International Poplar Commission

Environmental Applications of Poplar and Willow Working Party Workshop meeting

June 5th - 8th, 2007, Montreal, Quebec, Canada

Programme

Abstracts and Field tour

Plant biology research institute of the Montreal University

The Institut de recherche en biologie végétale (Plant Biology Research I Institute) is located within the Montreal Botanical Garden, one of the largest and most prominent botanical gardens in the world. Initially known as the Institut botanique de l'Université de Montréal (University of Montreal Botanical Institute), it was founded in 1920 and was moved to the administration building of the Montreal Botanical Garden in 1939. The institute has grown out of a long-standing collaboration between the University of Montreal and the city of Montreal. In March 1996, newly renovated offices and research laboratories at the IRBV were inaugurated along with the new reception centre of the Montreal Botanical Garden. Aside from being a very pleasant and stimulating working environment, this setting provides an outstanding source of plant material for research. The Institute's staff consisted of thirteen professors from the Department of Biological Sciences of the University of Montreal and seven scientific researchers from the Research and Development division of the Montreal Botanical Garden. The research activities at the IRBV are focused on cellular mechanisms of development and plant defences against adverse environments and pests, the biodiversity of flowering plants and the management of ecosystems in populated areas. Scientists also supervise the research projects of numerous graduate students, spanning areas of biology from the molecular to the ecosystem levels. Finally, the IRBV participates in scientific education for the general public, particularly through the Friends of the Montreal Botanical Garden.

Today, the IRBV is a major leader in research on national and international levels, in academic training, and in scientific outreach activities.

Workshop Programme

Monday, June 4 2007

17h00 -19h00 Registration and Ice breaker – Reception Center, Montreal Botanical Garden

Tuesday, June 5 2007 – Oral presentations and poster session. Room B-354, Institut de recherche en biologie végétale

8.00 – 9.00 **Registration**

9.00 – 9.20 Welcoming words:
Michel Labrecque, Institut de recherche en biologie végétale, Montréal, Canada
Tim Volk, SUNY College of Environmental Science & Forestry, Syracuse, U.S.A

9.20 – 9.40 **Ian Shield**. Rothamsted Research, U.K.
A willow breeding programme for the UK, part of the Biomass for Energy Genetic Improvement Network (BEGIN).

9.40 – 10.00 **Katrin Heinsoo**. Estonian University of Life Sciences, Estonia. Potentials and limiting factors of biomass energy: Estonian Experience of Short Rotation Forests.

10.00 – 10.20 **Terenzio Zenone**. Department of Forest Science and Environment, University of Tuscia, Italy. Carbon sequestration in short rotation forestry (SRF) and traditional poplar plantation: the JRC Kyoto experiment.

10.20 – 10.40 *Coffee Break*

10.40 – 11.00 **Alistair McCracken**. Agri-Food & Biosciences Institute, Ireland. Effect of applying sewage sludge to SRC willow.

11.00 – 11.20 **Ioannis Dimitriou**. Swedish University of Agricultural Sciences, Sweden. Swedish experiences from wastewater irrigation on large-scale Short-Rotation Willow Coppice plantations.

11.20 – 12.00 **Pajand Nejad**. Dept. of Forest Mycology and Pathology, SLU, Sweden. Pathogenic and ice nucleation active (ina) bacteria causing damages to energy forestry plantations (Willow, Poplar): from problem assessment, characterization, identification to solution.

12.00 – 12h20 **Angelo Massacci**. Istituto di Biologia Agro-Ambientale e Forestale del CNR, Italy. Salicacee species: identification of molecular functions and analytical descriptors involved in metal uptake and translocation

12.20 – 13.30 *Lunch*

13.30 – 13.50 **Jud G. Isebrands**. Environmental Forestry Consultants, U.S.A. Phytoremediation of a Wisconsin Brownfield with Poplars

13.50 – 14.10 **Kim Cameron**. SUNY College of Environmental Science and Forestry, U.S.A. Breeding shrub willow as a feedstock for bioenergy, biofuels and bioproducts

14.10 – 14.30 **Andrej Pilopovic**. Institute of Lowland Forestry and Environment. Serbia & Montenegro. Potential of Different Poplar Clones in Phytoextraction of Some Heavy Metals

14.30 – 14.50 **Richard A. Lord**. University of Teesside, U.K. Biomass, Remediation, re-Generation (BioReGen Life Project): Reusing brownfield sites for renewable energy crops.

14.50 – 15.10 *Coffee Break*

15.10 – 15.30 **Mauritz Ramsted**. Dept. of Forest Mycology and Pathology, SLU, Sweden. The use of willows in phytoremediation of PAH-contaminated soils.

15.30 – 15.50 **Jaconette Mirck**. SUNY College of Environmental Science and Forestry, U.S.A. Sap flow of willow varieties being used to develop an evapotranspiration cover for the Solvay wastebeds in Upstate New York.

15.50 – 16.10 **Michel Labrecque**. Institut de recherche en biologie végétale, Canada. The use of willows and poplars for environmental applications in the Montreal region: an overview of diverse projects.

16.10 – 17.30 **Poster session**

17.30 – 19.30 **Welcome Reception at the Montreal Botanical Garden**

Wednesday, June 6 2007

9.0	Meet at Montreal botanical Garden (MBG)
9.30 – 10.30	Visit of an experimental/demonstration site located on a contaminated land in downtown Montreal.
11.15 – 12.30	Living walls used as noise barriers in Laval and in Boisbriand (North of Montreal).
12.30 – 13.30	*Lunch at Boisbriand hosted by the City of Boisbriand*
13.30 – 15.00	River bank stabilization project in Boisbriand (to be confirmed)
16.15 – 18.00	Business meeting at MBG

Thursday, June 7 2007

7.30	Depart MBG for Syracuse NY
10.30 – 13.00	Custom procedures and lunch
14.00 – 15.30	Visit to Tully: willow breeding and genetics program, streambank stabilization and riparian buffers
16.00 – 17.00	The Solvay wastebeds project in Syracuse, a pilot project to develop an alternative ground cover using willows.
18.00	Arrive to Genesee Grande Hotel

Friday, June 8 2007

7.30	Depart Genesee Grande Hotel
11.30 – 12.30	Willow plantations and clone trials in Huntingdon, Quebec.
12.30 - 14.00	*Dinner at Domaine de la Templerie*
17.30	Return to Montreal

Note: This programme may be subject to alteration and the organizers reserve the right to do so without giving prior notice.

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ORAL PRESENTATIONS

A willow breeding programme for the UK, part of the Biomass for Energy Genetic Improvement Network (BEGIN)

Ian Shield, William Macalpine, Steve Hanley, Ming Pei and Angela Karp

Rothamsted Research, Harpenden, Hertfordshire, UK

The primary objective of the programme is the improvement of the willow crop for renewable energy. This is increasing in importance as the UK Government seeks to incorporate into law it's aspirations for a 60% reduction in CO_2 emissions (over 1990 levels) by 2050. Suitable willow cultivars are available for growing in the UK but great potential for improvement remains. We bred two new varieties before joining Svalöf Weibull AB, Sweden, and Murray Carter, UK, to form the European Willow Breeding Partnership, which bred 8 more varieties. During Institute re-structuring in 2003 the partnership was dissolved, but UK willow breeding continued through "BEGIN", funded by the Department of Environment, Food and Rural Affairs (Defra). Yield improvement is the main target, followed by durable resistance to diseases and insects. More recently, wood quality is also being investigated. Advantage is taken of the wide genetic diversity present in the National Willow Collection held at Rothamsted, which has *ca*. 1,300 accessions, including 100 pure species. Five promising improved genotypes have already been identified from BEGIN's efforts. The main disease of SRC willow is rust caused by the *Melampsora* fungus. New lines are tested against rust pathotypes and willows with new sources of resistance are being used in the crossing programme. The pathogenicity of new isolates is continually tested and any potentially new pathotypes tested against willow varieties and elite lines. Pest studies have concentrated on the blue and brassy willow beetles *Phratora vulgatissima* and *P. vitellinae*, which are the main insect pest. Subtle but important differences in host preference were detected and found to be determined by a combination of specific leaf volatiles and secondary metabolites in the leaves. Crosses have been made to breed for insect resistance. The programme is underpinned with molecular genetics and genomics research. A dense willow genetic map has been constructed from a uniquely large (*ca*. 1000 progeny) genetic mapping population grown at on two contrasting sites. Six robust quantitative trait loci (QTLs) for biomass yield and three QTLs for rust resistance have been identified. More recently, the willow genetic map has been aligned with the poplar genome and the macro-syntenic relatedness of willow and poplar has been demonstrated. Dissection of the QTL to determine the underlying genes is underway and markers for use in marker-assisted selections are being developed.

Potentials and limiting factors of biomass energy- Estonian experience of short rotation forests

Katrin Heinsoo and Bert Holm

University of Tallin, Estonia

The willows production potential studies in Short Rotation Forest have revealed a lot of interesting information during last decades. The pioneer country of willow plantation for energy purposes, Sweden reported in 80ths that in South-Sweden climate it was possible to achieve annual willow production of 20 t of dry shoot biomass per ha per year (e.g. Christersson, 1986). Later on these figures decreased by one fourth even for plants without any visual damages (e.g Verwijst et al. 1995). Furthermore, in plantations with sufficient water and nutrient supply next to wastewater outlet points the annual production of willows was reported to be around 10 t ha $^{-1}$ (e.g. Aronsson et al 2002). Such large variability is mainly caused by the age and environmental conditions of plantation under investigation. Even if the study methods and research areas are the same throughout the years the yield numbers vary from year to year and depend on the planted clone. The highest annual productivity of 24 t ha $^{-1}$ in Estonia was estimated one year for one clone of *Salix dasyclados* (for details Heinsoo et al 2002). However, the average annual production of the same clone/plot over the whole rotation cycle was 15 t ha $^{-1}$. Overall average productivity of the same plantation during the same rotation cycle was 11 t ha $^{-1}$ and 5 t ha $^{-1}$ for fertilised and non/fertilised plots, respectively. Therefore we conclude that willow shoot production in Short Rotation Forests depends on different biological factors with the most important by our expertise listed (not in the order of importance): plantation size, plantation age, planting material suitability for local climate conditions, water availability, nutrient supply, pathogens existence, soil type. Beside biological barriers for biomass production in the SRF there exist also a lot of other non-technical barriers having an influence on the feasibility on the biomass production for energy purposes. Quite many of them are typical not only for Estonia but according to our practice with different international applied research projects in the other countries starting with SRF management as well (for example www.biopros.info). Some of these can be overcome with the longer expertise on regional level by producers themselves and some need special support activities from authorities: uneven availability of planting material, absence official cutting producers, high planting cost, fertilisation limitations do not give any specifications to energy crops, unequal land usage subsidies available, lack of training system for farmers, know-how dispersion, uncertainty of the production market. There are also some technical barriers that limit today acceleration the biomass production from Estonian SRF that should be solved by the local scientists and specialists with the support on governmental level: lack of local planting material, absence of suitable machinery for both harvesting or planting. Despite all this problems and shortages in SRF management of Estonia, we have got a great public interest so far, both by the farmers and the entrepreneurs of distant heating systems. Furthermore, in January 2007 Estonian Parliament approved the biomass and bioenergy usage development plan for 2007-2013 where the role of SRF for biomass production is pointed out. Therefore we assume that the area of SRF in Estonia will continue rapid growing during next years and in the long-run it has a future in the Estonian energy market.

