on 12-year-old trees; its main results are described in this work. Forty-five genotypes in three trials were compared with the most traditional clone, *Salix babylonica* var. *sacramenta*, preferred for the paper industry because of its high quality for the technological process, but characterized by very low volumetric yield and other negative factors, such as non-straight branches, with numerous ramifications. The species involved in the new evaluation were: *Salix matsudana* Koidtz, *Salix alba* L., *Salix nigra* Marsh, *Salix amygdaloides* Andersson, *Salix babylonica* L, *Salix bonplandiana* H.B.K. and *Salix humboldtiana* Willd. Growth assessment showed satisfactory performance of 15 genotypes that originated from two controlled crosses : *Salix matsudana* × *S. nigra* (3 genotypes), *S. matsudana* × *S. alba* (7); open-pollinated progenies of *S. alba* (1), *S. amygdaloides* (3) and *S. matsudana* (1). As part of the study, wood samples (logs 1.5 meters long) of these genotypes were analyzed in the laboratory and analysis of pulp-paper was conducted; as a result, a wide variation in the parameters of the experimental clones was observed and outstanding technological properties were detected in 10 genotypes, characterized by high values of relevant parameters involving in the pulp process. This preliminary technical wood characterization makes the selected new material comparable to the reference clone of the *Salix babylonica* var. *sacramenta*, but with the strategic advantage of high growth rates.
In addition, tests developed in the sawing industry showed very satisfactory results.

Keywords: Salix, paper industry, wood properties

IMPLEMENTATION OF GENOME EDITING TECHNOLOGY TO IMPROVE BIOTIC STRESS TOLERANCE IN WHITE POPLAR

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Abstract
Due to severe climate change, plant genomes adapt to constant changes of synergistic and antagonistic environmental signals. Their capability to reprogram their transcriptome patterns in dynamic and temporal manner lead to adaptive plasticity of plants in highly variable environments that is mainly achieved by networking of various transcription factors (TFs). There is plethora of regulatory proteins that are involved in various biotic and abiotic stresses and one of them are WRKY TFs. It was found that WRKY TFs are large protein family that are evidently associated with plant immune responses. Recent research shown that their key mechanisms triggering strong immune responses appear to be based on the inactivation of defense suppressing WRKY proteins. Based on the previous research results, our goal was to silence WRKY73 gene in *P. alba* genome by applying one of gene editing technology, CRISPR/CAS9 system, to prove its ability to positively or negatively affect regulation of the defense system in poplar. The WRKY73 was knocked out via CRISPR/Cas genome editing technology (NHEJ approach). The sequences of WRKY73 gene from *P. trichocarpa* sequenced genome was retrieved from the popgenie.org database and processed with CRISPR-P 2.0 software to find out the guide RNAs (gRNA) that can target exon parts of particular gene. Many PAM sequences containing gRNAs were selected according to their appropriate GC content and off-target capacity in poplar genome. By this way, two gRNAs targeting one gene (WRKY73) were determined and synthesized. Two gRNAs were then transferred into a Cas9 containing Agrobacterium plasmid (pHSE401) by using golden gate cloning. The plasmids were then inserted into Agrobacterium GV3101 strain by electroporation. The confirmed Agrobacterium were grown in the same liquid selection media for overnight and after centrifugation the bacterial pellet was prepared for transformation step. Plant preparation was from "*in vitro"* grown plants. After inoculation with Agrobacterium and co-cultivation, the explants were placed on selective callus induction media, then once gained calluses the tissue were transferred into shoot induction media and after several passages on shoot elongation media. Agrobacterium mediated transfer of the gRNA/Cas9 constructs into poplar explants and optimization of the tissue culture regeneration of *P.alba* were done successfully. In the future studies, T0 mutant plants will be tested and used to understand the effects of WRKY73 TF on gene regulation in poplar response to biotic stress factors. The knowledge obtained in the study can be used to develop new tree cultivars that can cope with adverse effects of climate change in years to come. Genome editing CRISPr/Cas9 technology were successfully established in the molecular and tissue culture laboratory of the Institute of Lowland Forestry and Environment, Novi Sad, Serbia. Fruitful collaboration with Ondokuz Mayıs University, Samsun, Turkey and University of Glasgow, United Kingdom were established.

Keywords: Genome editing, WRKY TFs, CRISPR/Cas9, *Populus alba* L.

Acknowledgements
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Session 2:
New Bio Economics – Livelihoods, Ecosystem Services, Carbon Credits

Oral presentations

EARLY DYNAMICS OF CARBON SEQUESTRATION RELATIVE TO SPACING OF AN EASTERN COTTONWOOD PLANTING

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Abstract
Fast-growing trees such as the poplars (*Populus* spp.) show great utility for quick initiation of forest ecosystem services on sites removed from agricultural production. The rapid growth and development of poplars result in carbon accumulation and a transformation of the carbon storage status of a restoration site that exceed those of slower-growing tree species. Accordingly, eastern cottonwood (*P. deltoides* Bartr. ex Marsh.) currently is being planted to catalyze restoration of native forests and their functions in the southeastern United States, especially on sites appropriate for pursuing a financial return on carbon sequestration. We studied the early dynamics of above-ground carbon accumulation in an eastern cottonwood planting established on former agricultural land located in the Lower Mississippi Alluvial Valley, USA. The planting was established on 4 replicated spacings (0.9 × 0.9 m, 2.1 × 0.8 m, 2.7 × 1.8 m, 3.7 × 3.7 m) suitable for forest restoration objectives ranging from tightly-spaced buffer strips to widely-spaced stands of a framework species. Measurements of tree survival, number of stems, height, and diameter were collected annually in permanently established plots for 9 years. These measurements were applied to published equations to estimate above-ground biomass, and biomass values were converted to carbon mass for analysis at the tree- and stand-level. Our presentation will report on above-ground carbon sequestration from establishment through year 9 for each level of spacing, drawing management implications from the annual and total accumulation of carbon through the study period.

Keywords: forest restoration, ecosystem services, planting density, above-ground carbon

THE MULTI-FACETED POTENTIAL OF POPLAR IN THE DRIVE TOWARDS A CIRCULAR BIOECONOMY

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Abstract

The use of biomass as a natural renewable resource is postulated as one of the key factors in generating a circular bioeconomy given the associated economic, environmental and social benefits. Short rotation coppice (SRC) plantations are conceived specifically for supplying biomass destined for the production of bioenergy as well as new bioproducts. This source of biomass is highly manageable in terms of the time and land required; therefore it may be especially recommendable to stabilize the market in areas of low biomass availability. The main aim of these plantations is to achieve higher, more efficient production, maximizing the benefits by combining economic viability with environmental sustainability. Improving the sustainability of biomass production depends on different factors such as: selection of the genotypes which are best adapted to each environment, design and management appropriate to each circumstance, evaluation of production and the development of accurate estimation tools, characterization of the biomass in order to adapt the crop according to the desired end use, as well as the economic analysis of all these factors. However, while the end product of these plantations is biomass, they provide numerous added benefits, which until now have not been adequately quantified and which would increase the final value of these plantations. The ecosystem services associated with these plantations include carbon sequestration, increasing biodiversity in agricultural environments, soil decontamination, mitigation of erosion or flooding, regulation of clean air or water with phytoremediation, among others. The need for irrigation to make these plantations viable under Mediterranean conditions, coupled with current and future water constraints, makes this ecosystem service one of the most important for the future of these plantations. The opportunity to transform a weakness such as the need for irrigation into a benefit such as the reuse of a resource as precious as water enhances the viability of such plantations both in environmental and economic terms. If we add to this the possibility of obtaining other types of economic contribution, such as carbon credits, as well as the possibility of occupying marginal areas not suitable for other uses along with favoring the use of renewable energy under the current scenario of energy crisis, then these crops clearly present us with an opportunity to address the imminent challenges arising from global change.

Keywords: *Populus*, Short rotation coppice (SRC), Biomass, Ecosystem services

WILLOWS AND POPLARS AS HABITAT TREES – PIONEER TREE SPECIES IN NATURAL RIPARIAN FOREST HOST RICH AND DIVERSE ASSEMBLAGE OF TREE-RELATED MICROHABITATS

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Abstract

Protection and restoration of forest biodiversity are among the most important task of modern forestry. One of the tools used for this purpose is the determination and protection of large, old, dying or dead trees, which are rich in tree-related microhabitats (TreMs) i.e. structures such as cavities, cracks, sap flows or fungi fruiting bodies etc., and thus which are specified as habitat trees. Pioneering, fast growing tree species such as Willows and Poplars being capable of achieving considerable dimensions during their relatively short lifespans, have the potential to harbor large numbers of TreMs and therefore, have the potential to be a valuable habitat trees. However, the TreMs occurring on Willows and Poplars have yet to be identified. We investigated the assemblage of TreMs in natural Willow-Poplar *Salici-Populetum* riparian forests and analyzed the tree-related and environmental factors that influence their qualitative and quantitative compositions on individual tree and study plot level. A total of 90 sample plots were selected at random in old-growth riparian forests that have survived along a large unregulated river, the Vistula (Poland). A total of 62 TreM types were identified on 1541 individuals from 12 tree taxa, in which the dominants were Willows *Salix sp*., Black poplar *Populus nigra* and White poplar *Populus alba*. The highest mean number of TreM types (TreM richness) on living trees were found on Willows and Black poplar. TreM richness and probability of occurrence of different TreM forms was increasing with the diameter and number tree trunks, however its value on trees with a given diameter or number of trunks were taxa-specific. The richness, density and diversity of TreMs found on a plot depends on the structural characteristics of the forest stand such as density of living trees, the basal area of living or dead trees, percentage of multi-trunk trees and characteristics related to tree species composition such as the number of tree species and the percentage of Willows. Our study records for the first time the assemblage of TreMs in natural riparian forests revealing that Willows and Poplars are likely to have large numbers of TreMs and single trees of these genera reach high TreM richness compared to other broad-leaved tree species. Our results indicate also that multi- species forests influenced by natural waterflow-related disturbances are hot-spots of TreM richness and abundance, and highlight the urgent need for the protection or restoration of these vanishing habitats.

Keywords: tree microhabitats, wildlife trees, biodiversity, saproxylic species, alluvial-forests

Session 2:
New Bio Economics – Livelihoods, Ecosystem Services, Carbon Credits

Posters

LOOKING FOR *Salicaceae* PLANT MATERIAL SUITABLE FOR CULTIVATION WITH INDUSTRIAL WASTEWATER: TOWARDS MULTIPURPOSE PLANTATIONS

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Abstract
This work is housed within a general context of wood production as a raw material of interest for the bioeconomy, at the same time that secondary wastewater from the agri-food industry is used for irrigation, while regenerating in a commitment to the circularity of resources. Under Mediterranean conditions, irrigation is a common practice for biomass production in short-rotation coppice. At the same time, the brewing industry uses a significant amount of water that must be treated after use to finally be discharged into the sewage network or into the environment. This treatment follows a cycle of processes in such a way that it gives rise to an effluent characterized by high N and P contents, high DOB and COD, and high salinity.
Salicaceae (*Populus* spp. and *Salix* spp.) have excellent characteristics that make them suitable for soil and water phytoremediation, as has been substantially referred to in the literature. The suitability of the plant material is a key issue in the development of this nature-based solution. Therefore, twenty-four genotypes of different species or hybrid groups of both genera were tested under greenhouse conditions using hydroponic culture. Both productive hybrids whose adaptation was tested for Mediterranean conditions and autochthonous material are included. The treatments applied were a control solution and secondary wastewater from the brewing industry. Five replications per treatment and genotype were randomly installed in 55l containers. Trials were maintained for 60 days.
The behavior of the plant material was evaluated through the analysis of traits linked to growth and production (height, diameter, different fractions of the biomass-root, stem and leaves-, root/shoot ratio) as well as physiology (net photosynthesis, A, stomata conductance, gs, and transpiration, E). Additionally, the composition of the influent and effluent wastewater was recorded and analyzed weekly to determine water quality.
During the test period, survival in control conditions was 96.5%, decreasing to 86% in the wastewater. Irrigation with wastewater significantly affected all the evaluated parameters, both productive and physiological. Stomatal closure, less transpiration and a drop in the net photosynthesis rate were observed, which implied a lower biomass production. However, not all genotypes were affected in the same way. Differences in tolerance were identified in each taxonomic group. In terms of wastewater treatment, sampled water show higher attenuation values for SS, followed by BOD, COD, TN and TP, respectively.
The possibility of contributing to the regeneration of industrial waters at the same time that necessary raw material is produced will depend, among other factors, on the adequacy in the choice of plant material.

Keywords: wastewater from brewing, phytotechnologies, poplar, willow, biomass production

MICROELEMENT ACCUMULATION IN VEGETATIVELY PROPAGATED POPLAR CLONES DEPENDS ON PROPORTION OF BIOGAS DIGESTATE AND WOOD ASH IN FERTILIZER TREATMENTS

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Abstract
The use of industrial byproducts as soil conditioners is a way to reduce the problem of organic byproduct production. Although organic byproducts are rich in nutrients needed for plants, plant accumulation capacity for them can differ compared to mineral fertilizers. Biogas digestate is a byproduct created during the production of biogas. Scientific interest for biogas digestate has increased over the last decades because it can be potentially used as fertilizer for plants. Wood ash obtained during the cogeneration of woodchips has already been used as a fertilizer and liming agent for plants, including in tree plantations. Although the positive effects of digestate are observed on annual crops, studies of its effects on tree plantings are lacking.
The purpose of this study was to determine the most effective fertilizer combination for poplars by measuring the uptake of macroelements by poplar clones fertilized with digestate and ash in a variety of proportions. Two poplar clones were selected as the subject: the widely used 'OP42' and the local clone 'AUCE', which were selected in Latvia. The digestate and ash ratios (digestate:ash) used in the fertilizer were 1:1; 1:2; 1:3; 1:4. Soil chemical analyses were carried out at the beginning of the growing season before planting the poplars, and after harvesting poplars at the end of the growing season. The amounts of nitrogen, phosphorus, and carbon were determined in different parts of the plant (i.e., leaves, roots, shoots). Higher levels of phosphorous in all parts of the plant were observed in trees fertilized with the 1:1 digestate:ash mixture. The amount of carbon and nitrogen in the shoot was lower in groups with lower ash proportion, whereas leaves and roots had lower carbon and nitrogen in groups with higher ash proportion.

Keywords: By-products, Phosphorus, Nitrogen, Fertiliser, *Populus* spp.

Session 3:
Physiology and Ecophysiology

Oral presentations

THE PROTECTIVE EFFECTS OF PUTRESCINE LEAF SPRAY IN THE RESPONSE OF HYBRID POPLAR (*Populus nigra x maximowiczii*, clone NM6) PLANTS TO SALT STRESS

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Abstract

Polyamines regulate plant growth and development in a typical environment and biotic and abiotic stress, including salt stress. However, little information is available about how polyamines improve salt tolerance. This study aims to determine the role of putrescine, a primary polyamine, in the presence of salt stress in a greenhouse condition. The objective was to study the effect of exogenous putrescine in 3 months old hybrid poplar (*Populus nigra x maximowiczii,* clone NM6) under two different salt concentrations: 100 mM and 200 mM NaCl. Leaves were sprayed repeatedly with 1 mM putrescine after plants were treated with salt via roots in the greenhouse. Plant growth like height, stem diameter, and leaf number were recorded throughout the experiment. Gas exchange values like photosynthesis, conductivity, and transpiration rate of the leaves were measured on multiple days with a LICOR-6800. Leaf discs were collected on days 0, 3, 6, 13, and 20 to study total chlorophyll content, proteins, relative water content, and soluble sugars. Putrescine spray had a significant effect on chlorophyll a, carotenoid, and total protein content on various days in the presence of salt. Morphological growth and gas exchange values were decreased during salinity treatment but increased in the presence of exogenous putrescine. However, overall growth and gas exchange values were comparatively higher in 100 mM NaCl than in 200 mM NaCl. It was also seen that putrescine significantly increased soluble sugars like fructose, glucose, and galactose in the presence and absence of salt. However, putrescine significantly affected sucrose only in the presence of 100 mM NaCl. Their content was highest on day 6 in plants treated with 100 mM NaCl and putrescine. Although putrescine decreased the relative dry weight in leaf tissues from day 0 to 3 but dropped on day 13 and again a spike in the water content on day 20, showing no significant changes. The results demonstrate putrescine is more effective on the plants treated with 100 mM NaCl vs. 200 mM NaCl. While putrescine could become an approach to promote salt tolerance, it may also help to increase carbon and nitrogen assimilation. Current research involves studying the role of endogenous polyamine under constitutive and inducible promoters in transgenic poplars.

