WORKING PARTY ON POPLAR AND WILLOW DISEASES
HOST-RANGE STUDIES OF MELAMPSORA ON SALIX IN THE PACIFIC NORTHWEST REGION OF THE UNITED STATES

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*Melampsora* is known as an important cause of disease on willows throughout the world. Even so, little is known about the occurrence, distribution, and specific hosts of these leaf rusts in North America. Current classification places all willow rusts occurring in North America into the species complex *Melampsora epitea* Thuem. This is a result of supposed morphological continuity throughout the complex. Several lines of study have been initiated in an effort to understand what is represented by this complex on a regional scale.

Host-range inoculation experiments of *Melampsora* on *Salix* are the main components of this baseline study. Although wild collections are still in progress, a preliminary inoculation has been performed with rust from *Salix sitchensis*. Ten different *Salix* species that occur throughout the Pacific Northwest (PNW) region of the United States were used as test plants. It was found that this *Melampsora* isolate was able to infect across the range of its host plant, which represents six geographically distinct *S. sitchensis* populations throughout Washington and Idaho states. Three others: *Salix* spp., *S. wolfii*, *S. bebbiana*, and *S. scouleriana* were also susceptible to this inoculum, although both *S. bebbiana* and *S. scouleriana* displayed a hypersensitive response and prolonged latent period (> 15 days). Five other *Salix* species - *S. geyeriana*, *S. exigua*, *S. melanopsis*, *S. alba*, and *S. eriocephala* - from locations throughout this region were resistant to the inoculum. These preliminary results clearly demonstrate that *Melampsora* is host specific and that certain populations of *Salix* are resistant to this particular isolate.

The internal transcribed region of 42 rust samples was sequenced from eight different willow species of multiple populations throughout the PNW. The resulting phylogeny shows some exact matches to the *M. epitea* in GenBank, while others were a close match. A possible hybrid group also emerged with an isolate from *S. sitchensis* and one from *S. lasiandra*. This phylogeny also displayed a distinct group of three samples that lie far outside the larger *M. epitea* group. This group, consisting of two isolates from *S. sitchensis* and one from *S. piperi*, show relatedness more closely to *M. occidentalis*, poplar rust, than to *M. epitea*. This clearly demonstrates that *M. epitea* does not encompass all willow rusts in North America.

*Melampsora* morphology at the SEM level (>8,000X) reveals unique morphological features in the uredinal paraphyses. Some samples show smooth paraphyses whereas others show distinct reticulation on the paraphyses. These investigations are still in the preliminary stages, and further conclusions are forthcoming.

These initial investigations into *Melampsora* on *Salix* in North America display host-specificity, the presence of three different phylogenotypes, and morphological discontinuity within the *M. epitea* species complex. This clearly argues that there are specific taxa within this species complex and that the complex does not clearly represent what occurs at a regional level.

**Key Words:** *Melampsora*, *Salix*, host-specificity, willow rust.

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WATERMARK DISEASE OF WILLOWS IN AGRICULTURAL AREAS: A STUDY OF THE EFFECT OF ENVIRONMENT AND SOIL CHARACTERISTICS ON DISEASES EXPRESSION

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Willow has an historical value as a polyfunctional landscape tree in large agricultural areas of northwestern Europe which should be protected. In Flanders, Belgium, willows (Salix sp.) are typically seen alone or in rows bordering fields and meadows. There is also a new interest in willow growth for biomass production and soil sanitation. However, the general condition of willows in the agricultural environment is declining. Solitary trees or even total willow rows suffer disease and often die. Disease severity varies from year to year. The disease is not evenly spread on different locations and sometimes a healthy old willow stand or solitary tree is observed, surrounded by other diseased or dead trees. The disease is caused by the bacterium Brenneria salicis and is known as the Watermark Disease.

In this study, four test sites with old willow stands were selected. The history of watermark disease in these stands has been followed for several years by means of B. salicis monitoring and symptom observation. At two out of four locations watermark disease is present. On these four locations over a hundred isogenic willow trees were planted close to the old stands so that they were subjected to the same soil conditions. Both the old and the newly planted trees were studied over a 3-year period.