Carbon sequestration in short rotation forestry (SRF) and traditional poplar plantation: the JRC Kyoto experiment

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In recent years, the increasing funding of non-food crops provided by the Community Agricultural Policy (CP) has brought about a series of changes in the traditional land use. In this light, considering that Short Rotation Forestry (SRF) could allow Italy to increase its quota of renewable energy production, several Italian Regions, where biomass thermoelectric power plants are under construction, have included in their Programme for Rural Development (PRD) a series of financial incentives to support the establishment and maintenance of SRF plantations. For these reason, SRF for energy purposes is rapidly expanding in Italy. In order to evaluate the carbon sequestration in traditional plantation and Short Rotation Forestry (SRF) of poplar, we measure the CO_2 exchange between the atmosphere and canopy using eddy correlation (EC) technique during the years 2002-2005. EC allows to determinate the Net Ecosystem Production (NEP) in terms of carbon stored by the ecosystem. The study areas are located in the northwest of Italy inside the Basin of Ticino river: the poplar plantation (Populus x *euramericana* clones I-214 spacing 6 x 6 m density 270 plants ha^{-1}) occupies an area of 120 ha. The SRF (P *generosa* X P. *nigra* clone Pegaso) occupies an area of 80 ha: trees were planted in March 2004 using 1-year-old seedlings in a double row design with a spacing of 2.8 x 0.75 x 0.45 m corresponding to a density of 12.500 plants/ha. The three years observation in poplar plantations shows that the carbon uptake was stronger in 2003 than in 2002 for a period ranging from bud break up to around DOY 160 (early June). Afterwards, the 2003 Net Ecosystem exchange (NEE) was severely reduced. This trend may reflect the precipitation trends of the year 2003 that were comparable to year 2002 until April while, from May onwards, precipitation was much lower in 2003.The total annual sequestration has been estimated as 11.2 and 27.5 t CO_2 ha^{-1}, respectively for the first and second growing season. In addition, in order to calculate an overall GHG budget for the examined SRF plantation, CO_2, CH_4 and N_2O emissions from agriculture machinery use (ploughing, harrowing, planting, harvest, irrigation, fertiliser and pesticides spearing) were estimated according to IPCC methodology. The resulting annual GHG emission, in terms of tons of CO_2 equivalent ha^{-1}, has been estimated in 1.03 and 0.59 for the first and second growing season, respectively. To further extend the GHG budget analysis, we modelled the GHG emissions/absorptions for 10 years in SRF and in a conventional, non-coppiced, poplar plantation. Based on data obtained from experimental experience and from literature, two level of productivity were hypothesized based on the level of potential productivity and intensity of the cultural practices: high (H) and low (L) inputs. The resulting CO_2 uptake has been calculated in 130 (L) – 183 (H) t CO_2 ha^{-1} for the poplar plantation and 134 (L) – 235 (H) t CO_2 ha^{-1} for SRF. On the other hand, the CO_2, CH_4 and N^2O emissions from agricultural treatments and pesticide/fertilizer production resulted to 7.7 (L) – 11.5 (H) t of CO_2 equivalent for poplar plantation and 9.2 (L) – 23.4 (H) for SRF. Overall, these results indicate a very good GHG balance for both the cultivations and the different scenarios analyzed.

Effect of applying sewage sludge to SRC willow

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Short Rotation Coppice (SRC) Willow has potential for the bioremediation of high nutrient waste streams. Currently in Northern Ireland raw sewage sludge cake is being applied to growing crops of SRC willow at rates of around 74t ha^{-1}, normally during the first year of re-growth following coppicing. The sludge is about 30% dry matter and is applied using a specially adapted machine which injects the sludge into the soils to a depth of approximately 50 cms. In a replicated trial, sludge was applied to plots of one of six *Salix* spp. varieties – 'Stott', 'Parfitt', 'Olaf', 'Tora', 'Torhilde' and 'Sven', at rates equivalent to 37t ha^{-1}, 74t ha^{-1}, 118t ha^{-1} and 128t ha^{-1} between July 2005 and March 2006. The chemical analysis of the sludge was variable depending on the batch. However the dry matter was between 21 and 32% and had a pH of 5.6 to 7.0. There was 2.2 – 2.34% nitrogen in the dry matter. Levels of calcium (8158 mg Kg^{-1}), potassium (8334 mg Kg^{-1}), phosphorus (11,270 mg Kg^{-1}) and iron (4330 mg Kg^{-1}) were relatively high. Water samples were obtained from boreholes within each plot throughout a 2 year period. Soil samples were also taken for analysis annually. In December 2006, i.e. after two years growth the plants were harvested and dry matter yield obtained for each variety with each sludge application. All of the *Salix* spp. varieties produced a greater dry matter yield with the lowest application of sludge 37t ha^{-1}, compared to no sludge. However, with the exception of 'Tora' additional levels of sludge application, even up to 128t ha^{-1} did not result in significant yield increases. Analysis of borehole water was inconclusive but there was little evidence that there were elevated levels of total nitrogen (TON) in the plots receiving high levels of sludge. The implementation of the European Union Nitrates Directive (91/676/EC) in 2007 has meant that a maximum of 34t ha^{-1} of sludge may be applied to soil. This at least doubles the area of SRC willow required to treat the same quantity of material. Compliance with further legislation (Safe Sludge Matrix) requires a pre-treatment of the sludge in order to reduce the pathogen numbers. The required 99% pathogen kill can be achieved using lime (Ca0). Addition of 10% or 25% lime on a dry weight basis resulted in an immediate rise in pH to >11.5 with an associated immediate drop in numbers of *Eschericha coli.* When the sludge was mixed (25%) with cement kiln dust, a waste product from the local cement factory the pH was raised to ~10.0 with the required pathogen kill occurring within 4 days. Treatment with lime cement dust resulted in ammonia release, which has to be managed, and which also resulted in nitrogen loss. This potentially could permit higher levels of sludge to be applied to the willow. Treatment with lime or cement dust may achieve the necessary pathogen kill, but the application of such a high pH material to the soil may have an adverse effect on the plants. This will be monitored following lime treated sludge application in spring 2007. The use of cement kiln dust is an attractive approach as it is dealing with a second waste product, although it is necessary to ensure that it contains no potentially waste elements which may have an adverse environmental impact. Sites where sludge has been applied over a period of time are also being monitored for environmental changes which will indicate the sustainability of this approach for sewage waste management.

Swedish experiences from wastewater irrigation on large-scale short-rotation willow coppice plantations

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Short-rotation willow coppice (SRWC) is cultivated in Sweden to produce biomass for energy. The crop is commercially grown on agricultural land and the produced biomass is used in district heating plants for combined heat and power production. In recent years, nutrient-rich wastewaters, mainly municipal wastewater, landfill leachate, and industrial wastewaters as log-yard runoff, have been successfully applied to SRWC to reduce fertilisation costs and simultaneously increase biomass production and/or to facilitate alternative low-cost wastewater treatments. The content of pollutants and nutrients in waters and soils is reduced through plant uptake and microbial degradation. Simultaneously, biomass production is enhanced. There are around 30 operating SRWC plantations for utilisation and treatment of wastewaters in Sweden. Municipal wastewater contains nitrogen (N) and phosphorus (P) and is preferable to be used as a fertiliser on non-food, non-fodder crops such as SRWC for sanitary reasons. Municipal wastewater has been reported to be a suitable and well-balanced fertiliser for willow. During the 1990s, large SRWC plantations with drip or sprinkler irrigation systems were established adjacent to wastewater treatment plants in a range of cities in Sweden to improve the N treatment efficiency while producing biomass. It was assumed that if biomass production were 10 t DM/ha and the N concentration in the willow shoots 0.5%, then 50 kg of N/ha would be removed from the field at harvest each year. Research has shown, however, that N retention can be more than 200 kg N/ha/yr because of denitrification, *i.e.* the microbial transformation of nitrate to nitrogen gas, and long-term binding of nitrogen in the soil, namely the build-up of nitrogen-rich soil organic matter. In Enköping, a town with 20000 inhabitants in central Sweden, a novel system based on the above idea has been introduced; Sludge is dewatered after sedimentation and centrifugation, and the N-rich water produced, which formerly was treated in the plant, is now distributed to an adjacent 75-ha SRWC plantation during the growing season, as a part of an obligation to reduce the total N outflows in an adjacent lake by 50%. This water contains approximately 800 mg N/l and accounts for about 25% of the total N treated in the wastewater treatment plant. The water is pumped into lined storage ponds during winter and used for irrigating SRWC during summer irrigated for about 120 days annually (May to September), mixed with conventionally treated wastewater to promote plant growth. The system treats about 11 t N and 0.2 t P per year in an irrigation volume of 200000 m^3 of wastewater, of which 20000 m^3 is water derived from dewatering of sludge. Irrigation ceases automatically on rainy days. Irrigation rates reach a daily mean value of 2.5 mm during the growing season. Possible environmental hazards associated with such applications, e.g. N leaching and N_2O emissions into the atmosphere, are monitored; results so far from the field but also from lysimeter trials indicate minimal risks after wastewater application, and biomass growth has proved to be higher than the average for commercial SRWC in the area (unpublished data). Other N-rich wastewaters as e.g. landfill leachate, or industrial wastewaters with lower nutrient content but with sufficient amounts available during summer, e.g. log-yard runoff, are irrigated to SRWC in Sweden for utilisation of nutrients and treatment of hazardous compounds. At Ragnsells Avfallsbehandling AB landfill, located in Högbytorp, central Sweden, landfill leachate is stored and aerated in ponds and then pumped into a 5-ha SRWC field irrigated daily during the growing season with approximately 2-3 mm. At the Heby sawmill in central Sweden, where about 60000 m^3 per year of log-yard runoff is recycled to a 1-ha SRWC field. The field is irrigated with sprinklers during the growing season at a rate of about 4000 mm per year. A conclusion after monitoring and evaluating a range of such systems is that pre-testing of irrigation regimes in combination with plant material under local conditions is necessary to avoid failure, in connection with continuous detailed monitoring of parameters of concern is required for successful application. For e.g. landfill leachate, stress symptoms to the plants due to high chloride concentrations or low P supply can occur, therefore laboratory pre-trials testing tolerance of different willow clones under different leachate irrigation regimes are necessary before establishing a large scale system.