Keywords: Putrescine, leaf spray, salt stress, hybrid poplar

IS IT NECESSARY TO APPLY CHEMICAL WEED CONTROL IN SHORT-ROTATION POPLAR PLANTATIONS ON DEEP SOIL SITES?

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Abstract

Chemical weed control has been widely applied in plantation forests around the world. However, understanding the long-term influence of weed control on stand growth and its underlying mechanisms is still limited. We conducted a six-year (2016–2021; an entire rotation) weed control study in Chinese white poplar (*Populus tomentosa* Carr.) plantations established using tall planting stock on a deep soil site, which is a typical afforestation mode for poplars in north China. Three treatments of chemical weed control were implemented: no weeding (W_0), removing weeds within 50 cm distance from the tree row (W_{50}) and removing all weeds on the ground (W_{150}). The applied herbicide in this study was glyphosate. Different tree growth indicators, soil water content (q), tree root distribution, and soil physical properties were measured. There was no weed control degree effect on q in 2017 (P = 0.4408) but significant q difference (P = 0.0140) among treatments existed in 0–30 cm soil in 2021. Different degrees of weed control did not change soil physical properties (P > 0.05) after the long-term application of chemical weeding. There were no differences (P > 0.05) in all growth indicators. But the cumulative increments of tree diameter, height, stand volume index, and biomass at the end of the rotation in W_{150} were numerically 2%–27% lower than W_0 and 11%–26% lower than W_{50}. The total fine roots biomass increased with decreasing weed control degree, although the difference was non-significant (P > 0.05). The fine root distribution pattern indicated that there might be many fine roots below 200 cm depth. The tree growth response to weed control may be attributed to tall planting stock that avoids light competition from weeds and deep soil resource exploration by tree roots that alleviate competition by shallow weed roots. Consequently, it may be unnecessary to apply chemical weed control in poplar plantations established using tall planting stock on deep soil sites. Our findings provide guidance for the sustainable management of industrial plantation forests.

Keywords: Competitive vegetation management, Industrial plantation forest, Forest management, Chemical weeding, Populus

ASSIMILATION EFFICIENCIES AND GAS EXCHANGE RESPONSES OF FOUR *Salix* species IN ELEVATED CO_2 UNDER SOIL MOISTURE STRESS AND FERTILIZATION TREATMENTS

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Abstract: Assimilation to internal CO_2 (*ACi*) response curve and gas exchange parameters were quantified for four North American willows ((*Salix cordata* (COR), *S. discolor* (DIS), *S. eriocephala* (ERI) and *S. interior* (INT)) grown in a 2 x 2 factorial of atmospheric CO_2 and soil moisture treatments. After the first year of greenhouse growth, we saw no difference in aboveground stem biomass between CO_2 treatments. In the second year, a second experiment on a subset of well-watered, coppiced willows was conducted in a 2 x 2 factorial of atmospheric CO_2 and soil fertilization treatments. In both experiments, the maximum rate of carboxylation (V_{cmax}) significantly declined for all four species in response to elevated CO_2 (eCO_2). In response to a drought treatment (DRT), V_{cmax} declined, except for INT which increased V_{cmax}. In both experiments, INT had the greatest V_{cmax}, maximum rate of electron transport (J_{max}) and triose phosphate utilization, followed by COR and ERI, with DIS having the lowest values. Fertilization (FERT) reduced the species J_{max} to V_{cmax} ratio by one third, and FERT resulted in a strong increase in assimilation (A) and stomatal conductance (G_{wv}) by 92% and 119%, respectively. DRT increased the species A:G_{wv} ratio by a factor of three. G_{wv} is the primary driver and A is a minor driver of water use efficiency (WUE) under DRT. In addition, FERT mitigated the V_{cmax} and A downregulation in eCO_2, but eCO_2 did not mitigate the DRT downregulation effect. Differences between INT and the other three willows in a number of adaptive traits and responses related to drought may reflect the evolutionary origins of INT and the taxonomic group *Longifoliae* in the arid southwest USA and Mexico.

Keywords: ACi curves; elevated CO_2; gas exchange, soil fertilization; soil moisture stress.

VARIATIONS IN WATER-BALANCE COMPONENTS AND CARBON STOCKS IN POPLAR PLANTATIONS WITH DIFFERING WATER INPUTS OVER A WHOLE ROTATION: IMPLICATIONS FOR SUSTAINABLE FOREST MANAGEMENT UNDER CLIMATE CHANGE

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Abstract

Understanding the long-term variations of stand water balance and carbon stocks under different water inputs is crucial for sustainable forest management under climate change. However, due to the lack of in-situ data, how forest plantations respond to variation in water inputs during stand development remains poorly understood. We varied water inputs with distinct irrigation amounts and measured the water-balance components, carbon stock growth, and water productivity during a whole rotation (2015–2019) in poplar plantations. Furthermore, in 2020, soil water contents in our stand and an adjacent 37-year-old poplar plantation were measured. Under rainfed conditions, soil water storage of different layers decreased greatly year by year, especially at the 2–3 m depth, such that transpiration was curtailed in 2019, a dry year. By 2019, the 0–2 m depth layer became periodically dried, and the 2–3 m was persistently dried, which was further confirmed by observations in 2020. Additionally, serious soil desiccation occurred throughout the 0–6 m soil depth in the 37-year-old poplar stand. Increasing the water inputs avoided stand water stress and decreased the drying rate of the deep soil. Furthermore, the highest water inputs treatment brought great increases in groundwater recharge, carbon stock growth, and water productivity. This treatment also led to 67% higher soil water storage in the 0–1 m soil layer and 23% higher soil water storage in the 1–6 m layer by the end of 2019, as compared to the rainfed treatment. However, these advantages were small or disappeared if the water inputs were insufficient. Our findings will be helpful to predict water relations and facilitate sustainable forest management under climate change in water-limited regions..

Keywords: Transpiration, Groundwater recharge, Soil desiccation, Water balance, Afforestation

DIURNAL AND NOCTURNAL TRANSPIRATION BEHAVIORS AND THEIR RESPONSES TO ENVIRONMENTAL FACTORS OF *Populus tomentosa* IN THE NORTH CHINA PLAIN

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Abstract

For theories of forest water utilization over day-night cycles, it is important to have a reliable information on plant transpiration behaviors during daytime and nighttime and their environmental controls. With the help of thermal dissipation probes, our study investigated the seasonal variations in the diurnal (Q_{day}) and nocturnal stand water use (Q_{night}) of a *Populus tomentosa* plantation and explored their responses to groundwater table (*GT*) fluctuations and the meteorological factors. The results indicated that Q_{night} occurred on most nights during the growing season in the *P. tomentosa* plantation, but with a different seasonal dynamic from Q_{day}. A distinct increase in the ratio of nocturnal to daily water use appeared at the beginning and the end of the growing season. During the experimental period, stem refilling accounted for 61% of Q_{night}, indicating that *P. tomentosa* was able to draw on stored water in the stem to support transpiration. The Q_{day} and Q_{night} were positively and negatively related to *GT*, respectively. When the *GT* varied within 170 cm of the surface, the proportion of stem refilling accounting for Q_{night} increased with *GT*. The positive and negative correlations were found in meteorological factors with Q_{day} and Q_{night}, respectively. Among the single meteorological factors, vapor pressure deficit (*VPD*) and photosynthetically active radiation (*PAR*) explained the highest variation in Q_{day} in 2010 and 2011, respectively. In contrast, Q_{night} was dominantly affected by *VPD* in 2011. Nocturnal stand water-use could help *P. tomentosa* to recharge the consumption of water stored in the stem and overcome the seasonal drought. The compensation effect of groundwater on the nocturnal stand water-use of *P. tomentosa* could reduce its consumption of water stored in the stem. As the stand age of *P. tomentosa* plantation varied, the key meteorological drivers of both Q_{day} and Q_{night} also changed. Our results enable better understanding of the water-use strategies of poplars in the North China Plain and can lead to refinement of current silvicultural techniques.

Keywords: Sap flow; Nocturnal water use; Stem refilling; Groundwater fluctuation; Poplar

ADAPTING 3-PG PARAMETERS TO DECIDUOUS TREES: POPLAR SHORT ROTATION PLANTATIONS UNDER MEDITERRANEAN CONDITIONS

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Abstract
Climate change mitigation and adoption of a sustainable bio-based economy are key EU objectives targeted at ensuring smart, green development. Biomass plantations are expected to play a major part in promoting the bioeconomy strategy. The characteristics of poplar (*Populus* spp.) plantations grown under Short Rotation Coppice (SRC) are of interest due to their rapid growth and capacity for vegetative reproduction. Short Rotation Coppice contribute to the pool of woody raw material for biofuels and/or bioproducts, supporting rural economies and providing environmental benefits.
Growth and yield prediction is a valuable management tool. In this regard, this work aims to review the need to adapt the process-based model 3-PG (Physiological Principles Predicting Growth) to a deciduous species such as poplar, growing in SRC under Mediterranean conditions. Two different water scenarios were considered in order to take into account the impact of climate change. A total of 590 trees were sampled from a poplar short rotation plantation of the highly productive poplar hybrid *P.* × *canadensis* ('AF2') over a rotation period of three years and planting density of 10,000 trees ha^{-1}.
Based on this information, the specific objectives are not only to determine all the required species-specific parameters but also to adapt the 3-PG model architecture to the poplar SRC plantations, as well as identifying the parameters which present a significant response to restrictive irrigation water.
For deciduous trees, the 3-PG model has not yet been fully defined. The current problems arise from the lack of model adaptation for this type of plantation. As part of the adaptation of the model, an in-depth study of the foliar fraction in poplar SRC systems is necessary. Leaves develop progressively, reaching maximum foliage before senescing and falling (litterfall), a process that lasts at least 3 months. As the tree has no leaves during winter no transpiration takes place and spring flushing is dependent on reserves accumulated in the roots and stem.
This study aims to provide more realistic output variables, which would be of interest to end-users of the model, taking into account different water requirements of the studied species in order to ensure sustainable management of SRC plantations.

Keywords: 3-PG, Poplar, Short Rotation Coppices, Biomass estimation, Water reduction.

LEAF AND TREE WATER-USE EFFICIENCIES OF *Populus deltoides* × *P. nigra* IN MIXED FOREST AND AGROFORESTRY PLANTATIONS

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Abstract

In a global context where water will become a scarce resource under temperate latitudes, managing tree plantations with species associations, i.e. forest mixture or agroforestry, could play a major role in optimizing the use of this resource. Conceptual frameworks in community ecology suggest that, in mixed plantations, resources such as water may be more efficiently used for carbon acquisition and tree growth thanks to niche complementarity among species. To test the hypotheses behind these conceptual frameworks, we estimated water-use efficiency (WUE) for poplar trees grown in a monoculture, in association with alder trees (forest mixture), and in association with clover leys (agroforestry) in an experimental plantation located in northeastern France. The experience focused on the 7th tree growing season. WUE was estimated (i) at tree level with sap flow sensors and growth increment measurements, (ii) at wood level through carbon isotope composition, and (iii) at leaf level through both gas exchange measurements and analysis of carbon isotope composition. We hypothesized that WUE will be higher for poplars in mixtures as compared to the monoculture due to a reduction in competition allowing an optimized use of water and / or a facilitation effect due to the presence of the N_2-fixing species in mixtures benefiting to poplar trees.

At the tree scale, the poplars in both types of mixtures used water more efficiently than in the monoculture, and this difference was more pronounced between the forest mixture and the monoculture than between the agroforestry treatment and the monoculture. The transpiration efficiency was 6.0 g DW $kg^{-1}H_2O$ in forest mixture, 4.0 g DW $kg^{-1}H_2O$ in agroforestry and 2.5 g DW $kg^{-1}H_2O$ in monoculture (Figure 1). The different methods used to estimate WUE gave consistent information. However, the more WUE was integrated in time (instantaneous gas exchanges < leaf life span < seasonal wood core < whole tree), the more the differences among treatments were marked. Taken together, our results showed that differences in WUE between monoculture and agroforestry were explained by differences in leaf level stomatal conductance or tree level transpiration, while on the contrary, differences between monoculture and forest mixture were likely due to differences in carbon assimilation and biomass accumulation.

From an agronomic perspective, an agricultural system where poplar is grown in association with a leguminous crop is very promising since it provides higher biomass production, as we have previously demonstrated in the same plantation, associated with less water consumption.

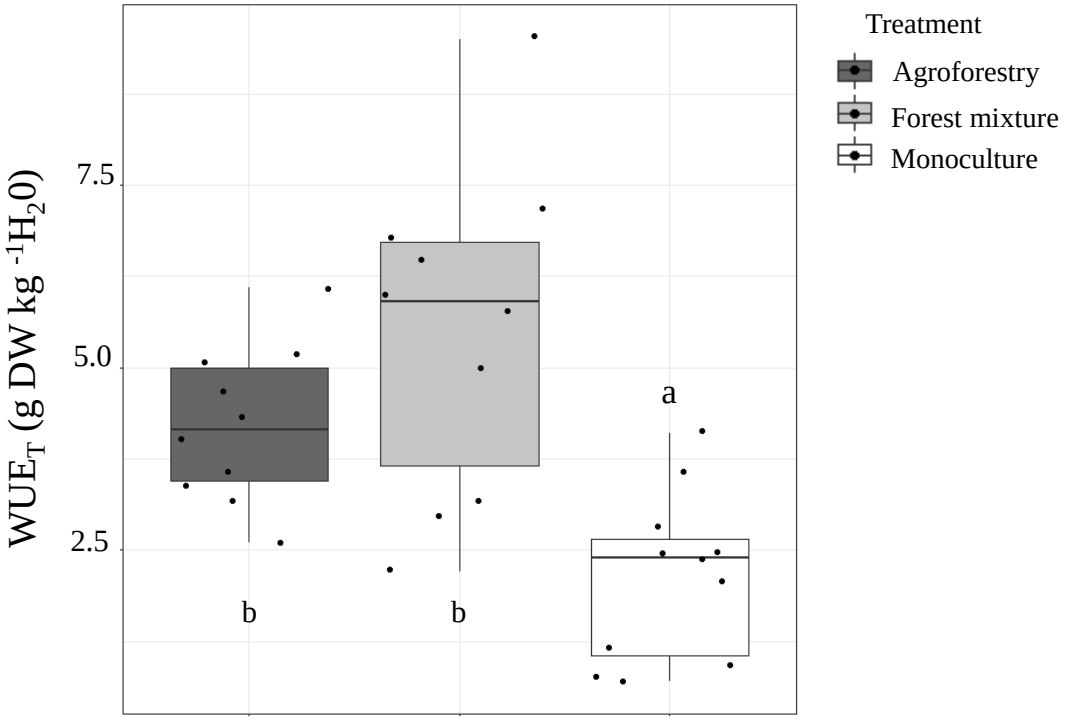

Figure 1. Transpiration efficiency (WUE_T, g DW $kg^{-1}H_2O$) for poplars in different treatments (n = 12) during the 2020 growing season (June to September). Different letters indicate significant differences between treatments.

Keywords: poplar, mixed tree plantation, agroforestry, water-use efficiency

CAN PREVIOUS EXPOSURE TO DROUGHT INFLUENCE FUTURE DROUGHT RESPONSES IN YOUNG MALE AND FEMALE ASPEN CLONES?