The genetic background of the old willow trees was visualised in AFLP and confirmed prior observations that especially the trees belonging to the Salix alba - S. fragilis complex are very susceptible to watermark disease. The planted isogenic willows are S. alba. The monitoring of B. salicis, by means of PCR, in the wood of the trees indicated that B. salicis can be considered a wide-spread wood bacterium which is predominantly present in the affected trees, also present in healthy willows and even detected in sampled poplar and alder wood. The evolution of B. salicis concentrations in relation to other wood bacteria is being followed during the different seasons with molecular DGGE profiles. The bacterial fluctuations and evolution to B. salicis dominance in the wood, which results in disease expression, vary during the seasons and also from year to year.

Research is focusing on the correlation with sugar and amino acid streams in the wood to explain temporary, selective and excessive growth of B. salicis. In addition, work is being done on soil components that have their direct influence on these nutritional fluctuations. At the same time, the typical weak pathogenesis of B. salicis is being researched to understand the impact and regulation of the wood-degrading enzymes and of the polysaccharide coat, which can obstruct the transport vessels under certain specific conditions in young or narrow wood vessels.

Key Words: B. salicis, bacterial ecology, soil composition, leaf components, wood formation, nutrient storage and transport.

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INFLUENCE OF TEMPERATURE AND LEAF WETNESS DURATION ON THE MONOCYCLIC COMPONENTS OF POPLAR RUST

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In Brazil, poplar rust is the most important disease in nurseries and in the field. Most of the clones are susceptible and the epidemic is more severe each year. The objective of this study was to quantify the influence of temperature and leaf wetness duration on the infection of *Melampsora medusae* on three poplar clones: Latorre – highly susceptible; SM – moderately susceptible; and SJ – resistant.

The effect of temperatures between 8 and 31°C was estimated in the germination of *M. medusae* and in the infection frequency, latent period and sporulation of the pathogen in poplar leaves. The evaluation of infection frequency was done by counting the number of pustules per 2 cm² of three leaves/replication of each treatment. The latent period was the period of time between the inoculation and the first pustule. The sporulation was assessed at the end of the experiment counting the number of uredospores in each pustule. The effect of wetness duration from 0 to 24 hours in frequency of infection was determined under controlled condition.

The optimum range of temperature for uredospores germination was 11 to 21°C (around 80%), although germination was also observed at 6 and 26°C (around 40%). At 31°C, only 10% of the spores germinated. The range of optimum temperature for infection was between 16 and 21°C. No symptoms of the disease were observed at 31°C. The generalized beta function fitted to infection frequency estimated the maximum temperatures of 27 and 26°C and minimum of 8 and 10°C for clones Latorre and SJ, respectively. The latent period showed small variation in function of the temperature between 15 and 26°C, varying from 6 to 8 days for clones Latorre and SM, and from 8 to 10 days for clone SJ. The interval of minimum temperature to sporulation was similar to the infection. The number of pustules increased in function of the increase in the leaf wetness duration in the susceptible clone. The minimum wetness period necessary for infection was three hours.

**Key Words**: *Melampsora medusae*, epidemiology, *Populus spp.*

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PROGRESS OF EPIDEMICS AND EVALUATION OF DAMAGE CAUSED BY RUST IN POPLAR CLONES

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Poplar rust (Melampsora medusae Thuem.) causes serious damage in stool beds and is frequent in commercial crops, mainly in susceptible clones. Clonal behaviour in Brazil as to this disease is unknown.

This study aimed at: (i) quantifying epidemic progress in the field in seven poplar clones: (ii) correlating disease with production using incidence, severity, leaf area index (LAI) and diameter at the breast height (DBH). The evaluation took place monthly, from November to April, during the 1998-1999, 1999-2000 and 2000-2001 cycles. The incidence and severity of rust, leaf area duration (LAD), integralization of LAI, and DBH were assessed.

Results were analyzed by ANOVA, Tukey tests, and linear and non-linear regressions. Latorre and Guarani were the most susceptible clones; SJ was resistant, and the others moderately susceptible. The logistic model had a good fit to severity in the three cycles assessed. The epidemic was more severe in the 1999-2000 cycle when the temperature varied from 26.5 to 15.5°C. There was no significant relation between severity and DBH. In the susceptible clones, LAD was related to DBH and the coefficient of determination in linear regression was 0.73 and 0.56 in Guarani and Latorre, respectively. Latorre clone was relatively tolerant to disease because, although there was high severity, the percentage of DBH reduction was only 5% while Guarani showed 17%.

Key Words: Melampsora medusae, epidemiology, Populus spp.