Pathogenic and ice nucleation active (ina) bacteria causing damages to energy forestry plantations (willow, poplar): from problem assessment, characterization, identification to solution

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To find out whether bacteria isolated from diseased plant parts can be the main causal agent for the dieback appearing in *Salix* energy forestry plantations in Sweden during the last few years, and if the joint effects of bacteria and frost injury are synergistic, extensive sampling of shoots from diseased *Salix* plants was performed. We performed several laboratory and greenhouse investigations and used evaluation techniques on the functions of the Ice-Nucleation Active (INA) bacteria.We carried out a comparison between spring and autumn bacterial communities isolated from within (endophytically) and surface (epiphytically) plant tissues of *Salix viminalis*. Seasonal variation of bacteria in willow clones with different levels of frost sensitivity and symptoms of bacterial damage was also investigated. We further focussed on possible effect of fertilisation and nutrient availability on the bacterial community in relation to plant dieback in Estonian willow plantations.The identification and detection of INA bacteria which cause damage in combination with frost to willow (*Salix* spp) plants in late fall, winter and spring was performed using BIOLOG® MicroPlate, biochemical tests, selective INA primers and 16S rDNA analysis. To distinguish the character for differentiation between these bacteria morphologically and with respect to growing ability different culture media were used.We studied the temperature, at which ice nucleation occurred for individual bacteria, estimated the population of INA bacteria, effect of growth limiting factors, andevaluated the effect of chemical and physical agents for disruption and possible inhibition of INA among individual bacterial strains. The concentration of carbon, nitrogen and phosphorus on INA is discussed. We demonstrate that among the bacterial isolates recovered from the willow plantations, there were many that were capable of ice nucleation at temperatures between -2℃ and -10℃, many that were capable of inducing a hypersensitive reaction in tobacco, as well as causing necrotic symptoms on willows exposed to frost treatment. The most frequently isolated types were found to be non-fluorescent *P. fluorescens* (biotype A, B, C, F, G) and/or *Sphingomonas* spp. *Erwinia* spp, *P. fluorescens*, *Xanthomonas* spp and *P. syringae* however, were considered to be the most important pathogens in the field.We conclude that diseases caused by INA bacteria in relationship with frost are a limiting factor in willow and poplar plantations in Sweden and most likely also in other temperate regions in the world. Possible solutions: Bacterial/rust fungus resistance in current clonal material, testing for pathogenicity, certified cuttings — sterilisation, studying factors influencing pathogens (chemical/physical, growth temperatures, growth limiting factors) and Quorum sensing (QS) and regulation of biological functions. *Key words*: *Salix viminalis*, INA bacteria, frost damage, ina gene primer, BIOLOG®MicroPlate, nutrient starvation.

Salicacee species: identification of molecular functions and analytical descriptors involved in metal uptake and translocation

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The potential for an enormous genetic diversity of poplar and willow has suggested searching among clones of an Italian germplasm collection of these species to select material with a high capacity for phytoextraction of specific heavy metals from soils or waters. To this scope, 20 clones, selected for contrasting phenotypic characteristics, identified in previous experiments, were exposed to toxic concentration of cadmium solution. Growth and physiological parameters of exposed plants, as well as, their ability to accumulate cadmium in roots and to translocate it to leaves were determined and six genotypes with the best characteristics for phytoextraction were further selected. Data for all ecophysiological and morfological parameters were subjected to analysis of variance (ANOVA) using the SPSS software supplemented with multiple-comparison test of the means using the Tukey-Kramer method with a significance level of $P < 0.05$. We also used hierarchical cluster analysis to classify the clones based on the results of our experiment. Results showed interesting aspects of the plant interaction with the absorbed cadmium. For example, clones with high net photosynthesis had higher total dry mass and cadmium content. Chlorophyll content was not related to the maintenance of a high photosynthetic capacity. It was also shown that *salix* can absorb per plant more cadmium than *poplars* and maintain it in roots up to 50% with respect to the 80% maintained in poplars. By means of statistical analysis clones were ranked for specific phytoextraction abilities including tolerance variation towards the amount of cadmium absorbed in roots or trasnlocated to shoots. The six selected genotypes are also under genetic analysis by selecting genes involved in the different steps of the process: cadmium uptake, sequestration, compartmentalisation, and translocation. A database has been developing for *Populus* species containing EST homologous to genes isolated from different plant species. The genes encode for proteins involved in tolerance to heavy metals: metallothioneins (Zhou and Goldsbrough 1994); enzymes for phytochelatin synthesis (Howden et al 1995); tonoplast propteins (Salt et al, 1998); proteins produced under high temperatures and oxidative stresses (Diderjean et a l 1996). Genes homologous to different transporters involved in heavy metal uptake will be also isolated from *Populus* genome (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html). Single nucleotide polymorphisms (SNPs) will be looked for by comparing sequences from different poplar clones and by identifying those sequences that may be potentially responsible for allelic variants with functional significance. This experimental pathway will eventually help in identifying possible molecular descriptors of plants performance in phytoremediation. Two poplar clones were also investigated for mapping metal concentration within roots through scanning electron microscopy (SEM) and energy dispersive ray-x (EDX). Control plants had metal concentration below the instrument sensitivity. From SEM-EDX analysis, clone 6K3 was found to take up higher Cd amounts than clone 14P11. Higher Cd concentrations were found in the apical portion or roots in clone 6K3, while in the intermediate portion in clone 14P11. Amongst the elements considered, only Fe and P were significantly affected by metal treatment in both clone, while Cu, K and S showed significant interaction clone x treatment.

Phytoremediation of a Wisconsin brownfield with poplars

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Phytoremediation is used throughout North America to clean up, enhance, and revegetate former industrial brownfield sites. We are using poplars for phased phytoremediation at a former industrial site in Menasha, Wisconsin that is contaminated with lead and a suite of hazardous volatile organic compounds. The first phase was a pilot test to determine if poplars would grow suitably on the harsh site. Then, a diverse group of cottonwoods, hybrid poplars, and aspen clones were auger-planted across the heterogeneous site with mulch; the site included a building demolition area. Native grasses and wildflowers were added for beautification and wildlife habitat near a public street and railroad crossing. After two years, growth of several of the poplars has been outstanding, and water table levels and hazardous chemical levels have decreased in on-site monitoring wells. In addition, public acceptance has been very favourable as the site has been converted from an unsightly abandoned property to an aesthetically pleasing green space. Project details and a progress report will be presented.

Breeding shrub willow as a feedstock for bioenergy, biofuels and bioproducts

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The development of perennial energy crops as feedstocks for biorefineries and bioenergy will combat global warming and contribute to petroleum independence, while stimulating rural economic development. Dedicated woody crops, such as fast-growing shrub willow (*Salix* spp.) will become an essential part of the feedstock supply. There is wide genetic diversity across the genus *Salix*, with great potential to produce improved varieties through controlled breeding. The genus *Salix* includes over 300 species, many of which can hybridize naturally or through controlled pollination. In assembling an extensive, genetically diverse breeding population of shrub willow, SUNY-ESF has collected representatives from more than 10% of the known species of *Salix*. Many of these individuals have been used as parents in our breeding program, producing over 300 new families representing over 25 species and species hybrids. Breeding compatibility was species- and gender-dependent with intra-specific crosses generally successful more often than inter-specific crosses. Typically ~75 to 100 individuals from each viable cross were planted in field plots, then the best families and individuals within families, based on height of the tallest stem, total stem diameter per plant, and rust resistance, were selected after the first or second season post-coppice. Selected individuals were then planted in replicated, randomized selection trials. After two 2-year rotations, yields in excess of 22 metric tonnes ha^{-1} have been recorded (based on 4-plant plots on one site). Individuals displaying exceptional growth have been planted in replicated yield trials in New York, Minnesota, and Alberta (Canada), with several additional trials planned for 2007.

Potential of different poplar clones in phytoextraction of some heavy metals

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This research involves investigation of potential of poplar clones for heavy metals phytoremediation. The experiment was conducted in controlled conditions in the greenhouse with hydroponically grown poplar cuttings of following poplar clones: *Populus* x *euramericana* clone EA 8, (*i*) *Populus deltoides* clone PD 3 and (*iii*) complex inter-section hybrid (P. nigra x *maximowitzii*) x P. nigra var. "Italica" clone NM 8. Plants were grown at containers with Hoagland nutrient solution containing various concentrations of cadmium (Cd), nickel (Ni) or lead (Pb) ranged from 0, 10 and 100 ppm each. During their growth enzymatic activity of nitrate reductase (NRA) was assessed. After the growing period plants were harvested and accumulation of heavy metals and nitrates together with heavy metal phytoextraction coefficient was assessed. Highest concentrations of heavy metals decreased both biomass production and NRA of plants. Contaminant accumulation showed different potential of investigated clones and their plant compartments for phytoextraction.

Biomass, remediation, re-generation (BioReGen life project): reusing brownfield sites for energy crops

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The Tees Valley, NE England, contains one of the largest heavy industrial clusters in W Europe, including oil refineries and petrochemicals, iron and steel, bulk inorganic and speciality chemicals. Recent industrial history included extensive coal-based heavy industry, while mining, smelting and metallurgy of ferrous and non-ferrous metals has been carried out over two millennia. The result is a legacy of derelict, "brownfield" (previously used), and contaminated post-industrial land. It is estimated that an area of 20,000 ha of contaminated land exists in North East England, some of which is likely to underlie the 3160 ha of currently derelict sites or vacant buildings. Development of the 30 MWe Wilton 10 biomass power station (SembCorp Utilities) has created a local market opportunity for energy crop production in Teesside. A consortium led by CLEMANCE has been formed to investigate the potential of derelict industrial sites for energy crop production to supplement production from agricultural land. The purpose of this paper is to present the initial results from our pilot sites and to announce the subsequent Life III Environment Programme demonstration project. In April 2004 an experimental field trial of four potential energy crop species was hand-planted on a former industrial site near Bishop Auckland as part of the CLEMANCE European Regional Development Fund Bioremediation Programme. The brownfield site was originally a brick clay pit, backfilled over 50 years ago with domestic coal ash, privy waste and incineration residues. The resultant heavy metal contamination includes phytotoxic levels of Zn (400-1000 ppm), Cu (100-500 ppm) and Ni (100-200 ppm) and levels of Pb (300-2000 ppm) and As (50-200 ppm) which are significant for human health concerns. Soil preparation included rotavation but no amendments, weed killer or rabbit fencing. Duplicate experimental plots were planted with willow short rotation coppice (variety Tora), miscanthus (*Miscanthus x giganteus*), reed canary grass and switchgrass (variety Cave-in-rock). After plant establishment the plot was hand weeded and mulched with green waste compost. The results of the first season indicate that miscanthus rhizomes grow well in contaminated soils at these northern latitudes, and, unlike the willow cuttings, are not affected by rabbits grazing young shoots. Both reed canary grass and switchgrass were established from seed but suffered weed competition and grazing. After fencing two subsequent seasons have shown good survival and growth rates for miscanthus, reed canary grass and willow. This paper will compare the relative yields, ash composition and contamination results for samples of biomass fuel prepared from each of these three species from this pilot site, and from a contrasting brownfield site, an industrial and a rural greenfield control site. The BioReGen project (www.bioregen.eu) will demonstrate the feasibility of reusing brownfield sites to grow biomass energy crops at a commercial scale on a variety of contaminated sites. In addition to generating heat and power from renewable energy sources this land use has a number of environmental benefits: Damaged land is restored with ecological and aesthetic improvement. Use of composted wastes as soil amendment achieves diversion from landfill, contaminant stabilization and promotes *in situ* bioremediation of organics. Long-term growth has the potential to achieve cost-effective remediation of metal contamination via phytoremediation. This avoids the need to resort to energy-intensive processed-based remediation or other practices, such as excavation and removal, which merely relocates and contains pollution as a challenge for future generations. Reusing derelict industrial sites provides an economic advantage, since the major capital cost of land is avoided compared to using productive agricultural land. Indeed, many contaminated sites have negative asset values reflecting the costs of future remediation, or ongoing maintenance costs, which can be offset provided that adequate growth can be successfully established. Consequently, the resulting biomass crop might be contaminated from plant uptake or soil adhesion, necessitating testing to establish adequate pollution control measures before firing. The project will conclude by examining the technical issues of embedded generation using local biomass powered CHP.