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Abstract
Pre-exposure to drought stress can enhance the tolerance of trees to subsequent drought. We assessed the impact of prior exposure to drought on the adaptive responses of male and female aspen (*Populus tremuloides*) to a subsequent drought stress. One-year-old male and female aspen seedlings from 11 families, originating from Peace River and Camrose in Alberta, Canada, were selected for study. In the first greenhouse experiment, trees were well-watered or experienced a prolonged drought during a period of five weeks in the summer of 2020. One complete block of seedlings from the first experiment were retained and after a dormancy period during fall/winter 2020/21, the roots were washed and placed in trays to promote suckering. As the suckers emerged, they were cut, dipped in rooting hormone and clonal replicates were taken from the original male and female seedling parent trees exposed to either well-watered or drought conditions. In summer 2021, a second greenhouse experiment was conducted with a subset of families, and clonal replicates were again exposed to well-watered or drought conditions for three weeks. During the second drought experiment, trees in the drought treatment were maintained at 75- 80% field capacity, while control trees were well-watered. Prior to the onset of the drought treatment, during drought and during the recovery period, all trees were assessed for growth (height (h), diameter (d), volume (v)), and gas exchange (net photosynthesis (A), transpiration (E), instantaneous water use efficiency (WUEi)) and biomass production at harvest (stem, leaf, and root dry matter accumulation, root:shoot ratio, specific leaf area, root length) was measured. Overall the second drought treatment resulted in significantly lower growth of h, d, v, biomass production, A, E and higher WUEi than in the well-watered control treatment. Interestingly, the previously droughted trees had longer root length and a higher root: shoot ratio in response to the second drought treatment. Previously droughted female trees outperformed male trees for growth when exposed to a second drought compared with previously droughted male trees. Regardless of region of origin, families showed variability in performance, where specific families appeared to be better adapted to drought stress than other families. Our findings provide evidence that previous exposure to drought can increase a trees' ability to respond to subsequent drought stress and that male and female clones showed a differential response to drought stress.

Keywords: *P. tremuloides*, stress response, drought, sex, greenhouse

LEAF MASS PER AREA, NITROGEN, AND $\delta^{15}N$ REFLECT WATER AND NITROGEN USE AND TRADE-OFFS ACROSS EASTERN COTTONWOOD AND SEVEN HYBRID POPLARS

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Abstract
Knowing the mechanisms and the trade-offs of *Populus* water and photosynthetic nitrogen use efficiency (WUE and PNUE, respectively) under different soil conditions are essential for breaking the bottleneck for bioenergy production using *Populus* under minimum input and water scarcity scenarios. We selected and planted over 100 clones of eastern cottonwoods and seven taxa of *Populus* hybrids in two different sites in the southeastern US. We measured the leaf gas exchange, leaf mass per area (LMA), leaf nitrogen content, nitrogen content per area (N_{area}), photosynthetic nitrogen use efficiency (PNUE), and stable carbon and nitrogen isotope ($\square^{13}C$ and $\square^{15}N$). We find that gas exchange and carbon isotope derived WUE (WUEiso) are positively and significantly correlated with each other with significant variation across eastern cottonwoods and hybrid poplar under different soil conditions. WUEiso is more sensitive to soil nitrogen availability and leaf nitrogen content. Pontotoc site has double soil nitrogen content (1.06 mg/g) as the Monroe site (0.49 mg/g). We find significantly higher LMA (13%), leaf nitrogen content (11%), N_{area} (26%), photosynthesis (24%), and WUEiso (17%) but slightly lower PNUE (3%) at the Pontotoc site than at the Monroe site. Eastern cottonwoods have significantly higher WUEiso under nitrogen-limited conditions thanks to their higher nitrogen exploitability or better symbiotic relationships with soil microbes than the hybrid poplars, while hybrid poplars show better performance under non-nitrogen limited conditions indicating their nitrogen remediation potential. The $\square^{15}N$ is found to be significantly correlated with WUEiso (all negatively correlated), and PNUE (positively for the Pontotoc site, but negatively for the Monroe site) and is maintained below 4 ‰ at the Pontotoc site, while above 5 ‰ at the Monroe site. These results may suggest that $\square^{15}N$ can be an index of soil nitrogen deficiency and trade-offs of leaf nitrogen reallocation. Trade-offs between WUEiso and PNUE of *Populus* happen when plants grow under resource-limited soil conditions. The physiological performance and trade-offs can be indicated by easily-derived variables such as LMA, leaf nitrogen content, and $\square^{15}N$.

Keywords: *Populus*; Hybrid poplar; Bioenergy; Photosynthetic nitrogen use efficiency; Water Use Efficiency; Isotope Carbon and Nitrogen

RESPONSE OF THE POPLAR ROOT SYSTEM ARCHITECTURE TO A LOCAL SOIL MECHANICAL STRESS

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Abstract
The development of the root system allows for soil resource foraging and its 3D spatial arrangement inside the soil is known as the root system architecture (RSA). Since soil is spatially and temporally heterogeneous in terms of chemical and physical properties, RSA plasticity contributes to better face local stresses and maintain resources acquisition. In poplar plantations, forest machinery and short rotations can lead to significant soil compaction and endanger wood production sustainability (Virto et al., 2015) highlighting the need to understand the physical limitations to root growth in soils.
In order to study the response of RSA of poplar woody cuttings to mechanical stress, we perform a split root experiment, in rectangular flat rhizoboxes (28 x 19 x 3 cm) with two contrasting substrates: a peat and fine sand mix (50/50 v/v), and a coarser substrate made of the mix added with 55% of quartz stones (3-7 mm). We hypothesized that 1) the mechanical stress due to the presence of stones would result in variations of the poplar RSA and that 2) a local stress would affect the RSA in the other compartment through a systemic signal.
The analyses of the root traits and RSA within each compartment showed that the root dry weight, the total root length and the mean adventitious root length were reduced but that the mean lateral root length was higher in the stony compartment. We also found that the presence of a local mechanical stress (presence of stones in the soil) affected the root growth and the RSA at a distance from the stress, in the "soft" substrate compartment, suggesting a systemic signal from one side of the root system to the other. A further description of architectural traits based on detailed image analysis such as root diameter and lateral root distribution and density will complete these results.
Such studies open the door on the understanding of the effect of mechanical stress on the root system development, thus paving the way for new solutions that would preserve the root system of trees under such stress and selecting plant cultivars that behave better in such situation.

Keywords: Poplar, root system architecture, split root, mechanical stress

CHARACTERIZATION OF SALT RESPONSE IN POPLAR PLANTS OVER-EXPRESSING A TONOPLAST AQUAPORIN

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Abstract

In recent times, one of the evolving abiotic stresses for plant survival is salinity. Response to salinity was investigated on *Populus alba* L. 'Villafranca' clone transgenic plants (lines 16, 22, and 24) over-expressing an aquaporin – *aqua1* - (GeneBank: GQ918138) of *P. x euramericana* 'I-214' and compared to *P. alba* wild-type (Wt). Plants were grown *in vivo* under control conditions (0 mM NaCl) and salinity (100 mM NaCl) for 28 days. *Aqua1* gene was over-expressed in leaves of transgenic lines 16 and 24 under 0 mM NaCl (+156 Fold Change - FC and 32 FC respectively) and 100 mM NaCl treatment (+185 FC and 74 FC), confirming that the treatment did not influence the *aqua1* up-regulation in the transgenic plants. No difference among *aqua1* over-expression was observed for line 22 in comparison to the Wt plants. Transgenic (*aqua1*) and Wt plants were phenotypically indistinguishable. Over-expression of *aqua1* did not influence net photosynthesis, stomatal conductance and chlorophyll fluorescence. At the end of the experiment, the control plants showed Fv/Fm values in the average of 0.83, 0.78, 0.82, and 0.76 in Wt, over-expressing lines 16, 22, and 24 control plants; in contrast, treated plants reduced the Fv/Fm ratio by 25, 15, 27 and 12% compared to each control. After 28 days of treatment, the shoot elongation was not different among lines (P=0.198). The control plants growth was on average 30.9, 33.4, 30.7 and 27.7 cm in Wt and over-expressing lines 16, 22, and 24, respectively. NaCl treatment induced a significant decrease in shoot elongation (P<0.0001) in all lines studied: data indicated a reduction –by 28%, 35%, 39%, and 32% in Wt and over-expressing lines 16, 22 and 24, respectively, compared to their corresponding control. The over-expression of *aqua1* determined a significant decrease in Na concentration in the roots of transgenic lines. Line 16 and line 24 showed the lowest Na concentration with a decrease of 40 and 16% in comparison to the Wt plants. On the contrary, in woody stem, stem and leaves these differences in response to excess Na were not significant when compared to Wt. In conclusion, the over-expression of *aqua1* did not affect physiological parameters but favoured a reduction in Na concentration at root level. These findings, combined with previous studies on aquaporins, suggest that *aqua1* could be involved mainly in cell growth or elongation.

Keywords: poplar, salt stress, *aqua1*, aquaporin, TIP

EFFECT OF HETEROGENEOUS SALINITY ON *Populus alba* L. 'Marte' CLONE USING A SPLIT-ROOT SYSTEM

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Abstract
Species and clones of *Populus* have shown a different degree of sensitivity or tolerance towards salinity. Soil salinity is often not uniform and, to our knowledge, only one study is currently available on poplar response to a heterogeneous saline environment. In this work, plants of the *Populus alba* L. ' Marte' clone were grown outdoors in pots in a split-root system for 26 days and their response to 100 mM NaCl was investigated. One group of plants was watered with no salt on both sides of the system (0/0 mM), one with salt on both sides (100/100 mM) and one with salt on only one side (0/100 mM). Four plants were assigned to each group and two single shoots were grown for each plant. At the beginning of the experiment plants had an average shoot length of 52.5 ± 13.8 cm. Several morphological and physiological parameters were monitored regularly during the experiment, and mineral analysis (Na, Ca, K, Mg) and leaf and stem relative water content (RWC) were evaluated at the end. After 26 days, plants watered with 100 mM NaCl (on one or both sides) showed noticeable symptoms on leaves, and the radical system showed an evident difference between sides in the 0/100 mM group. Shoot elongation, diameter increase, and number of new/lost leaves were not significantly different within and among each group. Leaf relative chlorophyll content and chlorophyll a fluorescence did not differ among treatments. On the other hand, there was a decrease in terms of net photosynthesis (Pn) and stomatal conductance (Gs) in plants treated with salt, though there was no variation in water use efficiency (WUE). In particular, when compared to the 0/0 mM group, the 0/100 mM group showed an average decrease of 40% and 41% in Pn and Gs, respectively, and the 100/100 mM group had reductions of 85% and 87%, respectively. RWC showed a slight decline in leaves in the uniform stressed group (100/100 mM). Mineral content analysis showed that Na of the 0/100 mM plants, which was absorbed by one side of the plants, was equally distributed in both shoots, and reached a concentration in aerial organs between that of the other two treatment groups, as a confirmation of the physiological results obtained. Even if not significant, we also observed a higher accumulation of Na in the non-stressed root of the 0/100 mM group compared to the 0/0 mM group, which could be due to Na re-translocation. Other potential long-distance response mechanisms will be evaluated in the near future.

Keywords: *Populus*, poplar, salt stress, split-root, heterogeneous salinity

EARLY EFFECTS OF TWO PLANTING DENSITIES ON GROWTH DYNAMICS AND WATER-USE-EFFICIENCY IN *POPULUS DELTOIDES* × *P. NIGRA* AND *ROBINIA PSEUDOACACIA* SHORT ROTATION PLANTATIONS.

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Abstract
As a renewable energy resource, woody biomass has a role to play in reducing worldwide fossil fuel consumption. Species in the genera *Salix* and *Populus* are widely used for this purpose through short rotation tree plantations under temperate latitudes. In warmer climates, common short rotation species include genera such as eucalyptus or black locust (*Robinia*) although this latter is also cultivated in temperate climates. The objective of our study was to compare the potential of poplar (*Populus deltoides* × *P. nigra*, clone 'Dorskamp') and black locust (*Robinia pseudoacacia*, provenances 'Nagybudmerii' and 'Nyirseg') for biomass production in the Northwest of France. We estimated biomass production and efficiency of water use of poplar compared to those of the two provenances of black locust, (1) at two planting densities (short rotation forestry: SRF, 1428 trees per ha *vs*. short rotation coppice: SRC, 7272 trees per ha), and (2) 2 and 4 years after planting. Only few effects of the initial planting density on plant growth and physiology were visible two years after planting. Stem height and circumference became higher in SRF than in SRC for both species after four years. Yield at plantation level of the first SRC rotation was almost twice as high for black locust than for poplar, while both species exhibited similar stem height or stem increase rate likely due to the development of more sylleptic branches in black locust. At the end of the second rotation, plantation yield was significantly higher for poplar due to black locust mortality. The combination of higher leaf N and greater WUE probably enhanced black locust performance under the dry conditions of our study site in 2010. During the fourth year (2012), the gap between the two species in terms of $\Delta^{13}C$, used as a surrogate of water-use efficiency, was less marked than during the first years due to the fact that poplar $\Delta^{13}C$ decreased (increase in WUE), while black locust $\Delta^{13}C$ slightly increased (decrease in WUE). To conclude, black locust appears as an interesting option for biomass production at sites prone to water shortage. However, at high planting density, poplar exhibited a lower mortality after harvest leading to an improved biomass production at the end of the second SRC rotation compared to black locust. The choice of species/cultivar must be adapted to the production system (SRC *vs.* SRF) and to the specific site conditions.

Keywords: Biomass production, Water-use efficiency, short rotation plantations, Poplar, Black locust

PHYSIOLOGICAL AND BIOCHEMICAL DRIVERS OF SOIL CARBON ACCUMULATION IN EASTERN COTTONWOOD AND HYBRID POPLAR TAXA

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Abstract

Although high aboveground productivity is important for bioenergy feedstocks, concurrent belowground carbon accumulation provides ecosystem services that can potentially be monetized by landowners to decrease the overall cost of poplar bioenergy production. However, belowground carbon accumulation is time consuming to measure making genotype-specific data scarce even though it would be useful to landowners who want to optimize this ecosystem service in addition to aboveground productivity. Therefore, our objectives were to measure tree- and genotype-specific soil carbon accumulation and determine if aboveground leaf or whole tree metrics correlate with belowground carbon accumulation in eastern cottonwood and hybrid poplar taxa. We planted two field trials in the southeastern United States that contained over 100 genotypes of eastern cottonwood and hybrid poplars. Over two to three growing seasons, we tracked soil carbon accumulation in the 0-10 cm and 10-30 cm soil depths around specific trees and measured physiological parameters of these trees including leaf gas exchange, biogeochemical properties and whole tree water use. We found that, across genotypes within sites, aboveground biomass was not correlated with belowground carbon accumulation, although our site with lower aboveground productivity exhibited soil carbon accumulation that was over an order a magnitude higher than our site with higher aboveground productivity. At the leaf level, transpiration rates and leaf carbon concentration were positively correlated with soil carbon accumulation. At the whole tree level, seasonal water use derived from sapflow data was positively correlated with soil carbon accumulation. Overall, these results suggest that there is not a tradeoff between aboveground productivity and belowground productivity and genotypes can potentially be selected that optimize both above- and belowground productivity. Likewise, sites that are potentially not optimal for aboveground productivity may exhibit high belowground productivity and high potential for soil carbon accumulation. Finally, leaf and whole tree water use parameters as well as leaf carbon concentrations were positively correlated with soil carbon accumulation, suggesting the importance of root production and leaf carbon in overall soil carbon accumulation in eastern cottonwood and hybrid poplar plantations.

Keywords: belowground productivity, *Populus*, tree water use, feedstock production, ecosystem services

AGRICULTURE AND THE ENVIRONMENT – BRIDGING THE DIVIDE THROUGH WILLOWS

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Abstract

Native *Salix* (willow) species can provide large reservoirs of natural phenotypic and genotypic variation relevant to industrially-important traits. Focusing on pan-Canadian native *Salix eriocephala* and hybrid willows, our specific goals were to thoroughly understand the genetic underpinnings of carbon sequestration and cell wall trait variations and to use this information to accelerate willow breeding and selection in Canada, and to understand the genetic basis of the willow species' adaptation to environment. In a large set of *Salix eriocephala* populations, we phenotyped several traits: seasonal phenology, biomass accrual, nutrient acquisition, health/disease traits, and wood physio-chemical properties. These data, in addition to single nucleotide polymorphisms, were used for unweaving the population structure and genetic diversity. Selected examples of the results of these analyses, and their potential applications within the context of agroforestry for nutrient-rich riparian site management, phytoremediation of salinity-prone marginal lands, phosphorus mitigation from ag-runoff, value-added biochar amendment to improve soil health and low-cost bioburner adoption in rural communities will be discussed. The focus of my presentation will be on how form and function interact with the environment.