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Endophytic fungi in poplar trees

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Poplars are frequently attacked by fungal bark necrosis, weakness pathogens that very often attack plants under stress. During the past years it has been demonstrated that several of these crown weakness pathogens show an asymptomatic phase in healthy plant tissues with unknown duration that produce disease only after heavy and prolonged stress periods. The phenomenon is generally included in the concept of "endophytism" and is showing a great importance in forest pathology. The main aim of this research was to determine the incidence of endophytic fungal organisms, particularly the pathogenic ones, on asymptomatic Populus x euramericana (Dode) Guinier trees.

A series of periodical investigations were carried out in Central Italy (province of Viterbo) in February (winter) and May (spring) 2002 considering four trees per period, and 20 branches of three years, 20 twigs and 20 buds per plant. Tissue fragments obtained from these organs were surface sterilised in H₂O₂, and then inoculated on PDA (Potato Dextrose Agar) supplemented with 0,06 g/l of streptomycin, 0,05 g/l of iron chloride and 0,02 g/l of L-Asparagine. Plates were incubated at 20°C, in the dark, for 7 days. At the end of the incubation period, newly-formed colonies were identified morphologically, and in a few cases, with molecular methods. Isolation frequency (percentage of colonized fragments on the total of fragments examined) per each endophytic taxon was calculated.

No differences between February and May isolations were observed. Fourteen species of fungi were detected, seven of which can be considered pathogens: Alternaria spp. (two species), Botrytis cinerea Pers., Verticillium sp, Entoleuca mammata Rogers et Ju. (=Hypoxylon mammatum [Wahlenb.] P. Karst.), Fusarium lateritium Nees and Phomopsis tirrenica Moriondo. Among these, the last three are known as bark necrosis agents on poplar. Many other not pathogenic endophytes were also detected: Aureobasidium pullulans (de Bary) Arnoud, Cladosporium cladosporioides (Fres.) de Vries, Epicoccum nigrum Link, Gliocladium roseum Bainier, Sordaria sp., Trichoderma viride Pers. Some of these fungi are known as possible antagonists.

The results showed that pathogenic endophytes are able, in certain conditions, to change from the endophytic to the pathogenic phase that contributes to increase the on-going phenomena of plant decline. However, in future research it will be necessary to clarify causes that lead endophytes to become pathogens and all other interactions between them and their antagonists.

Key Words: poplar, bark necrosis, pathogens, endophytism.

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IDENTIFICATION METHODS OF ICE-NUCLEATION ACTIVE (INA) AND PATHOGENIC BACTERIA IN WOODY PLANTS (SALIX) AS AN ENERGY CROP

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A study on the identification and detection of ice-nucleation active (INA) bacteria which cause damage in combination with frost to Willow (Salix spp) plants in late fall, winter and spring was carried out, using the diagnostic tools BIOLOG® MicroPlate with similarity index between 0.505-0.974, biochemical tests, selective INA primers, and also using 16S rRNA analysis.

After amplification of bacterial DNA extracts agarose gel electrophoresis was used for comparing and determining the band patterns of the DNA with that of control bacteria. The identified INA bacterial strains from willow belonged to at least eight different genera and 17 species and showed varying levels of aggressiveness, ice-nucleation activity and population density within the willows tested.

To identify and distinguish the character for differentiation between these bacteria morphologically and in their growing ability, different culture media were used. Taxonomic tools, especially phylogenetic analysis derived from 16S rRNA sequences, clearly distinguished bacteria isolated in several independent investigations from different regions in Sweden and Estonia, as causing a serious new bacterial disease problem producing dieback in willows cultivated in bio-energy plantations.

The report demonstrates that dieback in Salix has been associated with Bacillus spp., Erwinia rhapontici, Frigoribacterium sp., Pedobacter sp., Pseudomonas fluorescens, P. grimontii, P. graminis, P. trivialis and Sphingomonas/Pseudomonas fluorescens, non-fluorescent P. fluorescens (biotype A, C, G), Xanthomonas spp, and other related species.

Key Words: Salix plants, INA bacteria, Biochemical reactions; BIOLOG® MicroPlate, Molecular tools, bacterial genera.

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THE USE OF *SPHAERELLOPSIS FILUM* FOR BIOLOGICAL CONTROL OF *MELAMPSORA* SPECIES ON *POPULUS* SPECIES

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Poplar rust is found everywhere poplar species are grown. Even though it does not kill mature trees, it can lead to significant economic losses due to growth reduction and predisposition to secondary plant pathogens. Growth reduction occurs as a result of reduced photosynthesis due to reduced photosynthetically-active leaf area and premature leaf drop. However, heavily infected seedlings can be killed.