The use of willows in phytoremediation of PAH-contaminated soils

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Five different willow clones were compared with respect to growth and degradation of PAHs (and diesel) in contaminated soil, with and without application of additional nutrients.The aims of this study were to reveal possible clone-specific properties among *Salix* in degradation of soil contaminants and to study the effect of nutrients present, on biodegradation of PAHs and diesel.
Aged creosote and diesel-fuel contaminated soil originating from Resecentrum, Uppsala (Central Railway Station) was collected at Hovgården Deposit Plant. Three kinds of soil were included in this study: creosote and diesel contaminated soil (undiluted); mixed soil 1:1 (diluted with non-contaminated soil) and control soil (non-contaminated). The initial total concentration in contaminated soil was 10 ppm (mg/kg) of PAHs and 1,150 ppm of aliphatic and aromatic hydrocarbons (i.e. diesel components). The *Salix* clones used were Tora, Björn, Orm, 78138 and 78112 and as nutrient additive Blomstra® fertilizer was used. During four months, no obvious difference was found between the five willow clones regarding shoot growth except that Orm grew less well in creosote and diesel contaminated soils. Root biomass increased and the Shoot/Root ratio (S/R) decreased for most clones at the higher concentrations of creosote and diesel contaminants. Degradation was almost similar for all clones although 78112 and Orm had somewhat lower PAH degradation capacities as compared to the three other. None of the five clones, however, showed higher degradation than treatment without plants in this experimental layout. On the contrary, the presence of plants in the creosote and diesel contaminated soil, retarded the degradation of most PAHs as compared to the treatment without plants. This was probably due to interference of the root exudates with the use of diesel as carbon source for the microorganisms. The latter hydrocarbons could act not only as carbon sources but also co-substrates that are needed in the co-metabolic degradation of PAHs. In the choice between the two, many microbes probably prefer the former more easily metabolizable compounds. The degradation of PAHs in the creosote soil treatments without plants was even higher in treatments with nutrients added, especially for the high molecular weight PAHs, such as benzo(b)fluoranthene (94.7%) and benzo(a)pyrene (100%) as compared to the initial values and the control treatment. This is likely to be a result of stimulation of specific PAH degraders by the higher nutrient level. The bacterial counts were significantly higher in both the treatment with plants and the unplanted treatment with nutrients, as compared to the initial soil and the control treatment. Eight bacterial strains with the ability to degrade phenanthrene were isolated from the undiluted creosote and diesel contaminated soil. The value of planting *Salix* as compared to other treatments in different kinds of soils with single contaminants or a combination of several different will be further discussed.

Sap flow of willow varieties being used to develop an evapotranspiration cover for the Solvay wastebeds in Upstate New York

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The production of soda ash over a 100 year period using the Solvay process resulted in the creation of about 600 ha of wastebeds near Syracuse, NY that are up to 21 m deep. About 250 ha of wastebeds remain uncovered. Chloride leaching from these wastebeds is impacting nearby water bodies. As part of a project to develop an evapotranspiration (ET) landfill cover using shrub willows, sap flow rates are being measured. The objectives of our study were to measure sap flow at different points in the growing season and to calculate the impact on the water budget of a willow ET cover. Sap flow was measured on established willow plantings on a gravelly silt loam soil using the heat balance method on three willow varieties (*Salix miyabeana* (SX64), *S. eriocephala* (S25) and *S. dasyclados* (SV1)) in the fall of 2004 and the whole year of 2005. In 2006 sap flow was measured on four willow varieties (*S. miyabeana* (SX64), *S. sachalinensis* (SX61), *S. sachalinensis x miyabeana* (9870-23) and *S. purpurea x purpurea* (9882-34)) that were established on the Solvay wastebeds in 2004. Maximum single-stem sap flow rates for willow stems ranging from 10-35 mm in diameter on gravelly silt loam soils were 0.8, 0.4 and 0.3 liter per day for SX64, SV1 and S25 respectively during the fall of 2004 as compared to 6.0 and 2.5 liter per day for SX64 and S25 during the middle of the summer of 2005. Sap flow rates during the summer of 2006 on the Solvay wastebeds for willow stems ranging from 10-25 mm in diameter changed over time and were different between willow varieties. Maximum sap flow rates for single stems were 4.1 liter per day for variety 9870-23 in June and 2.0 liter per day for variety 9882-34 in July. Diameter distributions were used to calculate stand level sap flow rates. The sap flow rates we observed during the end of the growing season suggest that willow short rotation coppice continue to have an impact on a site its overall water budget at least until the end of October. Key words: transpiration, water-balance, *Salix*, Penman-Monteith, leaf area index.

Willows for environmental applications: new opportunities for regional development in Quebec, Canada

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Over the last ten years, the Plant Biology Research Institute has initiated the establishment of several hectares of willow plantations in southern Quebec with successful results. Parallel to this, demonstration projects in which willows were used for environmental applications have also been undertaken by the IVBV. Projects were conducted in urban and rural areas addressing different environmental issues, such as the treatment of aquaculture effluents, the recycling of wastewater sludge, etc. Willows have been also tested on brownfields and revealed to be efficient tools for restoration and particularly well adapted to the poor, damaged, compacted and polluted soils which characterized these sites. In some experiments, willow performance was compared to various hyperaccumulators and/or other tree species and willows were found to establish and grow in such poor conditions relatively more efficiently. They have also shown a capacity to accumulate heavy metals, Zn and Cd in particular, in their aboveground parts. To assess the potential of willows to perform on brownfields, recent research has involved the use of arbuscular mycorrhizal (*Glomus intraradices*). In an experiment where willows (*Salix viminalis*, clone 5027) were compared to a hybrid poplar clone (*Populus* × *generosa* A. Henry 'Unal'), it was shown that the willow clone presented higher metal contents in stems and foliage tissues than those of the poplar. The presence of the inoculum seemed to have increased metal content in willow leaves but not in the poplar tissues. The IRBV has also conducted studies on the use of willow branches to construct living noise barriers along highways in urban areas. These projects have been tremendously successful, as well as attracting a great deal of public attention and generating new projects and partnerships. This has had a catalyzing effect resulting in the establishment of an increasing number of willow plantations in several regions in the province of Quebec. It has also stimulated regional initiatives by private or governmental enterprises to develop new economic activities based on willows.

POSTERS

Hybrid poplar stem dieback the year of planting

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Each year, stem dieback is observed on newly planted bare-root hybrid poplars trees produced by the provincial tree nursery of Trecesson. Trees often loose more than half of their height and later develop multiple leaders, which greatly affects their quality and productivity. This study aimed at understanding the causes leading to stem dieback of newly planted poplar trees. We tested the 5 following hypotheses:

(1) Bare-root trees are lifted too early in the spring, before they are completely hardened
(2) Trees are planted too late in the spring
(3) Storage temperature is not cold enough
(4) Fertilization rate is too high, preventing them from hardening
(5) The root-to-shoot ratio of trees is too low at planting

In spring 2005, 2,430 stem cuttings from clone 915319 (clone which usually shows a lot of stem dieback) were stuck in 3 adjacent beds at the provincial tree nursery of Trecesson, Abitibi. The 3 beds were fertilized separately during the growing season (not fertilized, fertilized until July and fertilized until the end of August (the later corresponds to the usual practice of the nursery). At the end of the summer, each bed was lifted at three different times (2 weeks prior to the usual lift time, at the usual lift time, and 2 weeks after the usual lift time, under the snow). The stem of each bare-root tree was cut back according to three types of planting stock (rootstock (the entire stem removed), half-length stems, or full-length stems). The trees were then stored during the whole winter (usual storage: - 3° C warmed to +2° toward the end of spring, or stored outside in trenches). Finally, trees were planted at three different dates (as early as possible in May, in June and in July). A sub-sample of trees was destructively harvested at the end of July to measure leaf area and roots and stems biomass. Growth of trees and stem dieback were measured at the end of the first growing season. Results show that stem dieback increased with fertilization, and that the trees fertilized until July or August had to be lifted earlier in the fall to reduce it. Unfertilized trees, on the other hand, had little stem dieback, regardless of the lift or planting dates. The results suggest that fertilization in the nursery prevents the trees from hardening before winter storage. Keywords: hybrid poplar, stem dieback, fertilization, fast-growing plantation.