Keywords: willow, photosynthesis, biomass, nutrient uptake, biochar

INTRINSIC AND EXTRINSIC FACTORS INFLUENCING *POPULUS* WATER USE: A LITERATURE REVIEW

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Abstract
Poplars (*Populus* L. spp.) are versatile, productive trees that are used in environmental systems worldwide to provide a variety of benefits. Though poplars are recognized for their high water use, summaries of existing data on poplar water use, and its influencing factors, are lacking. We sought to 1) summarize the sap flow methodologies used to quantify poplar water use and 2) assess the effects of different intrinsic (plant variables) and extrinsic (environmental variables) factors on poplar water use. We conducted a standardized literature review of *Populus* sap flow studies using the Web of Science database. We identified 133 articles containing information on the methodologies used to measure poplar sap flow. Of these, the thermal dissipation method was used in a majority (55%) of the studies. Poplar water use data were reported in 51 articles, which represented 13 countries, and spanned the time period of 1992 to 2018. Hybrids were studied in 18 articles and included 17 genotypes, while non-hybrids were studied in 33 articles, which included eight species. All water use data were converted to mm d^{-1} on a ground area basis and were classified into categories within different intrinsic (genetic background, tree age) and extrinsic (planting density, experimental context, water availability) factors.
Hybrid poplar water use ranged from 1.1 to 11.3 mm day^{-1}, with an average of 3.1 ± 0.3 mm day^{-1}. Non-hybrid water use ranged from 0.2 to 11.8 mm day^{-1} with an average of 2.5 ± 0.3 mm day^{-1}. Hybrid poplar water use differed significantly among tree age classes (P = 0.0124), with young hybrid *Populus* having significantly greater water use than mature hybrids (P = 0.0107). Hybrid water use also was significantly different among water availability classes (P = 0.0057). Hybrids grown with high water availability had significantly greater water use than those grown with medium (P = 0.0067) and low water availabilities (P = 0.0209). Non-hybrid water use, on the other hand, was significantly different among species (P = 0.0011), experimental context (P = 0.0226), and water availability classes (P = 0.0005). For these three factors, the categories with the highest water use were *P. fremontii*, shelterbelts, and high water availability, respectively. While we focused on poplar water use derived from sap flow methodologies, this review builds the foundation for a comprehensive summary of the available poplar water use information. Our results can be used to aid in the decision-making process when designing poplar-based environmental systems.

Keywords: Poplar; Water Use; Transpiration; Sap Flow

Session 3:
Physiology and Ecophysiology

Posters

MORPHOLOGICAL AND PHYSIOLOGICAL STABILITY IN TWO *Populus deltoides* COMMERCIAL CLONES: IDENTIFICATION OF DIFFERENT ACCLIMATION STRATEGIES

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Abstract
The stability in morpho-physiological traits influences the capability of *Populus* spp. genotypes for acclimating to contrasting environmental conditions. In Argentina, *Populus deltoides* 'Australiano 129/60' (A129) is the most widespread genotype in the low-diversed poplar plantations of the Delta del Río Paraná. This clone is a stable genotype that has good yields at different sites. Conversely, *Populus deltoides* 'Guayracá INTA' (GUA) is a genotype obtained few years ago from the Salicaceae breeding program developed by INTA. This new clone is potentially as productive as A129, but it is relatively unknown in its stability and capacity for acclimating to heterogeneous sites. The present study aimed to compare the morpho-physiological stability of GUA across 6 different environmental conditions representing a gradient of water and salt soil contents. One of the most common methods to evaluate stability is the regression coefficient *bi* (derived from a joint linear regression analysis). The comparison was made between GUA and A129 one-year-old plants, growing in 20 L pots under controlled conditions. The regression coefficient *bi* was calculated for 15 traits: total dry weight increment ($TOTAL_{DWI}$), stem dry weight increment ($STEM_{SWI}$), leaves dry weight increment ($LEAF_{DWI}$), roots dry weight increment ($ROOT_{DWI}$), leaf area (FA), specific leaf area (SLA), stem-roots ratio (SR_{ratio}), slenderness index (SI), Dickson quality index (DQI), hydraulic conductivity (kh), specific hydraulic conductivity (ks), leaf hydraulic conductance (K_{leaf}), maximum stomatal conductance (gs_{max}), maximum electron transport rate (ETR_{max}) and chlorophyll content (SPAD). GUA was identified as a genotype similar to A129 with respect to the stability of the $STEM_{DWI}$, with higher than average daily increment values and a *bi* index greater than 1. However, clear differences were observed in the *bi* values of the $TOTAL_{DWI}$ and $LEAF_{DWI}$. The clones were similar in the stability of water-use traits such as kh, ks, and K_{leaf}, but were completely different in ETR_{max} stability. While A129 was identified as a stable genotype in terms of ETR_{max}, GUA was observed as a clone that increases its photosynthetic capacity as growth conditions improve. These differences could be traduced in different above and belowground site occupations because of the variation in biomass partitioning, and in different nitrogen use derived from clonal photosynthetic variability. Both aspects are important to achieve a more diversified forest system in the region, sustaining or improving the level of productivity.

Keywords: Poplar, Delta del Río Paraná, phenotypic plasticity, regression coefficient.

EFFECTS OF CLONE, IRRIGATION AND PLANTATION DENSITY ON XYLEM HYDRAULIC CONDUCTIVITY IN A SHORT ROTATION COPPICE WILLOW PLANTATION

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Abstract
Short rotation coppice (SRC) plantations with willows are important for the production of bioenergy and for environmental services because of their fast growth rates. The rapid growth implies high transpiration rates, that needs to be sustained by a concomitant water transport capacity of the xylem. The aim of this work was to analyze the effects of water availability, genotype and plantation density on xylem hydraulic conductivity in willows.
The field trial was situated in La Plata, Argentina, and included two irrigation treatments (rainfed and irrigated), two plantation densities (13,333 and 20,000 plants ha^{-1}) and two willow clones (Barrett and Yaguareté), arranged in a split-split plot design. The plants were coppiced every year during winter. The sampling dates were December 15, 2021 and February 15, 2022. The stems were harvested in the morning, moved to the laboratory and the segment to be measured was re-cut under water. The hydraulic conductivity determinations were performed the same day by perfusion and weighing of the extruding water. The parameters determined were: hydraulic conductivity per unit stem length (kh), hydraulic conductivity per unit xylem area (ks) and hydraulic conductivity per unit leaf area (kl). Data were analyzed with a mixed model with irrigation, density and clone as fixed effects and block as random effect. The block and interaction effects were non-significant in all measurements.
We expected that a higher density would increase competition between the plants and affect the hydraulic conductivity, but the plantation density had no significant effect on any measurement.
There was no significant effect of any of the factors on ks. Nevertheless, the xylem conductive area was significantly affected by irrigation and clone. In consequence the total conductive area per plant was different, even if the conductivity per unit of xylem area did not change.
Clone and irrigation had a significant effect on kh in the first sampling date, but only clone was significant in the second date. Irrigation had a significant effect on kl in the first date, but not in the second one. Clone and irrigation had a significant effect on leaf area per branch, irrigated plants had a higher leaf area than rainfed ones, and clone Barrett had a higher area than Yaguareté.
From previous studies, we know that Barrett is more tolerant to drought than Yaguareté, probably because they have different susceptibility to cavitation. This point will be addressed in future studies.

Keywords: *Salix sp.*, leaf area, xylem vessels.

DISTINCT MECHANISMS CAN PROMOTE *Populus deltoides* ACCLIMATIZATION UNDER ZINC STRESS

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Abstract
Increasing metal inputs in the environment are of great concern since exceeding level of trace elements can trigger physiological and biochemical processes in plants. Apart from being an essential cofactor of several enzymes driving various metabolic pathways in plants, and having essential role in plant growth, elevated zinc (Zn) concentration can provoke detrimental alteration of plant metabolism, and hinder plant growth and development. Poplars, as fast-growing trees are deployed as effective phytoremediation tools since different genotypes can exhibit distinct tolerance levels and heavy metal accumulation patterns.
Here, we tried to unravel the underlying mechanisms involved in *Populus deltoides* acclimatization to elevated Zn concentration on the basis of physiological changes, ionomic and transcriptomic analyses. Poplars (clone PE19/66) were grown in hydroponic culture and exposed to optimum (1 μM) and excessive (1 mM) Zn level.
Plant growth and biomass were mainly affected on roots levels, by higher Zn dose applied. Elevated Zn content provoked a decrease in leaf gas exchange parameters, including net photosynthetic rate, transpiration and stomatal conductance, with the exception of substomatal CO_2 concentration. At the same time, various parameters related to the chlorophyll fluorescence were not affected by high Zn level. Macronutrients (potassium, calcium and magnesium) and trace elements (zinc, iron and sodium) distribution were determined and showed in plant organ-depended manner. Zn partitioning has followed the decreasing trend: roots > stem > mature > developing leaves. Antagonistic interaction between Zn and K, Ca, and Mg level was observed in roots, where higher Zn level has induced lower nutrients uptake. Efficacy of antioxidant machinery against oxidative stress damages was more evident in roots, where glutathione-S-transferase and glutathione peroxidase were significantly increased, as well as, proline content, while levels of reduced glutathione and catalase activity were slightly elevated in leaves. Additionally, transcriptional levels of different heavy metal transporters, which possess pivotal role in maintaining metal homeostasis, were assessed in meristem and elongation zone, versus differentiation root zone. NRAMP1 gene was downregulated in meristematic and elongation zone, while it was upregulated in differentiation zone, indicating its important role in adsorption and transport of heavy metals. Contrary, the prooxidative gene RBOHD was overexpressed in both root zones under high Zn level, due to its role in stress signaling, while GAUT4 showed an opposite trend.
The findings of this study give more in depth information of the mechanisms involved in poplars acclimatization to high Zn level, supporting its usage in phytoremediation process.

Keywords: poplar, trace elements pollution, photosynthesis, abiotic stress and antioxidative response, gene expression

DIGGING DEEPER INTO POPULUS CUTICULAR WAX COMPOSITION

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Abstract
The cuticle is the first line of defence against the environment and is composed of a cutin polymer and a mixture of lipids referred to as cuticular waxes. The cuticle plays significant roles in environmental adaptation, protecting plants against biotic and abiotic stress. The aliphatic lipids of the cuticle are derivatives of very-long-chain fatty acids that are functionalized as distinct chemical classes. Previous research found that natural accessions of *Populus trichocarpa* vary in their leaf wax chemical composition. In this study, we examine the wax diversity in stems and leaves of *P. trichocarpa* and *P. balsamifera,* two sister species found under contrasting environmental conditions in Canada. We hypothesize that the chemical composition of the cuticular wax reflects the adaptation to their particular habitats.
The wax in leaves and stem tissues from four accessions of *P. trichocarpa* and four accessions of *P. balsamifera* was characterized using gas chromatography-mass spectrometry. This survey found variation in wax composition depending on the accession, tissue, and developmental stage. Different chemical classes of waxes were identified including alkanes, alkenes, alcohols, fatty acids, aldehydes, phenolics, triterpenoids and alkyl hydroxycinnamates. Some of these compounds had tissue-specific accumulation, for example, alkenes accumulated only on the leaf. Others were species-specific, e.g., aldehydes were only detected in *P. trichocarpa*. Due to poplars' long life-span and widespread range of distribution, the metabolic diversity observed in these accessions might be an adaptation to the local environment.

Keywords: Cuticle, wax, *Populus* species, adaptation

THE IMPACT OF DIFFERENT ENVIRONMENTAL CONDITIONS DURING VEGETATIVE PROPAGATION ON BIOCHEMICAL CHARACTERISTICS IN POPULUS HYBRIDS IN CLONAL FIELD TRIAL

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Abstract
This study investigates epigenetics phenomena, phenotypic plasticity, genotypic variation, heritability, ecovalence of growth and total phenolics compounds of *Populus* hybrids in field trials which may be predisposed by the simulated contrasting temperature conditions at their vegetative propagation phase. The clonal test plantation in Jonava was established using vegetatively propagated plants rooted and grown under contrasting environmental conditions simulated in the Phytotron of LAMMC Institute of Forestry. The largest impact of rooting and growing treatments in greenhouse under vegetative propagation stage on amount of phenolic compounds in transplanted to field trial trees was observed for hybrid *P. trichocarpa* × *P. trichocarpa*. High estimates of hybrides' ecovalency indicate that this hybrid had ecological sensitivity and epigenetic phenomena can occur. Amount of total phenolics compounds is tightly controlled genetically, however rooting-growing temperature conditions had an impact on the heritability of traits. It was found that traits (the chlorophyll A and B, pigments content and the chlorophyll A/B ratio) of hybrid poplar clones grown in field trials, as well as their traits' genetic parameters, were affected by the rooting-growing conditions during vegetative propagation phase. Hybrids *P. balsamifera* × *P. trichocarpa*, and *P. trichocarpa* × *P. trichocarpa* have shown the most substantial changes of biochemical traits across vegetative propagation treatments in field trial. Rooting-growing conditions during vegetative propagation had also an impact on coefficients of genotypic variation and heritability in hybrid poplar clones when grown in field trials. The results suggest that certain environmental conditions during vegetative propagation not only have a short-term effect on tree viability, but also can help to adapt to climate change conditions and grow successfully in the long-term.

Keywords: phenolic compounds, chlorophyll, epigenetics, ecovalency, *Populus* hybrids

Session 4:
Pests, Diseases and Climate Change Impacts

Oral presentations

VOLATILE ORGANIC COMPOUNDS IN DEFENCE RESPONSES OF HYBRID POPLAR TO PHYTOPHTHORA CACTORUM AND PHYTOPHTHORA PLURIVORA

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Abstract

End-products of plant metabolism, such as volatile organic compounds (VOCs), are important molecules released from plant tissues during abiotic or biotic stresses. Active emissions of VOCs play important roles in priming defence responses, repelling herbivores, attracting their predators or beneficial microbes for plant immunity. In this study, the emissions of VOCs in response to *Phytophthora cactorum* and *Phytophthora plurivora* inoculations were studied in field-grown 10-year-old hybrid poplar, *Populus tremula* × (*Populus* × *canescens*). After initial well development of the pathogens within the bark tissue, typical cankers and lesions were observed together with a slight flow of dark exudates. At day 9 post-inoculation (p.-i.), 23 VOCs were identified in the control plants and both *Phytophthora* treatments. Three VOCs were present each in more than the 10% relative proportion, namely 2-hexenal, 2-ethylfuran and hexanal, whereas the remaining 20 compounds were present in lesser amounts. At day 98 p.-i., 32 VOCs were identified in all treatments. Twenty-two compounds were emitted constitutively, whereas 10 season-specific compounds were found only at the end of the growing season. The emissions of both α-cubebene and germacrene D were induced solely by the two *Phytophthora* treatments, whereas these compounds were not identified in the volatile blend of the control plants. Three compounds such as 2-ethylfuran, 3-hexen-1-ol, and 2-hexenal were found among the major volatile components, each of them showed higher than the 10% relative proportion. Significant positive relationships were found between both the axial and the tangential development of bark cankers and the emissions of α-cubebene and β-caryophyllene, respectively. Four years following inoculations, for the majority of the inoculated plants the callus tissue had already closed over the bark cankers, and neither of the *Phytophthora* species colonizing the bark tissues could be reisolated.

Keywords: Bark canker, β-Caryophyllene, α-Cubebene, Germacrene D, Tolerance to disease
Acknowledgements: This work was supported by the Slovak scientific grant agency VEGA (1/0450/19).

POPLAR APHIDS AND THEIR INFLUENCE ON PHYSIOLOGICAL STATUS OF DIFFERENT POPLAR CLONES

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Abstract

In recent years, there has been an increase in the prevalence of aphids as well as an increase in the number of aphid species in poplar growing zones in Serbia. The increased prevalence of aphids resulted in much more visible aphid attack outcomes, such as chlorosis, leaf deformation, various forms of galls on the leaves, stunted shoots, plant development, and, in severe cases, plant mortality. The distinctions between aphid species.