In recent years interest has been expressed in the use of the mycoparasitic fungus *Sphaerellopsis filum*, for control of poplar and willow rusts. *Sphaerellopsis filum* (Biv. Ex Fr.) B. Sutton has been reported from more than 360 different hosts on five continents. Given its compiled host range, *S. filum* is thought to be a common hyperparasite in uredinia of rust fungi generally with little or no host specificity. Its ability to thrive in both temperate and tropical climates makes it a valuable organism to study. There is very little known about its biology and genetics. Based on a few studies using leaf-disc assays, *Sphaerellopsis filum* reduces spore production of uredinia of willow rust (*Melampsora epitea*). Since *S. filum* is found around the globe, it would be a valuable tool to control poplar rust (*Melampsora* sp.) in poplar plantations in both developed and developing countries.

Greenhouse experiments and observations in the field showed that there are some limitations and problems associated with the use of the hyperparasite *S. filum* for biological control which have to be considered and may render it useless. Data from experiments using whole-plant assays will be presented that clearly show that host specificity of *S. filum* exists and questions in relation to this issue will be discussed. Because of the host specificity, any given *S. filum* isolate which may be used to control various rusts would have to be tested for its ability to infect and control a particular rust.

An even more important question that surfaced during greenhouse experiments on host specificity will also be addressed. The question is whether the hyperparasite may actually increase the amount of rust on poplars. Experiments to answer this question are in progress and will continue. The data of these experiments will be presented as well.

The host specificity and potential ability to increase, rather than decrease, the amount of rust would make *Sphaerellopsis filum* useless for biological control. The fungus would indirectly do more harm than good to the tree.

**Key Words:** *Melampsora* sp., poplar, biological control, *Sphaerellopsis filum*, host specificity.

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INVESTIGATION OF THE RESISTANCE OF SOME POPLAR CLONES TO THE RUST FUNGI *MELAMPSORA ALLI-POPULINA* KLEB. IN TURKEY

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Rust caused by *Melampsora* is the most serious disease of poplars in Turkey. The rust fungi leads to defoliation in poplar plantations, and predisposes plants to attacks by secondary pathogens. Rust was not previously a problem for commercial plantations, because trees were infected only towards the end of the growing season. But since 2001 rust attacks have begun earlier causing severe defoliation in stoolbeds and poplar plantations in the Marmara regions.

The incidence of *Melampsora* rust is linked to defined ecological conditions and depends on genetics of the host clones. The genetic diversity of the rust fungi is high because of the nature of its host-pathogen interaction.

The main purpose of this study was to determine the resistance of selected poplar clones at different spacings to the fungi called *Melampsora alli-populina* Kleb. The trial was located in the nursery area of the Poplar Research Institute at İzmit. Planting was done in the first week of March 2003. In this study, experimental plantations were established with *P. x euramericana* and *P. deltoides* clones [I-214, 45/51, 77/51 (Samsun), S.307.26 and 89 M.060] at four different spacings (3m x 1.5m, 3m x 2m, 3m x 3m and 3m x 4m). Rust appeared at the end of July. In 2003, rust disease surveys were carried out three times during the growing season. Severity of rust infection was assessed on 24 September 2003. The reaction to natural rust infections was scored on the basis of the actual percentage of leaf area infected. Rust severity was assessed using a 5-step scale as follows: 0= highly resistant, no sign of rust; 1= resistant (only one pustule or a single leaf infected); 2= moderate susceptibility (leaves bore a few conspicuous uredinia or more, but barely recognizable rust pustules); 3= susceptible (leaves frequently bore rust pustules, up to an average of 1-5% of leaf area covered by uredinia); and 4= high susceptibility (most leaves bore numerous pustules).

Analysis of variance was used to determine the differences in resistance among the five selected poplar clones at the four different spacings to the fungi called *Melampsora alli-populina* Kleb. Rust infection was calculated by clone and spacing. In this paper, one-year old results of trial sitre were reported. According to the results of variance analysis, significant differences in rust infection were found among clones, but not between different planting espacements. Among the five clones, 89 M.060 clone was found to be highly resistant, 45/51 and S.307.26 clones were found to be resistant, and 77/51 (Samsun) and I-214 clones were found to be susceptible.

**Key Words**: poplar, rust fungi, *Melampsora*, resistance.

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