Poplar and willow short-rotation coppice response to fertilisation in a lysimeter-based trial- Results of the first rotation

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The main goal of cultivating SRC is represented by their potential role as a renewable energy source. Several studies have recently focused on willow and poplar growth and productivity, but most of them have never been conducted in lysimeters where plants can usually grow under optimal environment conditions with particular regard to water and nutrient supply. A lysimeter allows a specific crop to grow throughout the season without risk of water shortage. Therefore, growth and biomass production obtained may indicate the potential for a specific crop grown under specific climate conditions. This research was carried out in San Piero a Grado, Pisa (north-central Italy) (43ºN, 11ºE; altitude 5 m a.s.l.) under typical Mediterranean conditions. In winter 2002, twelve drainage lysimeters were set up in the field. Each lysimeter was provided with an automatic system that maintained soil humidity around field capacity. In March 2003, two 0.2 m cuttings of *Salix alba* (clone SI62-059) and *Populus deltoides* (clone Lux) were planted 0.40 m apart in each lysimeter (10.000 plants ha^{-1}). All the stems were harvested at the end of the first year. Lysimeters of each species were assigned either a solution of only tap water (NF) or of the nutrients of N and P (F), in order to estimate the effect of fertilisation on biomass production. The annual amount of fertiliser applied for the three years was respectively: 120 kg, 200 kg, and 300 kg ha^{-1}. Height and diametric growth, as well as number of shoots per plant were registered every 15 days throughout the duration of the trial. Biomass production was determined at the end of the establishment year and at the end of the second year after coppice. During the first year, poplar stem size was, on average, higher than willow for both treatments. It ranged from 305 (NF) to 347 (F) cm, whereas in willow it ranged from 186 (NF) to 229 (F) cm. Significant differences were only due to the effect of the species and not the treatment. Average diameters ranged respectively from 28.3 (NF) to 35.4 (F) mm in poplar and 16.7 (NF) 20.4 (F) mm in willow. Statistical differences were due both to the species and treatment. Number of shoots per plant was instead higher in willow (both treatments) than in poplar. Oven dry biomass at the end of the season was respectively 3.13 (NF) and 7.72 (F) t ha^{-1} in willow and 3.70 (NF) 8.24 t ha^{-1} in poplar, and differences were mainly due to the effect of nutrient supply. At the end of the first year after coppice, average stem height ranged from 227 (NF) to 254 (F) cm in poplar and 149 (NF) to 294 (F) cm in willow, but no significant differences were found either between species or between treatments. Diameters ranged from 15.4 (NF) to 25.7 (F) mm in poplar to 8.9 (NF) to 25 (F) mm in willow, showing differences determined by the treatment. Number of shoots per plant was still higher in willow than in poplar, and such differences increased after coppice. Average stem dimensions at the end of the second year after coppice showed a general increase in differences among fertilised and non-fertilised plants. Height ranged between 330 (NF) and 430 (F) cm in poplar and 173 (NF) 446 (F) cm in willow, whereas average diameters ranged from 30.1 (NF) and 54.7 (F) mm in poplar and 11.5 (NF) and 41.2 (F) in willow. Number of shoots per plant did not change and was still determined by species. Aboveground biomass at the end of the second year was respectively 8.9 (NF) 44.4 (F) t ha^{-1}in poplar and 6.28 (NF) 63.8 (F) t ha^{-1} in willow. Main conclusions: Fertilisation positively affected poplar and willow growth and biomass production; nevertheless, at the end of the second year after coppice, the two species showed a different response to different fertilisation levels; under fertilised conditions (F) willow performed better than poplar.

Growth and productivity responses of willow and poplar in SRIC for treatment of aquaculture effluents in southern Quebec: preliminary results

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Following recent environmental applications of willow and poplar for remediation of urban wastewaters, SRIC plantations have also been proposed as a means for treating effluents of aquaculture plants. However, compared with other wastewaters (i.e. urban sewages), effluents from aquaculture on average present low concentrations of both nitrogen and phosphorus. This is mainly due to the fact that water flow rate in these plans is kept high to reduce free ammonia concentrations originating in excreta and undigested fodder. Therefore, when irrigated with such effluents, plant growth may be affected by related nutrient shortage or partially flooded soil conditions. Moreover, the high water flow may lead to a leaching of nutrients from soil and consequent loss of fertility. The aim of this study was to evaluate the performance of willow and poplar in SRIC grown under such conditions in southern Quebec (Canada). In this poster we present the preliminary results of the project. The plantation was established in spring 2004 close to a fish farm, on a surface of about 0.25 ha. In May 2004, 20 cm long cuttings of *Salix viminalis* and *Populus maximowiczii x P. nigra* (NM5) were planted at a density of 20.000 plants per hectare in a single row. At the end of the second growing season, all the plants were cut down in order to ensure good establishment. In spring 2006, the experimental field was split into four main treatments per species as follows: fertilised-irrigated (F-I), fertilised-non irrigated (F-NI); non fertilised-irrigated (NF-I), non fertilised-non irrigated (NF-NI). Sprinkler irrigation started on July 1st and ended on September 30th. About 42 mm of effluent was provided daily for a total of about 3750 mm throughout the growing season. Fertilisation was performed by applying 200 kg ha^{-1} of nitrogen (urea) once in spring. The height of the main stem, its basal diameter, the number of stems per stool as well as the oven dry biomass were evaluated. Leaf samples were collected in July from well-developed leaves 20 cm from the top of the main stem for chemical analysis and SLW measurements. Analyses of variance followed by multiple comparisons of means according to Tukey's method were performed using SAS software, to determine significant differences among the various treatments.Most growth parameters, including biomass yield, were negatively affected by high irrigation treatment (I). Fertilisation (F) did not seem to affect most growth parameters (i.e. height and diameter) in either species. Fertilised willow (F), as compared to unfertilised (NF), reached a higher level only in terms of biomass yield (respectively 4.3 and 1.9 t ha^{-1}). Poplar biomass yield, which ranged from 2.5 to 3.4 t ha^{-1}, showed no change due to fertilisation. This is probably due to the fact that nutrient requirements were generally satisfied in all blocks, a supposition also supported by the fact that N level in leaves and stems was almost constant in all treatments. This also suggests that willow is likely more sensitive than poplar to fertilisation. In terms of nutrient removal, neither species showed significant differences. However, within the same treatment, willow presented, on average, a slightly higher capability of retaining N than poplar. In F-I treatment, willow was able to uptake 13% of N supplied, poplar only 10%. The main differences were recorded in F-NI treatment, where willow could uptake 62% of N, poplar only 46%. Only in the NF-I treatment did poplar show higher N uptake than willow, respectively 36% and 32 %. In conclusion, the main results obtained from this trial are as follows. Water volumes involved in this trial likely exceeded water requirements of both species. N-content in both leaves and stem was fairly constant in all treatments and did not differ from other previously reported data for the same species. Therefore, reduced biomass yield is likely due to excess water, which probably created a flood environment in which plants could not develop normally. A more calibrated irrigation will probably allow for better results in terms of growth and productivity. Fertilisation might, in some cases, positively affect growth and productivity. In particular, compared with poplar, willow seemed to take more advantage of N application. Both species seemed to show a good response in terms of N-uptake, although poplar in some cases presented the greatest ability to remove N.

Willow research program at the University of Saskatchewan

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The Saskatchewan government has committed to deriving one-third of its electricity from renewable energy sources by 2030 and short-rotation woody crops (SRWC) will play a key role in meeting these targets. Consequently, in 2005 the provincial government proposed an ambitious 1.6 million-hectare (i.e., 10 per cent of the province's arable land) afforestation initiative, with the intention of addressing not only the expected increased demand for woody biomass as differential markets develop, but also to mitigate the increasingly unfavourable agricultural sector within the province. The establishment of SRWC, such as willow, therefore, represents a legitimate option for diversifying farmers trying to maintain an economically viable operation in the face of historically decreasing commodity prices, along with increasing input and transportation costs, especially in the northern regions where annual crops are grown on marginal agricultural soils. Before there is widespread incorporation of willow into Saskatchewan agroforestry practices, however, a clear economic advantage for producers to grow willow must become apparent. In order to achieve this goal, a number of important agronomic, economic, and environmental questions need to be addressed, from both operational and empirical perspectives, which will be the focus of the willow research program at the University of Saskatchewan over the next several years. Specifically, these research objectives will aim to answer the following: i) What willow clones demonstrate the best survival and growth characteristics for use in Saskatchewan, ii) What key cultural practices (and their associated costs) are required for realizing optimal high density willow plantation establishment and productivity, iii) What is the effect of environmental gradient on willow biomass yield, iv) What diseases and pests within willow age sequences affect above- and below-ground biomass production, v) Does intercropping willow with caragana (i.e., N-fixer) improve soil nutrient availability within these plantations, vi) What are the rates of greenhouse gas emissions from soil in willow and willow/caragana plantations following various cultivation practices, vii) How does a three-year rotation of high density willow affect biogeochemical cycling and the rhizosphere microbial community, viii) How much carbon is sequestered above- and below-ground in these SRWC plantations and using the ECOSYS model what are the constraints for carbon accumulation, and finally ix) What molecular techniques could be effectively used to explicitly distinguish among the currently available willow clones for developing a molecular fingerprint library. This work will help to fill the current knowledge gap regarding cultivating short-rotation willow plantations in the prairie provinces and, therefore, should benefit farmers looking to diversify their production system, the renewable energy industry looking for reliable biomass feedstock, and government policy makers developing strategies to meet Canada's Kyoto commitments.

Triple-mission riparian buffers: environmental stewardship, profits in the off-season and youth involvement

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Emphasis of this project is on multi-use plantings with the incorporation of horticultural enterprises and new crops that offer multiple benefits for the farmer and his family, including effective runoff management, financial returns during off-season time, and the involvement of children. Willows are an efficient component of riparian buffers due to their high transpiration rates and extensive root systems that effectively trap runoff from fertilized agricultural fields thus assisting with runoff management on the farm. Willow species with ornamental stems for winter-spring harvest incorporated into riparian buffers can be a unique production niche for farmers offering opportunities to supplement incomes during the dormant season. Due to its relative ease of cultivation and visual appeal, willow is an engaging crop for junior members of the family that encourages children's involvement in the production cycle while teaching basic agricultural practices. This project seeks to select ornamental species suitable for incorporation into riparian buffers and to replace traditional varieties that are used for cut-stems with varieties offering higher yields, exhibiting improved stress resistance and are more suitable to the consumer's taste; to integrate willow riparian buffers into existing agricultural settings; to develop effective production practices using a short-rotation coppice system based on the principals of sustainability. The project also aims to develop a new appealing product - living structures and mini-villages for children constructed from brightly colored willow whips - that can be sold in kit form in late spring thus broadening the market window and supplementing farm income. The project is meant to include junior members of the family into product development and production cycles in order to provide a steady supply of interested, skilled and trained farmers to sustain future agriculture in the U.S. Field trials, integrated on-farm demonstrations, extension publications and workshops will be developed to introduce farmers to these value-added off-season products.