A preference for various poplar species and clones was discovered amongst autochthonous black and white poplar clones were the most vulnerable. The aphid species shown were gathered and identified through inspection. According to the literature and the findings of this study, the total number of plant aphids documented on poplars in Serbia is 22.

Chaitophorus and *Pemphigus* are the most numerous genera. The influence of aphids on host plants was explored in the context of plant physiology research by measuring their effect on the physiological parameters of researched plant species under laboratory circumstances. The factors studied were net photosynthesis, dark respiration, pigments, nitrogen, carbon, and hydrogen content. The influence of aphids on growth and development was studied in the field using measures of photosynthesis, transpiration, and stomatal conductance.

Obtained results of photosynthesis, respiration, transpiration, and stomatal conductance lead to the conclusion that the reaction of plats on aphid attack either decreased or increased measured parameters. Obtained results of multiannual investigations showed a significant impact of aphids on host plants through their influence on investigated physiological processes.

Keywords: Poplar, aphids, clones, impact, physiology

BIOLOGICAL CONTROL OF POPLAR DEFOLIATOR *Clostera cupreata* (Butler) ON POPLARS IN INDIA

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Abstract:

Poplar is a fast-growing exotic tree species that has been extensively planted in India under various afforestation/reforestation programs. Poplar Timber is the backbone of the vibrant plywood, board, match, paper, and sports goods industries. The cultivation of poplar has generated huge employment in the rural sector in India and has overall improved the rural economy. Poplar is highly prone to insect attack, approximately, 108 insect species are causing damage. Out of these, Poplar defoliator- *Clostera cupreata* (Lepidoptera: Notontidae) is one of the most damaging pests of poplar which defoliate poplar plantations and often appears in outbreaks even causing the death of trees.

Novel, environment friendly chemicals for control of plant diseases, crop- damaging pests, and weeds are needed to replace pesticides that have been withdrawn from the market due to regulatory or economic reasons, or due to development of pest resistance. In addition, public concerns over the negative effects of synthetic pesticides on the environment and human health have heighlightened the need for biocontrol methods and pesticides derived from biological sources.

By keeping this into mind, environment friendly biopesticide was prepared from weed (*Calatropis procera*) for the management of Poplar defoliator (*Clostera cupreata)*. The 3rd instar larvae of *C. cupreata* were exposed to a wide range of concentrations (0.0625 to 2.00%). LC50 value of each sample and control was recorded simultaneously. After repeated experiments, a herbal formulation (Biopesticide) was developed and it showed almost 70% efficacies against *Closteracupreata* (poplar defoliator). Sustained experiments under outdoor cages and laboratory demonstrated 70% efficacy against poplar defoliator *(Clostera cupreata)*. Field trials of developed biopesticide were also carried out in northern part of India at farmer's field. BioPesticide is safe and economic alternatives to the synthetic insecticides and it will improve farmer's livelihood through increased productivity of poplar.

Keywords: Poplar, Poplar defoliator, *Closteracupreata* (Lepidoptera: Notontidae), environment-friendly chemicals, larvicidal efficacy

LEVERAGING *POPULUS* FIELD TRIALS TO MANAGE ENVIRONMENTAL AND HUMAN-INDUCED CHANGE

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Abstract

Introduction

Tree improvement research is a long-term commitment that is subject to challenges from environmental and human-induced change. Tree health, culture, and economics can be negatively affected by changes including climate change, pests and/or diseases, regulatory actions, and shifting economic circumstances. In the current paper we describe strategies to leverage short- and long-term field trials for managing different types of change that impact tree improvement. Field trials like those presented here could be used to test new materials and processes, as well as serve as an early warning system that measures how various changes may be affecting local and regional tree health. We highlight three main strategies of *Populus* plantation culture for establishing field trials: 1) forest conversion, 2) low-resource, and 3) Intensive Culture Hybrid Poplar (ICHP). In addition, we provide a field trial case study on the low-resource culture type. Replicated *Populus* plantation scenarios could incorporate a mix of native and proven hybrid poplar materials to assess the adaptive behavior of native and hybrid poplars across sites with varying soils, climatic conditions, and topography. Such field trials could also serve as a long-term archive and clonal/gene pool resource for accelerated tree improvement.

Keywords: Aspen; Forest Conversion; Intensive Culture Hybrid Poplar (ICHP); Low-resource Culture

AN ONLINE SYSTEM BASED ON SENTINEL 2 IMAGERY TO DETECT AND MONITOR FOREST HEALTH STATUS IN POPLAR PLANTATIONS

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Abstract
Biotic and abiotic damages in clonal poplar plantations (Populus x spp) cause a decrease in growth, which has an impact not only on the economic value of the plantation due to the amount of wood that can be obtained, but also in the ecosystem services provided by the poplars. Moreover, poplar plantations can cover large areas, which cannot be monitored every week in the field by the owners. There is therefore a need to minimize the damage to these plantations by developing an early warning system for health monitoring. The aim of this paper was to develop a user-friendly online system based on satellite imagery to detect and monitor health status in poplar plantations, so it could be used by the poplar owners/managers without previous knowledge in remote sensing.
The study area to adjust and validate the system was in the province of León (Spain), which has more than 20.000 ha of poplar plantations. We used four multi-clonal plantations to monitor damages in four different clones: I-124, AF8, Raspalje and Beaupre (with different susceptibility to Melampsora spp (rust)). In each plantation, 8 trees of each clone were monitored weekly. Diameter growth was measured from April 2021 to April 2022, while rust was assessed at tree level using a visual key with 10 levels since August 2021 until October 2021. Only rust was detected as damage in the four plots, while one of the plots showed damages as well in 8 trees due to severe water stress. In addition, 4 trees of USA 184/411, 4 of USA 49/177 and 4 of Koster were used as a control in one of the plots, due to their high (USA 184, USA 49) and low (Koster) susceptibility to rust.
A dense time series of Sentinel 2 MSI imagery was created for each plot using Google Earth Engine, and several spectral indices were computed (GNDVI, NDVI) for each one of the trees in the sample. Multiple logistic regression was carried out between the values of the spectral indices/bands and the rust scores, to determine the most useful information to predict the level of rust per tree/group of trees. Results showed the ability of GNDVI to identify high levels of rust and its suitability to detect water stress during the growing season. The dense time series allowed to detect false positives (e.g. areas without trees in the plantation). To detect rust, a change in GNDVI (%) should be used instead of an absolute thresholding value, otherwise, in the areas where there is a dense herbaceous understory the rust would not be detected. These results were integrated into a free online tool developed using Google Earth Engine and Python, so that the user draws/uploads their plantation in the map and the time period to analyse and obtains a map with the likelihood of their plot to being under biotic/abiotic stress. As conclusion, this system is highly recommended for monitoring water stress in adult poplar plantations, and it is useful as well to monitor high levels of rust.

Keywords: Diseases, Monitoring, remote sensing, Melampsora rust, water stress

SOIL CARBON AND NITROGEN CHANGES RELATIVE TO BIOMASS PRODUCTIVITY IN *POPULUS* BIOENERGY PLANTATIONS

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Abstract

Short rotation woody crops such as *Populus* species and their hybrids are important components of the bioenergy plantation landscape in the US. These trees grow quickly both above- and belowground and have the potential to increase soil carbon storage and sequestration.

To understand the relationship between growth and soil carbon and nitrogen, two experimental plantations were established in Mississippi, USA in 2018 (Monroe plantation) and 2019 (Pontotoc plantation) with four *Populus* taxa (*Populus deltoides* × *P. deltoides* (D×D)*, P. deltoides* × *P. maximowiczii* (D×M)*, P. deltoides* × *P. nigra* (D×N)*, P. deltoides* × *P. trichocarpa* (D×T) representing 50 unique clones. Soil carbon and nitrogen was measured annually at 0-10 cm and 10-30 cm soil depths along with tree productivity. Half of the experiment was coppiced at the end of the second growing season.

Growth differences were observed among taxa at both sites. At the Monroe plantation D×M clones were 7-18% taller than other clones in year 1, 2-11% taller in year 2, and 5-17% taller in year 3 ($p < 0.001$). Following coppice in 2020, D×D taxa were 6-22% taller than other clones ($p < 0.001$). At the Pontotoc plantation, D×D and D×T clones were 5-27% taller than DM and DN clones after a single growing season of growth. At both plantations, soil carbon and nitrogen content increased +10% relative to establishment baseline values ($p < 0.050$) but differences among taxa were not evident ($p < 0.050$). However, following coppicing at the Monroe plantation, soil nitrogen content decreased by 5%, although this was still larger than pre-plantation values. Preliminary results suggest coppicing had no effect on soil carbon or nitrogen, which continued to increase relative to pre-planting conditions across all four taxa.

Experimental plantations demonstrate the potential to increase carbon and nitrogen storage in soils. Interestingly, aboveground productivity differences did not directly translate to differences in belowground soil changes among taxa. Results also suggest that coppicing has no immediate detrimental impacts to soil carbon and that plantations will continue to store carbon in belowground pools following normal biomass harvesting operations. As such, belowground inputs (e.g., root exudates, turnover) may be more important sources of carbon in bioenergy plantations compared to losses from aboveground biomass removal.

Keywords: carbon sequestration, Populus, bioenergy

SCREENING OF *Populus deltoides* CLONES FOR DISEASE RESISTANCE AGAINST FOLIAR PATHOGEN

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Abstract
Poplar is among the most preferred agro-forestry tree species in the temperate and subtropical zones of the world. Despite its wider utilization, it is susceptible to attack by various pathogens and insect pests. It was reported in earlier studies that foliar diseases caused by *Alternaria alternata* and *Melampsora medusae were* found to be prevalent in poplar nurseries or plantations, which causes severe damage to yield. Thus, resistance breeding is the most effective approach for minimising the damage induced by fungal diseases. In the present study, for the screening of disease resistance against *A. alternata* and *M. medusae,* the 25 promising *P. deltoides* clones were rated on the basis of infection scale. Diseased leaves *showing typical leaf spot* symptoms were collected from different locations in Uttarakhand, Punjab, and Haryana. 28 *A. alternata isolates were recovered and* the most virulent isolate has been identified by using DNA fingerprinting and pathogenicity testing. Then the selected virulent isolate was artificially inoculated on poplar clones to identify their relative resistance. Furthermore, for resistance screening against *M. medusae,* poplar clones were evaluated under natural infection conditions in the nursery. Results of this study revealed that poplar clones showed significant differences in resistance to leaf spot and leaf rust. Four clones were found to be resistant to *A. alternata* and five were resistant against *M. medusae*. Hence, it is suggested that these resistant clones could be deployed for future breeding programme to enhance poplar productivity in northern India.

Keywords: *P. deltoides*, *A.alternata*, *M. medusae*, resistance screening.

Session 4:
Pests, Diseases and Climate Change Impacts

Posters

RESPONSE OF NEW BLACK POPLAR CLONES IN SRC PLANTATION IN CHANGING CLIMATE

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Abstract

Populus nigra is an important autochthonous woody plant that can be grown as a renewable energy source. Biomass for energy continues to be the main source of renewable energy in the EU and can play an essential role in achieving the renewable energy goal in 2050. With intensifying climate change more intense effect of changing weather can be expected also on growth of SRC plantations. Its effect was studied in field trial.

Fourteen clones of *P. nigra* from our black poplar breeding programme including one *Melampsora larici-populina* susceptible clone were used in the field trial with one reference interspecific clone 'MAX 4'. The trial was set up with hardwood cuttings at a spacing of 2.9 × 0.6 m (i.e., a plant density of 5747 plants per ha) in spring 2014. A randomized complete block design was used with six blocks and seven or eight plants of each clone in each block. The trial was harvested twice in 3-year rotation cycles. Differences in health, growth and yield characteristics among clones were statistically evaluated.

Mortality of plants within two rotations was low and was observed mostly in year of planting. The highest mortality, around 12%, was observed in the rust susceptible clone. Remaining clones had mortality lower than 5 %. Significant differences among tested clones were found in all evaluated characteristics. The mean diameters of main shoots were 37.7 – 49.8 mm and 28.8 – 42.3 mm in the first and second rotation, respectively, with the highest mean having clone 'Hélios' and 'MAX 4', respectively. In second rotation, the highest main and average shoot diameter had presumably 'MAX 4' as it had the lowest number of shoots (6.4). Oppositely, clone 'Hélios' had the highest number, i.e. 14.3. In the first and second rotation, when the amount of precipitations corresponded to very dry area, two and nine clones had yields higher than reference clone. The highest yield had clone 'Hélios', i.e 5.6 and 7.4 $t.ha^{-1}.yr^{-1}$, respectively. Reference clone 'MAX 4' had 4.9 and 5.5 $t.ha^{-1}.yr^{-1}$, respectively. In forthcoming harvest, higher yields can be expected as mean total cross-section area, that is very strongly correlate with yield, is one third higher than in same time of second rotation and can be related also to more humid third rotation.

Obtained results show that intraspecific crossing of *P. nigra* makes sense and selection of promising pure black poplar clones suitable for changing weather pattern is possible.

Keywords: *Populus nigra*, biomass production, intraspecific crossing, drought

Session 5:
Environmental Applications

Oral presentations

GETTING TO THE ROOT OF THE MATTER – BIOENGINEERING WITH POPLARS AND WILLOWS IN A CHANGING CLIMATE.

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Abstract

Poplars and willows, particularly, tree willows have been the primary bioengineering tools used for soil stabilization in New Zealand. The key to their success is in ease of propagation, fast growth, establishment in a difficult environment and their soil binding capability. Managed environments in New Zealand include pastoral hill country and river berms, and these are the environments where poplars and willows are used most effectively. River engineers use these tools readily, but private landowners require persuasion since every innovation means adaptation. As elsewhere in the world, soil loss and sediment transfer are major economic and environmental issues in New Zealand, and the promotion of poplar and willow use is for conserving soil and reducing sediment transfer. Sceptical landowners need supporting evidence of the effectiveness of these trees. Studies of the tree root behaviours have provided supporting evidence. Development of systems to manage tree growth and minimize impacts on farm income has been important in extension.

Good science is needed to underpin our messages. We must continue to do the more challenging field studies since findings from field studies make sense in the environments where we want practitioners to take up and apply this new knowledge. At the interface of science and application our role is more than a knowledge provider. We are also persuaders; at the level of policy maker and the level of practitioner. As persuaders we need to tell interesting stories, of which appropriate context is an important feature.

Keywords: soil stabilization, willow roots, soil binding

LOOKING FOR *SALICACEAE* PLANT MATERIAL SUITABLE FOR CULTIVATION WITH INDUSTRIAL WASTEWATER: TOWARDS MULTIPURPOSE PLANTATIONS

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Abstract
This work is housed within a general context of wood production as a raw material of interest for the bioeconomy, at the same time that secondary wastewater from the agri-food industry is regenerated, while used for irrigation, in a commitment to the circularity of resources. Under Mediterranean conditions, irrigation is a common practice for biomass production in short-rotation coppice. On the other hand, the brewing industry uses a significant amount of water that must be treated after use to finally be discharged into the sewage network or into the environment. The first steps of this treatment cycle give rise to an effluent characterized by high N and P contents, high DOB and COD, and high salinity.
Salicaceae have excellent characteristics that make them suitable for soil and water phytoremediation, as has been profusely referred to in the literature. The suitability of the plant material is a key issue in the development of this Nature-Based Solutions. Therefore, twenty-four genotypes of *Populus* spp. and *Salix* spp were tested under greenhouse conditions using hydroponic culture for 60 days. Both productive hybrids whose adaptation was tested for Mediterranean conditions and autochthonous material are included. The treatments applied were a control solution and secondary wastewater from the brewing industry. Five replications per treatment and genotype were randomly installed in 55 l containers. Trials were maintained for 60 days.
The behavior of the plant material was evaluated through the exploratory analysis of different variables linked to growth and production (height, diameter, different fractions of the biomass-root, stem and leaves-, and root/shoot ratio) as well as physiological (net photosynthesis, A; stomata conductance, gs; and transpiration, E). Additionally, the composition of the influent and effluent wastewater was recorded and analyzed weekly to determine water quality.
During the test period, survival in control conditions was 96.5%, decreasing to 86% in the wastewater. Irrigation with wastewater significantly affected all the evaluated parameters, both productive and physiological. Stomatal closure, less transpiration and a drop in the net photosynthesis rate were observed, which implied a lower biomass production. However, not all genotypes are affected in the same way. Differences in tolerance are identified in each taxonomic group. In terms of wastewater treatment, sampled water show higher attenuation values for SS and BOD, followed by COD, TN and TP, respectively.
The possibility of contributing to the regeneration of industrial wastewater while that necessary raw material is produced will depend, among other factors, on the adequacy in the choice of plant material.