Grow potential and heavy metal accumulation in poplar and willow plants inoculated with an arbuscular mycorrhizal

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Many large municipalities in the world are characterised by the presence of quantity of abandoned sites formerly used for industrial activities and which now presented a certain degree of contamination by heavy metals or organic compounds. These so calls brownfields, represent a complex issue for urban managers because of the low economic values of many of these sites which stayed without any status and destiny. In this situation, phytoremediation constitutes and avenue which can be envisaged to reduce the contaminants and, at the same time, restorate the ecological integrity of these sites. Plants of two fast growing woody species, one willow species (*Salix viminalis*, clone 5027) and one hybrid poplar (NM5 *Populus maximowiczii* X *Populus nigra*) were grown in pots of 30 centimetres using a soil contaminated with heavy metal (As, Cd, Cu, Pb, Zn) and collected from a brownfield from downtown Montreal, province of Quebec, Canada. A split-split plot experimental design, which includes two types of soil, the two species and two treatments of inoculation with an arbuscular mycorrhizal (*Glomus intraradices*) was set up on the site of the Montreal botanical Garden. Plants were cultivated for a period of four months and irrigated when needed. The study aims to evaluate the capacity of phytoextraction of the two species and the impact of the arbuscular mycorrhizal. In comparison to the hybrid poplar, *S. viminalis* showed higher concentrations for all analysed heavy metals in its leaves, stems and roots. Zn and Cd were notably very high in the leaves of the willow species. In the opposite, the poplar species used in this experiment produced more biomass which can represent an interesting criterion for the restoration of polluted sites. Observation on the degree of mychorization showed that two trees species were colonized with *G. intraradices* at a suitable level (50% of colonization for *Populus*, 19 % for *S. viminalis*) considering the type of soil in which they were grown. The mycorization however did not have any effect, neither on biomass production nor on heavy metals absorption.

Application of wastewater from municipal wastewater treatment works to SRC willow and poplar

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Short Rotation Coppice (SRC) willow has the ability to utilise large volumes of water and can also accumulate nitrogen, certain heavy metals and to a much lesser extent, phosphorous. It is therefore a potentially useful candidate for the biofiltration of wastewater streams including effluent from municipal water treatment works. The Water Treatment Works (WTW) at Culmore, Londonderry, Northern Ireland, is a state of the art WTW serving the city of Londonderry, which has a population of approximately 105,000. Working in collaboration with the NI Water Service, primary sewage effluent is drawn from a well within Culmore WTW and piped to an experimental site approximately 500m away. The effluent is trickle irrigated onto replicate plots of poplar (Hassendens and Hoogworst), willow (*Salix* spp.) mixtures plots (comprising seven *Salix* spp. varieties planted as random short runs – 'Tora', 'Stott', 'Beagle', 'Olaf', 'Resolution', 'Endeavour' and 'Terra Nova') and plots of grass. Irrigation commenced in September 2006 when the plants had been growing for two complete seasons. The rate of application was determined by nitrogen loading – in year 1 it was applied at an approximate level of 200Kg ha^{-1} and in subsequent years the amount of N applied will be doubled. The idea of the trial is to operate the site to 'breaking point' in order to determine where the top limit of nitrogen assimilation may be for these types of system. Effluent application started as the plants were approaching senescence and continued until December 2006 when the irrigation was stopped, due to heavy rain and water-logged soils. Soil water is collected at two-weekly intervals using soil-water sampling probes. Ground water is collected at monthly intervals from each of five deep boreholes positioned around the plantation both upstream and downstream of the naturally drainage direction. Soil was sampled annually. All samples were analysed for total nitrogen (TON), total phosphate (TOP), and a range of minerals and trace elements including aluminium, boron, calcium, cadmium, cobalt, copper, iron, potassium, magnesium, manganese, molybdenum, lead, selenium and zinc. The effluent had a pH of around 6.9 and a BOD ranging from 2 to 331 over a twelve month period. TON ranged from 2 – 40 mg l^{-1} and TOP from 0.2 – 9.0 mg l^{-1}. With the exception of calcium, potassium and manganese all of the other elements were present in the effluent only as traces. The E_c of the effluent was around 2000 uS cm^{-1}. To date (March 2007) no significant differences have been detected in nutrient levels in soil water between irrigated and non-irrigated plots of any of the crops. In irrigated plots of poplar TON in the soil water was elevated compared to grass and willow, from October to December and in the non-irrigated plots from September to December. TON from the grass and willow plots remained consistently low throughout the sample period. In none of the boreholes was there any indication that nutrients or metals were being washed into the ground water. E_c was consistently low at every sample date, even following heavy rain. Application of effluent will re-commence in April 2007 at a significantly higher rate and monitoring will continue at the same level to attempt to determine at what point the biofiltration system fails.

Evaluation of gene expression responses in poplar roots following N fertilisation

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Members of the Salicaceae, such as poplar species, have a high demand for water and nutrients. This characteristic makes them especially suitable for the removal of soluble pollutants from the soil. The systematic planting of poplars as riparian buffers to remove the nitrates released from fertilized agricultural land is a promising approach. Hence, the aim of the present work was to document the effect of nitrogen supply on root metabolism, focusing on the gene level. Rooted cuttings of *Populus trichocarpa* X *P. deltoides* H11-11 were maintained under standard greenhouses conditions during the experiment. The treatments consisted of daily applications of a water-soluble fertilizer where nitrogen (N) supplied as ammonium nitrate. We applied 0 g/L as the low N control and 5 g/L for high N; in order to evaluate the effects of contrasted conditions. Rapid physiological and developmental changes were induced after N fertilization. Gene expression analyses were conducted using an in-house cDNA microarray comprising a total of 3400 genes. After seven days of nitrogen treatment, 56 genes were identified as differentially expressed in poplar roots. Several genes coding for enzymes involved in primary carbon and nitrogen metabolisms showed an increased level of transcripts after N fertilization. Interestingly, we found a second group of sequences with a modified expression that were related to enzymes involved in detoxification and osmotic regulation. A third group of genes represent enzymes participating in cell wall formation and lignin synthesis. Finally, several transcription factors had altered levels of transcripts which suggest that their activation or repression is affected by nitrogen availability. Our results show that patterns of gene expression in roots of poplars are influenced by nitrogen supply. Our data may help to identify specific physiological pathways that are activated in response to high N availability. As poplars are becoming part of multi-purpose agrosystems, the plasticity of their responses for water and nitrogen conditions may provide evidences of unique adaptative mechanisms.

Environmental applications of poplar and willow germplasm in Italy: experiences and trends

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River restoration, rehabilitation of degraded sites, the re-establishment of forests in fluvial areas, the reversion of farmlands into natural forest areas through cereal-surplus reduction policies have all become common goals for central and regional government agencies at a European level. This has greatly increased the importance of poplar and willow in a field that is different from traditional intensive cultivation (i.e. poplar cultivation and short rotation forestry). Over the last decade the Institute has promoted and gained relevant experience on environmental applications of poplar and willow biodiversity. In co-operation with Natural parks and local State-run organizations it has carried out several pilot trials on the Po river basin testing plant materials, planting methods and cultivation techniques. Up to now, over than 100 hectares have been restored in different sites. Many were at the basis of the restoration pilot trials: promoting the use of poplar and willow in river restoration, separating areas subject to frequent erosive events from intensive agricultural cultivations, reducing the pollution of waters by introducing buffer strips, restoring flood areas for recreational purposes, promoting economical land use integrated with river restoration. As conservation efforts for river forests is a high priority in the European Union, the main aim was to contribute actively to the conservation of native poplars genetic resources on restoring floodplain forests. The European Black poplar (*Populus nigra* L.) and White poplar (*Populus alba* L.), are two of the most representative and threatened forest tree species of the old natural floodplain forests in the temperate zone in Europe. *P. nigra*, in particular, is considered to be on the verge of extinction all over Europe so that many initiatives have been undertaken since 1994 to protect its germplasm and to implement conservation strategies (EUFORGEN programme). Since in Italy *P. nigra* is in serious decline, a dynamic evolutionary process in the gene conservation strategies is underway: artificial *in-situ* conservation units are established in the restored sites with a selected pool of unrelated genotypes.The pilot trials set up showed that native poplars and willow can be successfully used in establishing plantations in fluvial ecosystems and, generally, in damp areas or agricultural flooded areas, as they are typical pioneer species and can grow in poor soil and start the natural evolution of forests. In order to succeed with rehabilitation and avoid phytosanitary problems it is necessary to resort to forest material appropriate for the site characteristics, of good quality and of reliable origin and to ensure appropriate cultural practices over the first five years to reduce infestation by invasive weed species. In order to improve the *in-situ* dynamic conservation a network of artificial gene conservation units will be created in a short time, by means of new collaborations with rivers parks in river restoration activities and according to the conservation strategies defined within the EUFORGEN Programme. Further inventories, collection and characterization of the genotypes included in the *ex-situ* gene-banks is needed. The use of specific database and public awareness will allow to promote the environmental use of poplar and willow among the end users (riparian ecosystem managers, regional tree nurseries, farmers). Key words: poplar, willow, *Populus nigra, Populus alba,* EUFORGEN, river restoration, conservation strategies, biodiversity

FIELD TOUR

STOP 1

BROWNFIELD PHYTORESTAURATION

Allée des Tanneries (Tannery Lane)

Context

The Plant Biology Research Institute (IRBV) continues to seek solutions for reclaiming contaminated or abandoned urban sites, commonly called brownfields. The main objective of these studies is to develop methods to quickly restore brownfields by using plants that can be easily established on these sites, which are characterised by compacted soil, poor drainage and the presence of a variety of pollutants.

The Institute is responsible for the scientific and technological components of this current project, whose scientific and community-related aspects are supported by the Federation of Canadian Municipalities' (FCM) Green Municipal Fund.

Site localisation and history

The site being studied is situated in south-west Montreal, between a residential zone and an industrial sector, and was acquired in October 1982 by the City of Montréal from National Canadian Railway.

In the 17th century, it was occupied by a small tannery, which gave the site its name. During the 19th and 20th centuries, this sector of the city close to the Lachine Canal was host to intense industrial activity. At the end of the 19th Century, the Lachine Canal was dredged and enlarged and the sediments extracted were deposited on adjacent lands. These sediments contained a mixture of organic and inorganic compounds which characterise the site today (Table 1).

A visual screen made of willows and poplars photographed two years after the establishment.

In 2004, the City of Montreal undertook the restoration of this site. One of the first operations was to create a visual screen, composed of willow and poplar plants in two rows, as suggested by the Institute's scientists.

Site characteristics

Table 1. Main contaminants identified on the site.