Keywords: wastewater from brewing, phytotechnologies, poplar, willow, biomass production

SOIL ORGANIC MATTER QUALITY AND STABILITY ARE INFLUENCED BY SALIX SPECIES IDENTITY AND COMPOSITION

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Abstract
Soil organic matter (SOM) is a valuable natural resource, which provides multiple ecosystem services, including mitigating climate change, facilitating biodiversity and securing food production. The formation and composition of SOM strongly depend on the quantity and quality of input from plant litter. A growing body of research explores the relationship between plant diversity and aboveground productivity, but diversity effects on soil compounds and processes remain poorly understood.
Here we combined detailed assessments of SOM molecular composition and stability as well as estimates of soil carbon (C) and nitrogen (N) content to investigate the relationships between plant species and species diversity on the one hand, and soil respiration on the other hand in two 9-year-old experimental sites in Germany and Sweden comprised of pure and mixed stands of two species of biomass willows (*Salix* spp.). Pyrolysis-field ionization mass spectrometry (Py-FIMS) was used to assess SOM molecular composition and thermal stability while C and N pools were calculated from CN analyses and bulk density for the upper 10 cm of soil.
We found significant effects of species and mixing of species on various soil compounds that are relevant for SOM stability and soil C accumulation, and these effects partly varied considerably between the two study sites. The results on the composition, quality and stability of SOM assessed across both pure and mixed plots of two phenotypically and functionally dissimilar willow species will be discussed with regard to the influences of willow species identity and richness on soil properties including nutrient content, SOM quality and stability. Further analysis of the spectrometry data will reveal an evaluation of the influences of species identity and composition on compound classes selected to represent SOM quality, here equivalent to decomposability; phenolic compounds in general, lignin dimers and monomers and lipids. Site variables such as clay content, mean annual precipitation, and soil type will be used as covariates in the analyses to explain interactive effects of species x site.
The study contributes to a better understanding of the factors influencing the accumulation of SOM under biomass plantations, here represented by willows; and how these plantations and their composition affects the potential for climate change mitigation under different climate and soil conditions.

Keywords: Soil organic matter, Mixed tree plantations, *Salix*, Carbon sequestration, Thermal stability

ENHANCING ECOSYSTEM SERVICES WITH AGROFORESTRY PHYTOREMEDIATION BUFFER SYSTEMS IN THE GREAT LAKES BASIN, USA

Ronald S. Zalesny Jr.[1,*], Brent S. DeBauche[2], Elizabeth R. Rogers[1,2], Ryan A. Vinhal[1,2], Richard A. Hallett[3], Jeff Jackson[4], Chung-Ho Lin[2], Andrej Pilipović[5]

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Abstract
There is great potential for phytoremediation and associated phytotechnologies to mitigate economic, social, and ecological impacts caused by human activities throughout the Great Lakes Basin in North America and globally. With support from the Great Lakes Restoration Initiative (GLRI), we developed and installed the largest field-based phytotechnology monitoring and testing network in the world. In particular, we formed Great Lakes Phyto, an international partnership aimed at developing phytotechnologies to enhance ecosystem services in rural and urban areas, and established our network of sixteen agroforestry phytoremediation buffer systems (i.e., phyto buffers) to reduce potential surface runoff and subsurface water flow at landfills in the Lake Superior and Lake Michigan watersheds. We will highlight key milestones of this long-term, field-based phytotechnologies network, focusing on phytoremediation best practices (i.e., phyto BMPs) that are geographically robust, being regionally designed yet globally relevant.
In addition to describing our Great Lakes Phyto partnership, we detail phyto-recurrent selection that was conducted through a polycyclic evaluation process of survival, growth, physiology, and health to choose superior genotypes for outplanting. After three greenhouse selection cycles, Cycle 4 was field-established at six (2017), five (2018), and five (2019) phyto buffers.
We focus on current phytoremediation field research at the phyto buffers, including recent publications related to: 1) genotype × environment interactions, 2) promising new clonal material, 3) biomass production, 4) ecophysiology, 5) sapflow and water use, 6) a green tool for contaminant prioritization, 7) DeValix willow mats designed for ecological restoration, and 8) anthropogenic succession. We end the presentation with a synthesis of these collective efforts, focusing on optimization of ecosystem services and the development of sustainable pollution solutions to solve difficult environmental problems.

Keywords: Great Lakes Phyto; Phyto Buffers; Phytotechnologies; Poplar; Runoff; Willow; Water

PHYTOEXTRACTION POTENTIAL OF *POPULUS ALBA* L. AND *PINUS HALEPENSIS* MILL. GROWN IN SOILS CONTAMINATED WITH HEAVY METAL-CONTAINING MINING WASTE

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Abstract

Phytoremediation presents an interesting, eco-friendly way of treating soils contaminated by heavy metals and trace elements. This work aimed to study the phytoextraction capacities of *Populus alba* L. and *Pinus halepensis* Mill. grown in heavy metal-contaminated soils from the Jalta Mine of Northern Tunisia. These soils contain high levels of Pb, Cu, Zn, and Cd. To highlight the different adaptability responses of the species to soil contaminants, trees were established in a generalized randomized block design in plastic pots filled with the contaminated soils. Control soils were prepared using compost and uncontaminated sand. Physical and chemical analyses were conducted on the soil before and after the experiment. Four months after establishment, the height, diameter, number of leaves, and number of ramets were measured, and the plants' Pb, Cu, Zn, and Cd content were analyzed. The results showed that the levels of contaminants in the soil decreased significantly between the beginning and the end of the experiment. Regarding the plants' response to soil contamination, promising results were found. Leaf bioaccumulation factors of 1.03 and 1.09 were observed for Zn and Cd, respectively, and show the species' ability to accumulate contaminants. On a dry weight basis, poplar and pine leaves had Pb concentrations of around 349 mg/kg and 265 mg/kg of Zn, respectively. Results also showed that the concentrations of Pb in roots were 3 to 4 times greater than those in the leaves, with a transfer rate of 0.02, highlighting phytostabilization processes in the roots. Concentrations of Pb in roots were 214 mg/kg and 232 mg/kg on a dry weight basis for the poplar and pine respectively, representing the amount of Pb accumulated during the 4-month experiment. This work highlighted the efficiency of *Populus alba* L. and *Pinus halepensis* Mill. in the phytoremediation of contaminated soils in mine areas and the possibilities of their use for future restoration plans in contaminated areas.

Keywords: Heavy metals, *Populus alba* L., *Pinus halepensis* Mill., phytoremediation, contaminated soils, mine, Tunisia

EARLY GROWTH AND COPPICE PERFORMANCE OF HYBRID POPLARS GROWN FOR PHYTOREMEDIATION

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Abstract
The use of hybrid poplar for phytoremediation has been well documented, yet oftentimes height, diameter, and survival are the only parameters used to estimate establishment success. In order to better assess overall tree health and vigor, it is important to incorporate other parameters such as health metrics, total dry mass and coppice ability.
In January 2019, whole-tree harvests of poplar were conducted at a closed municipal landfill in Menomonee Falls, WI, USA following three years of growth. Dry biomass of boles and branches was determined for 17 'DN34' and 8 'NM2' trees. Mean annual increment (MAI) was calculated based on total (bole + branch) aboveground dry biomass, at a planting density of 1,682 trees ha^{-1}, and tree age of three years old. In addition, annual measurements of height, diameter, and tree health metrics were collected for both clones in 2017, 2018, and 2019.
Clones differed for third-year height increment ($P < 0.0001$) and height ($P = 0.0018$). During the 2019 growing season, 'NM2' grew 2.84 ± 0.03 m versus 2.40 ± 0.06 m for 'DN34', while overall height was 6.33 ± 0.09 m and 5.79 ± 0.10 m for 'NM2' and 'DN34', respectively. Though bole dry mass was similar for both clones ($P = 0.0953$), branch ($P < 0.0001$) and total ($P = 0.0018$) biomass differed, leading to significantly greater MAI ($P = 0.0018$) for 'NM2' (3.3 ± 0.04 Mg ha^{-1} yr^{-1}) relative to 'DN34' (2.2 ± 0.01 Mg ha^{-1} yr^{-1}). Meanwhile, tree health metrics did not differ between clones prior to coppice. Following coppice, the number of stems ($P = 0.0167$), height ($P = <0.0001$), diameter ($P = 0.0041$) and MAI ($P = 0.0025$) all differed significantly between clones. 'NM2' had higher values for all parameters including number of stems (18.50 $\pm$ 7 stems for 'NM2' vs. 14.41 $\pm$ 4 stems for 'DN34'), height (5.5 $\pm$ 1.96 m vs. 4.34 $\pm$ 1.05 m), diameter (34.69 $\pm$ 12.26 mm vs. 27.33 $\pm$ 6.63 mm), and MAI (4.73 $\pm$ 0.70 Mg ha^{-1} yr^{-1} vs. 2.86 $\pm$ 0.20 Mg ha^{-1} yr^{-1}). Overall, collecting a diversity of growth-, biomass-, and health-related field measurements provides much-needed information to better understand tree vigor and establishment success in poplar-based phytoremediation systems. Studies like this are important for improving clonal selection and making more-informed decisions when choosing contaminant-specific clones.

Keywords: *Populus*, biomass, phytotechnologies

WATER USE EFFICIENCY AND GROWTH PERFORMANCE OF POPLARS AND WILLOWS GROWN IN SOILS FROM LANDFILLS IN THE GREAT LAKES BASIN, USA

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The Great Lakes Basin of eastern North America is the largest surface freshwater ecosystem in the world, providing substantial provisioning, regulating, supporting, and cultural ecosystem services to over 34 million people in the United States. Aiming to reduce global losses of ecosystem services resulting from land use changes in this region, the Great Lakes Restoration Initiative (GLRI) funds projects related to mitigation of the influence of nonpoint source pollution and capturing untreated stormwaters. Landfills are one of the most relevant nonpoint contamination sources in the Great Lakes Basin. This research deals with testing the potential of different poplar and willow clones for landfill surface and groundwater runoff phytoremediation at different sites in the Lake Superior and Lake Michigan watersheds. For this purpose, three greenhouse phyto-recurrent selection cycles were used to choose and test fifteen poplar and nine willow clones (from a base population of 200 genotypes) for differences in water use efficiency and growth performance when grown in soils from five different southeastern Wisconsin landfills (Bellevue, Whitelaw, Slinger, Menomonee Falls, Caledonia). Testing included evaluation of water use efficiency through determination of carbon isotope accumulation ($\delta^{13}C$) along with assessment of chlorophyll fluorescence measurements, tree health metrics (e.g., leaf chlorosis, tree vigor, insect/disease resistance), and growth parameters (e.g., tissue biomass, numbers of leaves and roots, leaf area). Water use efficiency differed among clones, with $\delta^{13}C$ of poplar clones ranging from 32.54 to 35.64‰ and that for willows ranging from 32.03 to 33.42‰. Growth parameters showed significant soil and soil × genotype effects for $\delta^{13}C$ and growth parameters ($P < 0.05$). These results indicated that incorporating novel traits such as $\delta^{13}C$ and water use efficiency can enhance phyto-recurrent selection of appropriate poplar and willow clones for phytotechnologies such as phytoremediation and runoff reduction.

Keywords: *Populus*, *Salix*, landfills, biomass, chlorophyll fluorescence, $\delta^{13}C$

Session 5:
Environmental Applications

Posters

VARIATION IN REPRODUCTIVE AND PHYSICAL TRAITS OF *Pinus wallichiana* (A.B. Jacks) TREES ALONG ALTITUDINAL AND ASPECT GRADIENTS WITH RESPECT TO STRESS CONDITION INDEX IN NORTHWESTERN HIMALAYA

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Abstract
Blue pine does not produce good seed every year, so it is vital to harvest large amounts during favourable seed years. The current study examined the effects of elevation, aspect, and stress condition index (SCI) on reproductive and physical parameters of *Pinus wallichiana*. In 2019 and 2020, phenotypically superior trees were selected from four different altitudinal ranges [1800-2100 m.a.s.l. (A1), 2100-2400 m.a.s.l. (A2), 2400-2700 m.a.s.l. (A3), and >2700 m.a.s.l. (A4)] and three aspects [southern (As1), eastern (As2), and southeastern (As3)] of the northwestern Himalayas. The results showed substantial diversity in morphological, reproductive, and physical features along elevational ranges and slopes. Trees growing at >2700 m.a.s.l. had higher proportions of dead foliage (2.41), branches without foliage (2.33), dead crown (1.93), and stress condition index (6.67). Greater values for cone width (4.36-3.39 cm), cone length (18.2-13.6 cm), cone weight (105.8-86.1 g), seed weight per cone (2.16-1.38 g), number of scales per cone (139-89.3 scales), seed length (9.67-8.75 mm), seed weight (5.29-4.41 g), seed thickness (3.29-2.78 mm), seed weight (7.37-3.13gm), number of seeds per cone (78.4-62.3), needle length (14.31-10.59cm), were recorded in the lowermost altitudinal zone and declined towards upper altitudes ($P \leq 0.05$). Among aspects, the southern aspect showed maximum values for all the reproductive traits, followed by the southeastern aspect. The adverse relationship of all reproductive variables with SCI and altitude is shown by principal component analysis (79.8%). Regression analysis also demonstrated that as altitude increased, the value for reproductive qualities decreased. Aspect had a similar trend from southern > southeastern > eastern. So, based on early field screening, seedlings from lower altitudes and southern and southeastern aspects were identified as suitable for large-scale *P. wallichiana* plantings in this location.

Keywords: Altitude, aspect, stress condition index, *Pinus wallichiana*, reproductive traits

ESTABLISHMENT OF AGROFORESTRY SYSTEMS WITH COPPICED TREES FOR EROSION CONTROL

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The aim of our work is to evaluate promising agroforestry systems (AFS), which would combine conventional plant production farming with (narrow) belts of coppiced trees for production of biomass, which would also provide valuable ecosystem services namely erosion control.
For this purpose, we established a coppiced tree belt (CTB) at research station Průnonice-Michovky in 2020, which consists of three rows of fast-growing trees (poplars Max-4, 'Kaktu', and willow 'Rokyta') within an experimental agroforestry alley cropping system with slope declination of 7-12%. The total area of the agroforestry system is 1 ha (12% tree lines and belts, and 88% conventional crops; distances between tree lines and belts are 26 meters). We have successfully tested electric fences for the protection of trees against animal damage (hare, roe-buck). In 2021, we conducted 2 experiments with simulated rains in 11th June and 6th October to evaluate anti-erosion and infiltration ability of belts of coppiced trees. The principle of measurement was based on water splash on an area of 10.5 m² from which surface water flows away together with soil. Two simulated rains were applied in each experiment. Both rain events lasted 60 minutes (with a 15 min. break after 30 min.) with an intensity of 1.2 mm/min and total rainfall of 780 l (e.g. 72mm/m²). Trees were between 1-1.5 m tall and soil was covered with grass vegetation in tree lines. A rolled fallow land without plants served as control in both experiments. In the case of the coppiced tree belt, no surface runoff was observed and on fallow land surface runoff and soil loss were 496 l and 6,95 t/ha in June experiment, respectively, and 41 l and 0.58 t/ha in October experiment, respectively.
The experiments proved the very strong anti-erosion and infiltration ability of coppiced tree belts. We will continue with artificial rain experiments in different conditions in the coming years. We will also evaluate production of biomass and economic efficiency of AFS with coppiced trees.

Keywords: agroforestry, coppiced trees, erosion control, simulated rain, surface runoff

EVALUATION OF IMPROVED WILLOW CLONES FOR PHYTOREMEDIATION AT AN URBAN SOLID WASTE LANDFILL IN LAMARQUE (RÍO NEGRO, ARGENTINA)

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Abstract
In many developing countries, municipal solid wastes are dumped using non-preventive methods to avoid gas emissions and leachate generation, which poses a high risk to the environment and human health. Extensive evidence in several countries around the world demonstrate that vegetation planted in a sanitary landfill has an important role in erosion control, elimination of pollutants, and imparting aesthetic value.
This is the situation in the case analyzed in Lamarque (Río Negro, Argentina), where there is a municipal solid waste landfill located in a valley area unlike most regional landfills (39°36'38"S; 65°40'42"W). The closeness of the water table poses a potential contamination problem from leachate of dumped wastes. In order to start the phytoremediation experimental process, a willow (*Salix* spp.) clone trial was established to understand the real capacity of remediation. Survival in the first vegetative cycle and the annual growth are the main variables being evaluated. The planting consists of three rows of 200 m (4m x 2m) and includes five willow clones. The willows were planted in September 2021 using a method known as "deep-planting", a technique to make it easier for the willow poles to come in contact with the water table. One-year-old poles of 2.5-3.0 m long were planted in holes dug to the depth of the water table using a backhoe. The willow clones being evaluated were hybrids of *Salix matsudana* x *Salix alba* ('Los Arroyos INTA-CIEF', 'Agronales INTA-CIEF' and 'Carapachay INTA-CIEF'), an open pollination hybrid of *Salix matsudana* ('Géminis INTA-CIEF') and an open hybrid of *Salix alba* ('Yaguareté INTA-CIEF'). All genotypes were obtained from the INTA Willow Breeding Program.
After six months of establishment and before leaf fall, the survival rate of each clone was: 'Yaguareté INTA-CIEF' 88.2% ; 'Géminis INTA-CIEF' 86.1% ; 'Agronales INTA-CIEF' 86.0% ; 'Los Arroyos INTA-CIEF' 84.9% and 'Carapachay INTA-CIEF' 69.2%. In addition, the presence and absence of signs of chlorosis and necrosis in the leaves were observed, with 'Yaguareté INTA-CIEF' and 'Géminis INTA-CIEF' being the best performers.

Keywords: *Salix*, environmental remediation, Patagonia.

EVALUATION OF IMPROVED WILLOW CLONES FOR PHYTOREMEDIATION URBAN SOLID WASTE LANDFILL IN LAMARQUE (RÍO NEGRO, ARGENTINA)

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Abstract
In many developing countries, municipal solid wastes are dumped using non-preventive methods to avoid gas emissions and leachate generation which pose a high risk to the environment and human health. Extensive evidence in several countries around the world supports that the vegetation planted in a sanitary landfill has an important role in erosion control, the elimination of pollutants, and in addition to imparting aesthetic value. This is the situation in the case analyzed in Lamarque (Río Negro, Argentina) where there is municipal solid waste landfill located in valley area unlike most regional landfills (39°36'38"S; 65°40'42"W). The closeness of the water table conditions a potential contamination with leachates of damped wastes. In order to start a phytoremediation experimental process a willow (*Salix* spp.) clone trial were implanted to understand the real capacity of remediation. Survival in the first vegetative cycle and the annual growth are the main variables to register and evaluate. The experience is conformed to three rows of 200 m (4m x 2m) of five willow clones. It was implanted in September 2021 using a method known as "deep-planting", a technique to make it easier for the willow poles can be in contact with the water table. One-year-old poles of 2,5-3,0m long were planted in holes made with backhoe to water table depht. The willow clones in evaluation were hybrids of *Salix matsudana* x *Salix alba* ('Los Arroyos INTA-CIEF', 'Agronales INTA-CIEF' and 'Carapachay INTA-CIEF'), an open pollination hybrid of *Salix matsudana* (Géminis INTA-CIEF') and an open hybrid of *Salix alba* ('Yaguareté INTA-CIEF'), all genotypes obtained by the INTA Willow Breeding Program. After six months of established and before leaves fall the survival rate achieved of each clone was: 'Yaguareté INTA-CIEF' 88.2% ; 'Géminis INTA-CIEF' 86.1% ; 'Agronales INTA-CIEF' 86.0% ; 'Los Arroyos INTA-CIEF' 84.9% and 'Carapachay INTA-CIEF' 69.2%. In addition, the presence and absence of signs of chlorosis and necrosis in the leaves were observed showing 'Yaguareté INTA-CIEF' and 'Géminis INTA-CIEF' the ones with the best performance.

Keywords: *Salix*, environmental remediation, Patagonia.

ECONOMIC ANALYSIS OF AMOUNT IRRIGATION UNDER PRESSURE SYSTEMS (TAPE) AND PLASTIC MULCH ON WEED CONTROL IN THE NURSERY SEEDLING PRODUCTION OF *Populus* spp.

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Abstract

This project was carried out on an approximate area of 400 m² and used three *Populus* clones: *Populus nigra* '62/154', *P. alba* '44/13', and *P. euramericana* '92/40'. These clones had high wood production performance in compatibility experiments at the Alborz Research Station in Karaj, Iran. First, field preparation including plowing, discing, and leveling was performed. In March 2020, cuttings were prepared in the nursery from the mentioned clones (180 cuttings from each clone). This study was conducted in a factorial experiment based on a completely randomized block design with three replications. The main treatment included the use of white plastic mulch and control (without the use of plastic mulch) and the sub-treatment was three species of *Populus*. A total of 540 cuttings (3 clones x 2 treatments x 3 reps x 30 cuttings per rep), measuring 20-25 cm in length and 1-1.5 cm in diameter, were planted at a spacing of 1× 0.3 m. Drip irrigation system was using a strip type and plastic mulch was placed on the strip type. The amount of water consumed was measured by a water counter installed in the main entrance route. Weeding was done every 45 days until the end of the growing season, and after identifying the species, the weed samples were dried and then weighed. Vegetative characteristics of poplar seedlings were measured and evaluated at the end of the growing season. Then, all the poplar seedlings were cut from the collar. In March of the second year, vegetative characteristics of first-year sprouts of two-year-old root stock were measured, including the number of sprouts in each coppice, height, collar diameter, diameter at breast height and fresh and dry weight of the sprouts. Also, identification of weeds and their weighing were performed as in the first year. Economic comparison of treatments was carried out using cost-benefit ratios and the present values of net benefits. Profit was calculated based on the number of seedlings produced for each year, separately. The results showed that in the study of the average interaction of treatments, there were the highest height and fresh and dry weight of annual seedlings for clone '62/154' , block 1, and the lowest amount of weeds in mulch application treatment. On the other hand, the lowest fresh and dry weight of annual seedlings was exhibited by clone '44/13', and block 2. In the second growth year, it is obtained the highest height in '92/40', block 2 in application with mulch, and the largest collar diameter and diameter at breast height (DBH) in '44/13', block 1. The highest fresh and dry weight occurred in '92/40', blocks of 2 and 3, with the highest gross income in application with mulch. There are the highest fresh and dry weight and height of weeds of *Populus* stands in control treatment, and block 1, which is related to the *Conyza canadensis* and *Convolvulus arvensis* weed species.

Keywords: Evaluate growth rate, Nursery, Gross income, *Populus*, Weed control

PERFORMANCE OF THREE POPLARS AND ONE WILLOW TREE SPECIES ESTABLISHED ON SOIL IRRIGATED WITH WASTEWATER AFTER TWO YEARS OF GROWTH

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Abstract
Wastewater reuse for the irrigation of timber plantations as a market-driven action can contribute to the promotion of the circular economy by reclaiming poor quality waters and applying them to certain plant species such as poplar and willow trees. A greenhouse study was conducted to compare the performance of poplars (Populus nigra L. '62/154', P. alba L. '20/45', P. euramericana (Dode) Guinier '92/40') and willow (Salix excelsa S.G. Gmel) grown in soil irrigated either with wastewater (WW) or tap water (TW) after two years of growth. Results showed that WW had a positive impact on growth and biomass production in all the species. Poplars and willow under the WW treatment had significantly greater values for plant growth and biomass parameters including diameter, height, biomass (root, stem, leaf, and total), and leaf area as compared with the TW treatment. Likewise, phytoextraction and phytoaccumulation of macro- and micro-nutrients and heavy metals of trees treated with WW were significantly greater than those of trees treated with TW. Two-year-old *P. nigra* trees had the most growth and biomass production with the exception of leaf area, which was greatest in *P. alba* and *P. euramerican* trees. After two years of growth, under both experimental conditions (TW and WW), the greatest total content of Cu and Mn was observed in *P. nigra*, while the greatest Zn content was observed in *P. alba*. No significant differences among species were observed for Fe content. On the contrary, the greatest total contents of Ni and Cr were observed in *S. excelsa* followed by *P. nigra* and *P. alba* under both experimental conditions. In all the species and under both experimental conditions, translocation factor values (TF: leaf+stem/root ratios) for Zn, Cu, and Mn were greater than 1, whilst they were less than 1 for other metals (Fe, Ni, Cr and Pb). Therefore, phytoaccumulation of Zn, Cu, and Mn was higher in aerial tissues; in contrast, phytoaccumulation of Fe, Ni, Cr and Pb occurred in root tissue. As tolerance index values exceeded 100% for all two-year-old poplar and willow trees, it can be stated that all the species tested here can be considered for planting in soil affected by urban wastewaters with similar conditions as in the present study. However, along with greater total biomass, *P. nigra* trees showed high total contents of Cu, Mn, Ni, and Cr, and thus two-year-old *P. nigra* trees outperformed all other species overall.

Key words: Heavy metals, Phytoremediation, *Populus*, *Salix*, Wastewater

Session 6:
Production Systems-From Traditional Plantations to Modern Agroforestry

Oral presentations

DEVELOPMENT OF MERCHANTABLE VOLUME EQUATIONS FOR MAJOR HYBRID POPLAR CLONES IN SPAIN USING STEM DATA OBTAINED FROM TERRESTRIAL LASER SCANNER

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Abstract
Accurate and frequently updated tree volume estimates are required for poplar plantations, characterized by fast growth rate and a high commercial value of the individual trees. Stem taper equations are a standard method for estimating merchantable tree volume. These equations require multiple observations of stem diameters along the tree bole, which were traditionally taken from destructively sampled trees. Currently, techniques such as Terrestrial Laser Scanner (TLS) provide an alternative for obtaining quantity and quality of data for taper modeling.
Using TLS, we scanned 265 poplar trees (61, 54, 40, 55, 56 trees for 'Beaupre', 'I-214', 'MC', 'Raspalje' and 'Unal' clones, respectively) located in 14 sites. Stem diameters were calculated automatically in 0.1-meter-thick slices, so that diameters along the stem (d_i) and merchantable stand volumes (v_i) were obtained for different heights above ground level (h_i). Additionally, for one site, two trees of each clone were felled and field measurements of d_i were taken as reference data, allowing the discrepancies ("error") from TLS measurements to be obtained. The aim of this study was to develop a merchantable volume equation from the compatible volume system proposed by Fang et al. (2000) and TLS-derived data.
Regarding the TLS estimation of d_i for the 10 trees which were felled, the global RMSE obtained was 1.05 cm when considering the diameters were measured to a top diameter limit of 20 cm and 1.38 cm when considering the diameters were measured to the very top of the tree, which correspond to a global relative RMSE of 4.37 % and 9.78 %, respectively.
The compatible volume system of Fang et al. (2000) explained more than 99% of the variation in d_i and v_i for all the five clones and estimated total and merchantable individual-tree volume accurately. The goodness-of-fit metrics obtained are slightly better than those previously reported for the clones 'I-214' and 'MC' in Spain using data from traditional destructive techniques. The non-linear extra sum of squares method indicated differences in stem taper and merchantable stand volume equations for the five clones, so that a different model was fitted separately for each clone.
Our results support the reliability of the TLS technique for reconstructing the stem taper and subsequently obtaining total or merchantable stem volume for hybrid poplar clones. Moreover, the TLS allows this achievement at significantly lower costs and time requirements than traditional destructive methods. For clones 'Beaupre', 'Raspalje' and 'Unal' the models developed here are the first published to date.
Keywords: Terrestrial Laser Scanner, stem taper, merchantable volume, tree volume, modelling.

VARIATION IN WILLOW NUTRITIONAL FEED VALUE BASED ON CULTIVAR AND HARVEST REGIME

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Abstract

Biomass production and nutritional quality are the essential traits of any forage crop. Many tree species can be grown for forage production, but the ability to regrow after harvest results in long-term sustainability. Growth rates and recovery from harvest cutback are traits that are relatively easy to select for in breeding trials. Harvest regime are also likely to significantly affect yield. Integrating willows cultivars that can regrow vigorously as perennial fodder crops in agricultural lands could provide biomass as forage and potential improvements in the sustainability of crop production.

From 2020 to 2021, we investigated the effects of harvest regime based on one and two cutbacks per year and compared forage quality at each harvest time, cultivar response to mid-season cutback, and total seasonal biomass production. This work was performed on an existing cultivar evaluation trial in Geneva, NY containing 25 entries, replicated four times. Each plot was randomly split, with one row assigned a single cut and the other assigned two cuts per season. SPAD (chlorophyll content) measurements and growth (stem diameter and height) were measured, and nutritional value was analyzed by conventional forage analysis.

One-cutback per year resulted in significantly greater aboveground growth compared to two-cutbacks reflected in biomass (12.2 ±0.4 vs. 9.8 ±0.2 Mg ha-1 yr-1, respectively; P<.0001), mean stem, mean stem diameter, as well as stem: leaf ratio. Greater biomass from the one cut regime was explained by greater stem diameter and greater stem: leaf ratio. There was no difference in the SPAD reading between harvest regimes. The nutritional value was significantly greater from the two-cutback regime compared to one-cutback based on crude protein (CP) (12.2 ±0.6 vs. 7.4 ±0.2, respectively; P<.0001), acid detergent fiber (ADF), neutral detergent fiber with α-amylase and sodium sulfite (aNDF) and relative feed value (RFV). Nitrogen uptake was greater for the two-cutback regime. This may indicate the effects of low stem: leaf ratio with more leaves and small stem diameter in two-cutback vs. one-cutback regime. All the above traits varied significantly by genotype, indicating a potential for selection in a breeding program with varying responses to harvest regime. While biomass by the two-harvest regime was 24% lower, forage quality was 23% better, and nitrogen uptake was enhanced by 22%. In selecting improved genotypes for forage production, an index that accounts for biomass yield, leaf: stem ratio, and forage quality will be most effective.

Keywords: Salix, Forage, Nitrogen uptake, Growth indices, Regrowth.

CAPILLARY RISE IN A NON-IRRIGATED POPLAR DEEP-PLANTING AFFORESTATION

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Abstract

The existence of a shallow water table in Alto Valle of Río Negro and Neuquén causes a capillary rise that modifies the water content in the soil profile. Water table recharge is an anthropic process that has a seasonal pattern, with a remarked recharge in spring, a decline in summer and a raise in autumn. Capillary rise in non-irrigated plots is conditioned by soil texture, evapotranspiration, moisture in the topsoil and water table depth. The influence of the capillary rise on the water balance might be used in arid regions to develop deep-planting techniques to establish poplars. This trial included deep-planting of a poplar afforestation and the determination of the water table level and the volumetric water content (VWC) of the soil in a non-irrigated condition. The poplar plot was planted in September 2018 in four rows of 50 m, in INTA Experimental Station (General Roca, Rio Negro, Argentina). According to previous soil studies, the soil profile is heterogeneous, medium-fine textured, slightly saline. Two poplar clones were planted interleaved in each row: *Populus x canadensis* 'Guardi' and *Populus nigra* 'Jean Pourtet', with a raw spacing of 4 m and 2 m between trees. The implantation depth achieved was of 0.8 m, using a method known as deep-planting, a technique that makes it easier for the poplar poles to reach deep humidity of the soil profile. Water table level was measured in an open aquifer piezometer. Soil water content was measured with Frequency Domain Reflectometry (FDR) sensors at two depths: 0.40 m and 0.80 m. The trial was carried out in 2018-2019 and 2019-2020 growing seasons. Water table level of the growing seasons studied (from September to May) raged between 0.9 m and 1.3 m. According to FDR data, the VWC at 0.40 m (40 % m^3/m^3) was lower than the VWC at 0.80 m (50 % m^3/m^3. This can be explained by the presence of the shallow water table and its fluctuations, that causes a capillary water input into the root zone which favored the establishment and development of the evaluated non-irrigated poplar afforestation.