Parameter	Unity	Results	Criterion		
			A	B	C
pH		7,9			
Inorganic contamination:					
Copper (Cu)	mg/kg	36	40	100	500
Lead (Pb)	mg/kg	120	50	500	1000
Zinc (Zn)	mg/kg	160	110	500	1500
Arsenic (As)	mg/kg	17	6	30	50
Organic contamination:					
Anthracene	mg/kg	0,3	0,1	10	100
Benzo(a)anthracene	mg/kg	0,6	0,1	1	10
Benzo(a)pyrene	mg/kg	0,5	0,1	1	10
Benzo(b+j+k)flouranthene	mg/kg	1,2	0,1	1	10
Benzo(ghl)perylene	mg/kg	0,3	0,1	1	10
Chrysene	mg/kg	0,8	0,1	1	10
Fluoranthene	mg/kg	1,5	0,1	10	100
Indeno(1,2,3-cd)pyrene	mg/kg	0,3	0,1	1	10
Phenanthrene	mg/kg	1,05	0,1	5	50
Pyrene	mg/kg	1,2	0,1	10	100
2-Methylnaphtalene	mg/kg	1,1	0,1	1	10
1-Methylnaphtalene	mg/kg	0,9	0,1	1	10
1,3-Dimethylnaphtalene	mg/kg	0,6	0,1	1	10

Experimental design

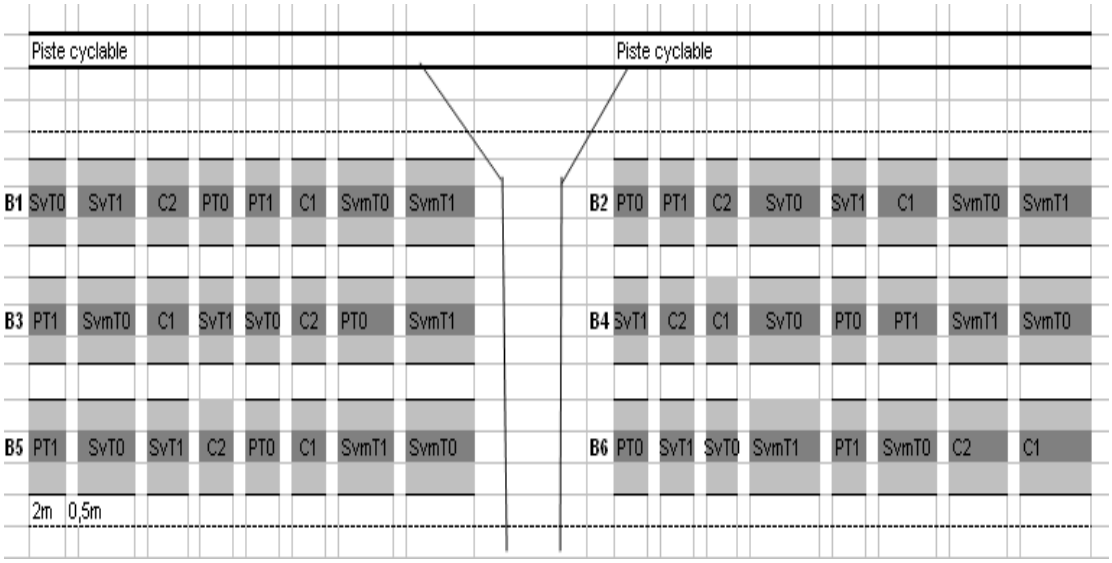
In 2006, a split-plot experimental design was set up to compare the development and growth performance of willows and poplars, as well as their capacity for phytoremediation of inorganic pollutants.

The experimental design is comprised of:

6 blocks

Each block comprises 2 species: 1 m long cuttings of *Salix viminalis* and *Populus × generosa* A. Henry 'Unal' planted at a 3.3 plants/m^2 density and 10 cm long cuttings of *Salix viminalis* at a density of 33.3 plants/m^2.

One treatment with *Glomus intraradices* inoculum was applied on one half of each plot.



Legend:

SvT0:	Long cuttings of Salix viminalis without inoculum
SvT1:	Long cuttings of Salix viminalis with inoculum
PT0:	Long cuttings of *Populus* without inoculum
PT1:	Long cuttings of *Populus* with inoculum
C:	Free area
C1:	Plant area

B1-B6: Blocks from one to six

Sampling campaign

Plant establishment and development in response to various treatments was documented by a measurement and soil sampling campaign carried out during the 2006 growing season.

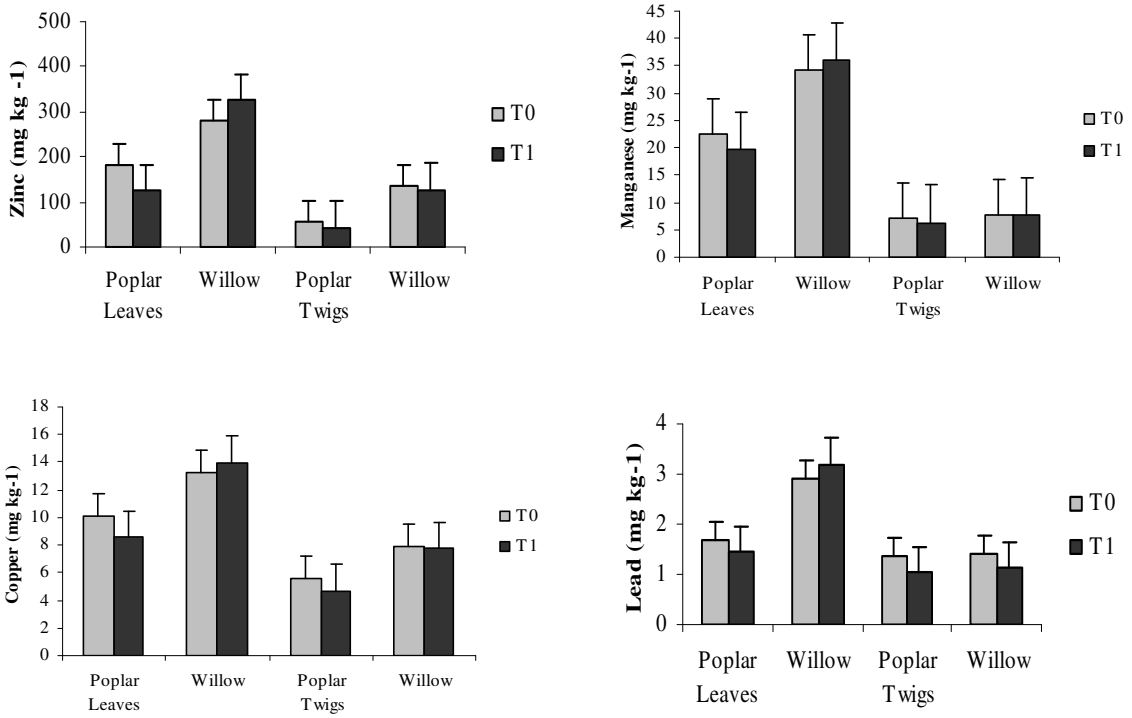
Results of the analyses

The textural analysis locates these soils in the class of loams, sandy on the surface and argillaceous with increasing depth, very rich in calcium, having an alkaline pH and a presence of 5% organic matter. Analysis of the soil contamination levels shows values located between A and B criteria specified in the Policy of Soil Protection and Rehabilitation of Contaminated Sites of the Ministry of Development, Environment and Parks.

The rate of plant survival under such soil conditions was up to 98% for willows and as high as 90% for poplars, with an insignificant difference between those with mycorrhizae and those without.

Results of analysis to evaluate the capacity of willows and poplars to extract and accumulate Zn, Mn, Pb and Cu in leaves and stems are presented in Figure 1.

Figure 1. Comparison of the heavy metals concentrations in leaves and stems of willows and poplars.



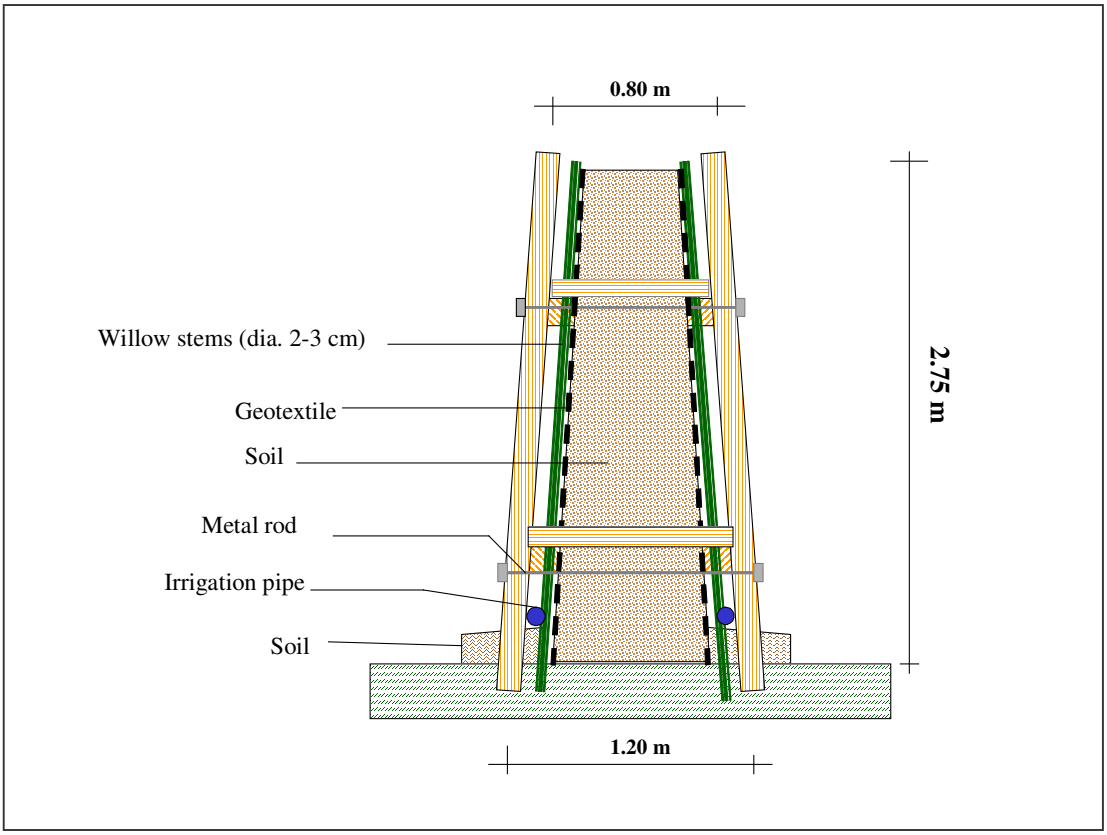
Legend:
T0: Without treatment
T1: With treatment (mycorrhizae)

STOP 2

LIVING NOISE BARRIERS IN LAVAL

This living noise barrier was established in 2006-2007 by *Beaupré and Associated,* a landscaping firm that has collaborated with our Research Institute on diverse projects. The structure was set up in the fall of 2006, and branches were planted in the spring of this year. This is our first experiment with the construction of such living walls in two steps.

The construction design is presented below:



STOP 3

LIVING NOISE BARRIERS AND VISUAL SCREENS IN BOISBRIAND

Diverse structures have been set up and are being tested in this regional park. Among the structures, there are:

- A 30 m long living wall barrier set up in 2003
- Visual screens made of long willow branches 4 to 8 years old, established in 2004 and 2006.