Keywords: *Populus*, soil water balance, shallow water table, Patagonia.

INVESTIGATION OF SUITABLE PLANTING DISTANCE ON WOOD PRODUCTION AND QUALITATIVE CHARACTERISTICS OF SUPERIOR POPLAR CLONES IN MARKAZI PROVINCE, IRAN

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Abstract
Nowadays, wood farming with fast-growing species such as poplars gain significance due to its fast growth rate, the potential of use for various manufactured products, and important role in mitigation of climate change effects using sequester carbon. Moreover, they supply raw material for industrial processing as pulp, paper, engineered wood products, lumber, pallets, and furniture) and valuable non-wood products. To determine the planting spacing that produces the greatest wood volume from superior clones of *Populus nigra*, we conducted a four-year experiment (2018-2021) at Agricultural Research Station of Markazi Province, Iran using a completely randomized block design with four replications of three planting spacings (1 m × 2 m, 2m × 2m and 2 m × 3 m) and five clones *of P. nigra* (‘*62/154*’, ‘*56/72*’, ‘*62/127*’, ‘*62/167*’, and ‘betulifolia 17/13’). Field preparation included plowing, disc and leveling. In March 2018, 30 cuttings (20-25 cm long) were planted for each replicate of the treatments. The management of the trials included hand weeding and drip irrigation system. Diameter at breast height (DBH) and tree height were measured annually and total wood volume was calculated after years of growth using height and DBH. Qualitative characteristics of trees including forking, vitality, crown/stem breakage and leaning were assessed. The effect of planting spacing on DBH, height, and mean increments of diameter, height and volume were significant at the level of 5% and on current annual volume increment and total volume (stocking) at the level of 1%. The main effect of the clone on all the studied vegetative characteristics were significant at the 5% level. The interaction effect of planting spacing x clone showed a statistically significant difference in DBH, height, and mean increments of diameter and volume at 1% level and on mean of height increments and total volume at 5% level. According to the comparisons of means with Duncan’s test, after four years, the highest wood production of the clones was obtained at the spacing of 1m × 2 m. The highest diameter growth was at the spacings of 1 m × 2 m (10.75 cm) and 2 m × 2 m (10.39 cm) and the highest height growth was at the spacing of 2 m ×2 m (11.33 m) and 2 m × 3 m (10.87 m) in clone ‘62/154’. Clones ‘62/154’ and ‘56/72’ had the highest total wood volume at the spacing of 1 m × 2 m with 137.29 m^3 ha^{-1} and 127.64 m^3 ha^{1-}, respectively. In qualitative assessment, clones of ‘*62/154*’ and ‘*56/72*’ had 100% smooth and straight trunks and the other clones showed a low curvature of up to 75%. Taking into account both wood volume production and the best rate of trunk quality at the interaction effect of planting spacing x clone, the ranking order of the study clones was ‘*62/154*’ *(best)*, ‘*56/72*’, ‘*62/127*’, ‘*62/167*’, and ‘betulifolia 17/13’ (worst). Therefore, to achieve high yield in wood production, clones of *62/154* and *56/72* would be recommended for mass cultivation in the region.

Keywords: Growth response, *Populus*, Short rotation, Suitable poplar clone, Wood production

YIELD DEVELOPMENT OF POPLAR, WILLOW, ASPEN AND BLACK LOCUST IN TWO TO SIX YEARS ROTATION LENGTHS FOR A TOTAL OF 15 TO 24 YEARS IN SIX DIFFERENT SHORT ROTATION COPPICE LONG TERM EXPERIMENTAL SITES

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Abstract
The dynamics of current (CAI) and mean annual increment (MAI) of short rotation coppice (SRC) plantation gives information about the total period of use of such a cultivation system. However, SRC-yield investigations over a long period of time are rare. Therefore, practical implications regarding yield development and profitability or carbon-sink performance of coppice systems lack information base. In Germany, several SRC experiments were established in the 1990s with varying tree species and clones and observed for longer periods of time. This study presents results of the development of CAI and MAI of the Thuringian experimental sites “Dornburg”, “Langenwetzendorf” (three and five years rotation) and “Bad Salzungen” (three years rotation) with an observation period of 21 and 18 years each (“Bad Salzungen”); of the Saxonian experimental site “Kalkreuth” (three years rotation) at an observation duration of 15 years; of the Brandenburgian experimental site “Potsdam Bornim” (two and four years rotation) at an observation duration of 24 years as well as of the Mecklenburg Pomeranian experimental site "Gülzow" (three and six years rotation) at an observation duration of also 24 years. At every experimental site the plots were fully randomized and had three to four repetitions per investigated species or clone.
Overall the biomass performance of the poplar clones averaged better than willow, aspen, and black locust. In particular, the clones of the “Max family” (*P. maximowiczii x P. trichocarpa*) showed top performances that significantly surpassed pure balsam poplar clones, especially with increasing root age. Individual willow clones - Zieverich (*s. viminalis*) and Tora (S. schwerinii x S. viminalis) - showed in single experiments performances equal to the strongest poplar clones. For poplar at sites with low vigor, a decrease in CAI was not observed until after 18 years root age, and first after 21 years at sites with stronger vigor. The MAI decreased only after two subsequent harvests with decreasing CAI. For willow, the decrease in CAI and MAI ocurred one or two rotations sooner than in poplar. The influence of rotation length on biomass performance was dependent of the chosen planting density. Yields were highest at sites with improved water supply. All in all, we observed that under German site conditions short rotation coppices show stable MAI, and therefore productive life, up to 20 to 25 years. We suggest validating these results with actual trials exposed to climate change conditions.

Keywords: Short rotation coppice, Long term experiments, Yield development, Germany, Poplar

Session 6:
Production Systems-From Traditional Plantations to Modern Agroforestry

Posters

FOREST MAP OF POPLAR PLANTATIONS OF SPAIN

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Abstract
The Forest Map of Poplar plantations of Spain has been created based on the best information available, in cooperation with the Autonomous Communities, Hydrographic Confederations and companies of the sector, among others. This information has been consolidated and harmonized in a common data model; it was also updated to the 2020 reference date to ensure that the result obtained reflects reality using remote sensing techniques and LiDAR. The maps obtained will serve as the basis for the implementation of the works of the first Forest Inventory of Poplar Plantations, which was designed within the framework of a specific working group made up of experts from different Autonomous Communities, companies in the sector, owners' associations and research centers. In addition, the first National Directory of Poplar Plantations has been prepared, as well as the first national-scale map of potentially viable areas for poplar cultivation.

Keywords: Poplar; Mapping; Forest inventory; Geographic information systems; Spain

VALUE OF INTRODUCED WILLOWS IN ATTRACTING POLLINATORS AND OTHER BENEFICIAL INSECTS

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Abstract

Willow plantations add structural diversity to landscapes while in turn enhancing biodiversity. Native willows are known to provide food and habitat to a plethora of wildlife species. *Salix* has more than 400 known species of shrubs and trees that use olfactory signaling, visual cues, and reward cues to attract bees and other pollinators. Different species of *Salix* act as primary hosts to a multitude of oligolectic bees including *Andrenids*. This study investigated insect preferences for a few species of willows and provided new knowledge about the overall value of native and introduced willows in attracting pollinators and other beneficial insects. The common garden experiment, originally planted in 2017, consisted of 3 native (*S. eriocephala, S. sericea*, and *S. lucida)* and 3 introduced (S. '*SX64'*, *S. '*Onondaga*'* and S. '*S365')* willows. In the spring of 2021, blue and white colored pans were utilized for collection. Different bee species were collected and recorded to subgenus levels. *Andrena* bees were the most prevalent pollinator species visiting all six taxa of willows. In addition to the collection of *Andrenids,* other bees such as *Colletinae* and *Hylaeus* were collected in significant quantities as well. In conclusion, there was no significant difference found in the willow preference by the pollinators when comparing native and introduced taxa.

Keywords: *Salix*, biomass, biofuel, plantation, *Andrena*

COVER CROP POTENTIAL FOR WEED MANAGEMENT IN AGROFORESTRY SYSTEMS

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Abstract

Introduction

Weed management is an integral practice for all plant production systems, whether it be in agriculture, horticulture, forestry, or phytotechnologies. Weeds have a unique ability to compete with crops and overrun these systems, causing losses through reduced crop quality and yields. Today's agricultural and horticultural systems typically manage weeds by chemical or mechanical means. While historical weed management techniques are effective for these practices, chemical and mechanical control require a great deal of labor and are very costly and detrimental to soil heath and water quality.

Materials and Methods

Agroforestry is defined as the intentional planting of trees or shrubs with crops and/or livestock in interacting combinations for multiple products or benefits from the same land management unit. In this improved cropping system, weeds create problems that are similar to that of agriculture and horticulture. A lesser known but unique weed management technique is the use of ground cover (i.e., cover crops) to control the spread of weed species and suppress weed development across the landscape.

Results and Conclusions

Cover crops are an alternative weed management technique that have great potential for weed suppression through competitive inhibition and allelopathic interactions. The use of cover crops as a weed management tool offers a sustainable practice that aligns with the intentional integration and sustainability aspects of agroforestry. In this presentation, we highlight the potential uses for cover crops as a weed management tool in all six major agroforestry practices: 1) alley cropping, 2) forest farming, 3) riparian forest buffers, 4) silvopasture, 5) windbreaks, and 6) urban forest farming, along with how these agroforestry principles translate to field-based phytotechnologies of inorganic and organic pollutants.

Keywords: Improved Cropping Systems; Alley Cropping; Forest Farming; Riparian Forest Buffers; Silvopasture; Windbreaks

EVALUATION OF GROWTH AND ECONOMIC PRODUCTIVITY OF *Populus euramericana* IN INTERCROPPING WITH AGRONOMICAL, FODDER AND MEDICINAL PRODUCTIONS

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Abstract

Intercropping fast growing trees with arable crops will improve the sustainability of farming systems, utilize the natural resources more efficiently, increasing productivity and poplar cultivation area increasing farmers income, and reduction of CO_2 in atmosphere. A four-year study was conduction at the Alborz Research Station in Karaj Iran to evaluate the growth and economic performance of *Populus euramericana* intercropped with barley (*Hordeum vulgare*), garlic (*Allium sativum*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*) and German chamomile (*Matricaria chamomilla*) in a completely randomized block design with four replications. *P. euramericana* and the other species were also were planted in a pure form. *P. euramericana* was planted at a spacing of 3 m × 4 m. The characteristics of trees height, diameter at breast height (DBH), stem volume, land equivalent ratio (LER), and economic advantage index were measured and evaluated at the end of the growing season. After four years, *Populus* trees in the pure cultivation reached a mean of height of 6.67 m, DBH of 10.03 cm and a volume of 22.78 m^3/ha. However, intercropped *Populus* trees had greater height in (*Populus*-sainfoin), (*Populus*-chamomile), (*Populus*-barley) and (*Populus*-garlic) systems, greater DBHs in (*Populus*-sainfoin) and (*Populus*-garlic) systems and greater volume in the (*Populus*-sainfoin), (*Populus*-barley), (*Populus*-alfalfa) and (*Populus*-garlic) systems than the pure-*Populus* system/cultivation. In the first year, yields of all crops were greater in intercropping cultivations than pure cultivations; however, in the second year, yields of sainfoin, alfalfa and barley were lower than in pure cultivations. In the third year, all crop yield were higher in pure cultivations than intercropping systems. LERs of all intercrop systems were more than one, which indicates the superiority of intercropping cultivation over their pure cultivation, and the highest LERs were in the (*Populus*-garlic) and (*Populus*-chamomile) intercrops. Based on LERs ratio, intercropping cultivation in barley with 96%, alfalfa with 78%, sainfoin with 103%, garlic with 128% and chamomile with 123% are more efficient use of land than their respective pure cultivations. The economic advantage index was positive in all crops, which indicates the economic benefit and advantage of intercropping cultivation and better use of available resources by both plants compared to the pure cultivation of each of them. The highest economic advantage indices under intercropping cultivation were in garlic (7.81) and chamomile (7.92) and the lowest index was in alfalfa (4.45). Due to the higher advantage index of garlic and chamomile plants, these products can be considered as a superior crop in intercropping with *P. euramericana* in the climatic conditions of Alborz Research Station, Karaj, in terms of agricultural and economic indicators.

Keywords: Agroforestry, Economic assessment, Evaluate the rate of growth, *Populus*, Production performance

GOOD SURVIVAL AND GROWTH AFTER APPLICATION OF INDOLBUTIRIC ACID ON UPPER CUT OF HARDWOOD CUTTINGS OF *POPULUS ALBA CL.* LBM

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Abstract
White poplar is characterized by relatively poor rooting ability of hardwood cuttings comparing to species from section Aigeiros (black poplars). One way to improve this trait is application of rooting stimulators. However, contemporary findings with application of indolbutiric acid (IBA) suggest that this effect depend on numerous factors, especially on clonal specificities, soil characteristics and nursery technology (terms of cutting preparation, storage conditions, term of cutting planting, tending, irrigation etc.).
In our trail, the hardwood cuttings of *Populus alba cl.* LBM, clone with relatively poor rooting ability, were prepared from stool bed one-year old whips (20th March 2022), treated with powdery formulation that contained 0,6% indobutiric acid and 50 µM $CoCl_2 \cdot 5H_2O$. After the treatment, the cuttings were stored in cool chamber at 2±2°C until the planting (26th March 2022). There were three treatments of hardwood cuttings: A – application of rooting stimulator on basal cut of hardwood cuttings, B – application of rooting stimulator on upper and basal cut and C – control treatment.
According to the results, there were inhibitory effect of application of IBA on basal cut on cuttings survival (probably due to the late term of cutting preparation for particular clone). However, the survival rate after application of IBA on both upper and basal cut was excellent (83.9%), but, according to Tukey's test, not significantly higher than in control treatment (60.5%). The effect of treatment with application of stimulator on both cuts on shoot height (SH) of plants measured on 30th June 2022, i.e. three months after the planting, was superior (SH=55.2cm) in comparison to application on basal cut (SH=32.0cm) and control treatment (SH=25.9cm). Results obtained in this study suggest excellent rooting of cuttings that were treated with examined powder formulation of IBA on both upper and basal cut. In that sense, further research should analyze and clarify the basis for such reaction and implications for further improvement of hardwood cuttings of difficult to root species.

Keywords: Rooting stimulation, White poplar, Nursery, Clonal technology

HOW FREQUENT CAN WE GO? INVESTIGATING THE RATE OF COPPICING ON THE WOODY AND NON-WOODY BIOMASS YIELD OF 8 DIVERSE *Salix* genotypes.

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Abstract
Rothamsted Research is currently investigating the use of woody biomass for a variety of uses including the generation of high value products and renewable materials. Adapting our management of short-rotation coppice willow is key to ensuring we can maximize the potential yield of woody and non-woody biomass for a variety of end-uses. Possibilities include decreasing the usual three-year coppicing cycle to annual harvests or even twice-yearly harvests, and utilizing both the non-woody/ leafy biomass and woody/ stem biomass for a variety of industries and products. Here, we show in a field trial of 8 diverse *Salix* genotypes 1) how the seasonal phenotypic and destructive yield measurements varied between twice-yearly and annual harvests, compared with the standard three-year coppice cycle during their first 4 years of establishment, 2) how the woody and non-woody dry weights and percentage of the yield varied between the 8 *Salix* genotypes with season and 3) how simple non-destructive measurements of biomass correlated with yield. In conclusion, the diverse *Salix* genus offers flexibility in its coppicing regime which can allow for its biomass to be used for a variety of industries and products.

Keywords: *Salix*, coppicing, phenotyping, yield