STOP 4

THE BOISBRIAND WILLOW PLANTATION FOR ENVIRONMENTAL APPLICATIONS

A seven hectare willow plantation has been established in partnership with the Municipality of Boisbriand. Sub-plots on the plantation range from one to four years old, and include the following clones:

- *Salix viminalis* 5027 - Established in 2004
- *Salix miyabeana* SX64 - Established in 2006
- *Salix miyabeana* SX67 - Established in 2006

Soil characteristics

Charactersitics		
Sand	%	22
Loam	%	29
Clay	%	49
Organic matter	%	6.5
pH		6.7
P available	kg/ha	43
K available	kg/ha	185
Ca available	kg/ha	4840
Mg available	kg/ha	1230
Saturated P	%	2
Saturated K	%	1.1
Saturated en Ca	%	55.5
Saturated en Mg	%	23.5
Saturated (K+Mg+Ca)	%	80.1
E.C.	m Eq/100 g	19.4

Clone trial

One clone trial including 26 varieties was set up in the spring of 2007 in partnership with the Department of Forest and Natural Resources Management, State University of New York.

Clones included in the test

Clone ID	Variety epithet	Species	Gender	Montreal
S365		S. caprea hybrid?	MS	325
SV1		S. dasyclados	F	325
S25		S. eriocephala	F	325
00X-032-094		S. eriocephala	Unk	325
9837-77		S. eriocephala	F	325
9832-49		S. eriocephala	F	325
SX64		S. miyabeana	M	325
94001		S. purpurea	M	325
9879	Oneonta	S. purpurea x S. miyabeana	M	325
99217-015	Millbrook	S. purpurea x S. miyabeana	F	325
99217-023	Saratoga	S. purpurea x S. miyabeana	F	325
9980-005	Oneida	S. purpurea x S. miyabeana	M	325
9882-034	Fish Creek	S. purpurea	M	325
9882-041		S. purpurea	F	325
99113-012	Onondaga	S. purpurea	M	325
99239-015	Allegany	S. purpurea	F	325
SX61		S. sachalinensis	F	325
9870-1	Cicero	S. sachalinensis x S. miyabeana	F	325
9870-23	Marcy	S. sachalinensis x S. miyabeana	F	325
9871-31	Sherburne	S. sachalinensis x S. miyabeana	F	325
9970-036	Canastota	S. sachalinensis x S. miyabeana	M	325
99201-007	Otisco	S. viminalis x S. miyabeana	F	325
99202-004	Fabius	S. viminalis x S. miyabeana	F	325
99202-011	Tully Champion	S. viminalis x S. miyabeana	F	325
99207-018	Owasco	S. viminalis x S. miyabeana	F	325
99207-020	Truxton	S. viminalis x S. miyabeana	F	325

STOP 5 Friday June 8
Guillon site

Characteristics

The Guillon site has a clay texture and its organic matter content is high on the surface, but it decreases further down. Exchange capacity shows moderate fertility on the first layer decreasing proportional to depth further down. The pH is slightly acidic (Table 3).

Soil characteristics

Component	Units	Clay site depth 0-20 cm
Sand	Wt %	2
Silt	Wt %	48
Clay	Wt %	50
Texture		clay
Organic matter	Wt %	9,1
pH		5,7
Available P	kg/ha	30,2
Available K	kg/ha	256
Available Ca	kg/ha	7650
Available Mg	kg/ha	2118
CEC	mEq/100 g	32,4

The plantation was set up in the spring of 1999 and includes a dozen new willow and poplar clones, listed below. The majority of the clones were provided by SUNY - Syracuse, New York State, a partner in this research.

Designation	Names
SV1	*S. dasyclados*
S301	*S. interior x eriocephala*
S25	*S. eriocephala*
S365	*S. discolor*
SX61	*S. sachalinensis*
SX64	*S. miyabeana*
SX67	*S. miyabeana*
S546	*S. eriocephala*
S625	*S. eriocephala x interior*
SVQ	*S. viminalis*
NM5	*Populus maximowiczii x nigra*
NM6	*P. maximowiczii x nigra*

Experimental design

The experimental design was set up in the spring of 1999. Several willow and poplar clones were planted on four blocks, each divided into twelve plots corresponding to the twelve clones involved in this trial. The surface of each plot measured 124.8 m² and the density of plantation corresponded to 18,000 cuttings per hectare (Figure 2). During the experiment, the plantation was not irrigated and no chemical treatment to control diseases or insects was performed.

This site also includes a collection of 57 willow clones from various countries all around the world donated in 1999 by the Québec Ministry of Natural Resources Wildlife and Parks (Figure 3). A follow up of all clones has been conducted to evaluate growth characteristics and their pest and disease resistance.

Coppicing and fertilization

At the end of the 2002 growing season, the willows were coppiced for the first time. In the spring of 2003 wastewater sludge equivalent to 90 kg "available" nitrogen per hectare was applied.

In the fall of 2005 the plantation was coppiced for the second time. In the spring of 2006 the plantation was chemically fertilized with 100 kg nitrogen per hectare (30-15-15).

Currently the root system and shoots are respectively 8 years and 1 year old.

Biomass yield

The highest annual biomass production was achieved by the NM5 poplar clone (19.85 t/ha) followed by the SX64 willow clone (19.81 t/ha) (Table 4).

Comparison of biomass yields

Clones		Biomass production (DM t/ha)			
		Cycle1 (99-02)	Cycle2 (03-05)	Total (99-05)	Annual
P. maximowiczii x P. nigra	NM5	66.48	72,47	138,9	19,85
Salix miyabeana	SX64	67.58	71,12	138,7	19,81
Populus maximowiczii x P.	NM6	72.20	62,24	134,4	19,21
S. sachalinensis	SX61	62.34	61,25	123,6	17,66
S. discolor	S365	54.48	64,10	118,6	16,94
S. dasyclados	SV1	46.64	64,08	110,7	15,82
S. eriocephala X S. eriocephala	S546	56.52	48,49	105,0	15,00
S. eriocephala X S. eriocephala	S25	44.38	59,36	103,7	14,82
S. miyabeana	SX67	37.74	59,00	96,7	13,82
S. eriocephala x S. interior	S625	37.20	40,14	77,3	11,05
S. viminalis	SVQ	35.84	37,44	73,3	10,47
S. interior x S. eriocephala	S301	24.84	19,08	43,9	6,27

Figure 2. Plantation design.

Planted: spring 1999:

Two coppicing cycles: 2002 and 2005

Fertilization: 2000 (wastewater sludges) and 2006 (chemical fertilizer)

Plantation density: 18940 cuttings/ha

Clones: NM5 (Populus maximowiczii x nigra); **NM6** (P. maximowiczii x nigra); **SV1** (S.dasyclados); **S301** (S.interior x eriocephala); **S25** (S.eriocephala); **S365** (S.discolor); **SX61** (S.sachalinensis); **SX64** (S.miyabeana); **SX67** (S.miyabeana); **SVQ** (S.viminalis); **S546** (S.eriocephala); **S625** (S. eriocephala x interior).

Experimental design: split split plot, 4 blocks of 1540.8 m² ; the blocks were divided into 12 plots of 9.6 X 13 m each one.

BLOC 1

SV1	SVQ	NM5	S301	S325	NM6	S365	SX61	S546	SX67	S625	SX64

BLOC 2

NM5	SV1	SX61	S625	SX67	SVQ	SX64	S301	S25	NM6	S365	S546

BLOC 3

SVQ	S546	NM5	SV1	NM6	S625	S25	SX67	S365	SX64	SX61	S301

BLOC 4

SX61	SX64	S546	S625	SVQ	SV1	NM5	S301	SX67	S365	NM6	S25

Figure 3. Collection of 57 willow clones.

Plantation: spring 1999
First rotation: 1999-2002
Second rotation: 2003-2005
Roots age: 8 years,
Shoots age: 1 year

New Erin road

No.	Clone Number	Latin name	Origin
1	5016	*S. alba*	Italy
2	5077	*S.dasyclados*	Germany
3	5095	*S. purpurea*	Canada
4	5047	*S. alba*	Yugoslavia
5	5087	*S. humboldtiana*	Argentine
6	5083	*S. glatfelteri X S.alba*	Yugoslavia
7	5008	*S. nigra*	Quebec (Ste. Scholastique)
8	5061	*S. alba*	Yugoslavia
9	5020	*S. alba*	Romania
10	5052	*S. alba*	Yugoslavia
11	5089	*S. hastata*	England
12	5005	*S. nigra*	Quebec (Aylmer)
13	5082	*S. glatfelteri X S.alba*	Yugoslavia
14	5085	*S. glatfelteri X S.alba*	Yugoslavia
15	5057	*S. alba*	Italy
16	5019	*S. alba*	Italy
17	5098	*Salix rubens*	Germany
18	5090	*S. Matsudana tortuosa X S. alba*	Yugoslavia
19	5017	*S. alba*	Italy
20	5096	*S. purpurea*	Canada
21	5056	*S. alba*	Italy
22	5013	*S. nigra*	Quebec(Quebec)
23	5021	*S. alba*	Romania
24	5070	*S. adenophylla (Hook)*	Poland
25	5102	*S. nigra*	Quebec
26	5050	*S. alba "Coerula"*	Yugoslavia
27	5055	*S. alba*	Italy
28	5066	*S. alba*	Italy
29	5049	*S. alba*	Yugoslavia
30	1200	*S. viminalis*	Quebec (Lotbinière)
31	5086	*S. glatfelteri X S.alba*	Yugoslavia
32	5027	*S. viminalix*	Quebec (Luceville)
33	5003	*S. nigra*	Quebec (Aylmer)
34	5075	*Salix (Hibride)*	Canada
35	5015	*S. nigra*	Quebec (Quebec)
36	5099	*Salix smithiana*	Yugoslavia
37	5067	*S. alba*	Italy
38	5084	*S. glatfelteri X S.alba*	Yugoslavia
39	5046	*S. alba*	Yugoslavia
40	5006	*S. nigra*	Quebec (Montebello)
41	5101	*S. dasyclados*	Canada
42	5051	*S. alba*	Yugoslavia
43	5007	*S. nigra*	Quebec (Montebello)
44	5044	*S. alba "sanguinea"*	Yugoslavia
45	5045	*S. alba*	Yugoslavia
46	5064	*S. alba "sanguinea"*	Yugoslavia
47	5018	*S. alba*	Italy
48	5062	*S. alba*	Yugoslavia
49	5094	*S. nigra*	Canada
50	5022	*S. alba*	Romania
51	5011	*S. viminalis*	Quebec
52	5002	*S. alba*	Quebec
53	5088	*Salix helix*	Germany
54	5069	*S. acutifolia*	Hungary
55	5068	*S. alba*	Romania
56	5012	*S. nigra*	Quebec
57	5048	*S.alba*	Yugoslavia