

Linking nucleotide polymorphisms in candidate genes and quantitative resistance to Melampsora larici-populina Kleb. in Populus nigra L.

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ABSTRACTS

Abstracts of oral and poster presentations given at the 4th International Rusts of Forest Trees Conference, Florence, Italy, 3 –6 May 2010

The 4th International Rusts of Forest Trees Conference was held in Florence, Italy, from May 3 to May 6, 2010. Florence is located in Tuscany, Central Italy, a region which was historically plagued by tree rust outbreaks. More than 40 tree rust specialists from 11 Countries from the northern as well as the southern hemisphere attended the meeting and gave 35 presentations on a broad array of topics, including phylogeny, distribution, biology, epidemiology, genomics, genetic resistance, and management.

Opening Lecture

Trends in tree rust research. R.C. Hamelin. *Natural Resources Canada, Laurentian Forestry Center, Ste-Foy, QC, and Faculty of Forestry, University of British Columbia, Vancouver, BC, Canada.*
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In the past 4 years since the last IUFRO meeting of the Tree Rust Working Group, there have been significant advances in tree rust research. In this presentation, I will briefly review the trends in tree rust research and highlight some of the most exciting new developments. Even after a century of research on rusts there has been significant progress in areas that have been addressed in the past, such as rust taxonomy, epidemiology, etiology, resistance screening and breeding, and studies of alternate hosts susceptibility and distribution. But maybe one of the most significant recent advances is the application of genomics to tree rust research. The genome of the poplar leaf rust (*Melampsora larici-populina*) is the first tree pathogen genome to have been completed. Currently, several rust genomes are being sequenced, including re-sequencing of strains of *Melampsora* as well as sequencing of *Cronartium quercuum* f. sp. *fusiforme* and *C. ribicola*. The availability of these genomes and gene catalogues will allow comparisons of rusts with different life cycles and host specificities and help us elucidate the complexities of rust life cycles. This will provide invaluable resources to our research community.

Phylogeny

Phylogenetic species delineation of *Melampsora* spp. on poplars. A. Vialle[1], N. Feau[2], L. Bernier[1], P. Frey[3] and R.C. Hamelin[4]. *[1]Centre d'Étude de la Forêt, Pavillon Abitibi-Price, Québec, QC, G1V 0A6 Canada. [2]INRA, UMR BioGeCo, 69 Route d'Arcachon, F-33612 Cestas Cedex, France. [3]INRA, Nancy-Université, UMR1136, Interactions Arbres – Microorganismes, 54280 Champenoux, France. [4]Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, 1055 du PEPS, PO Box 10380, Stn. Sainte-Foy, Québec, QC, G1V 4C7 Canada.*
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Over 25 species of *Melampsora* on *Populus* have been reported from around the world. Up to now, the poplar rust taxonomy and new species description were based

on morphometric characteristics and host specificity. However, wide variation and overlap in morphological characters have led to confusion in species identification and denomination. In this study, we sampled from about a hundred herbarium specimens representing 18 *Melampsora* species, including taxa from the *Melampsora populnea* complex that are morphologically similar. This material has been collected and identified by urediniologists during the last century, on poplars and alternate hosts from around the world and included some description type specimens. The large ribosomal RNA subunit (*28S*), the internal transcribed spacer (*ITS 1&2*), two mitochondrial genes encoding for the cytochrome oxidase 1 (*CO1*) and the NADH dehydrogenase subunit 6 (*NAD6*) respectively, and two single copy nuclear genes (*MS277* and *MS456*) were sequenced from these specimens. A phylogenetic approach using the six genes sequences was applied to recognize the *Melampsora* species. The host-specificity groups correlates with our results of phylogenetic clades and we suggest a revision of the taxonomy of these species, especially within the *M. populnea* complex. Based on data from evolutionary genetics we are proposing a revision of the poplar rust taxonomy.

Phylogeography and population genomics of the white-pine-blister-rust fungus, *Cronartium ribicola*. R.C. Hamelin[1, 2], M-S. Kim[3], B.A. Richardson[4], D. Joly[1], S. Brar[2] and N.B. Klopfenstein[5]. *[1]Natural Resources Canada, Faculty of Forestry, University of British Columbia, Vancouver, B.C., Canada;[2]Natural Resources Canada, Laurentian Forestry Center, Ste-Foy, Qc, Canada; [3]Department of Forestry, Environment and Systems, Kookmin University, Seoul 136-702, Republic of Korea; [4]USDA Forest Service- RMRS, Provo, Utah, USA; [5]USDA Forest Service-RMRS, Moscow, Idaho, USA.*
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The white pine blister rust fungus, *Cronartium ribicola*, was introduced into North America in the late 1800s and has caused significant mortality of five-needled, white pines. Although the introduction and spread have been well documented historically, few studies have used genetic and phylogenetic approaches to reconstruct the global dispersal and spread of the pathogen. To improve our understanding of this epidemic, genetic markers were developed to examine global and North American genetic variability in *Cronartium ribicola*. SNP genotyping at 8 polymorphic loci confirmed the presence of a barrier to gene flow between populations located east and west of the Prairies and the Great Plains of North America. Nucleotidic diversity was approximately 3-fold higher in eastern than in western populations, which is consistent with multiple introductions into eastern North America and a single introduction into the West. Ongoing studies are examining phylogenetic relationships among *C. ribicola* collected from eastern and western North America, northern Germany, Korea, Japan, and northeastern China. Preliminary phylogenetic analyses suggest *C. ribicola* comprises at least three distinct clades. Isolates from Korea and China formed one clade, and isolates from Japan formed a second clade that was intermediate with a third clade comprising isolates from USA and Germany. Identifying the evolutionary relationships and potential origin(s) of *C. ribicola* that spread through Eurasia and North America, and determining the phylogenetic relationships of its hosts are critical toward evaluating risks of cryptic introductions, contributing to the development of biological controls, identifying sources of host resistance, developing appropriate regulatory practices, and developing methods to predict other potentially invasive pathogens before they are introduced.

Distribution

The diversity of rusts of forest trees in India. G. Bagyanarayana. *Department of Botany, Osmania University, Hyderabad-500 007, A. P., India.*
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India, being a tropical country, has different forest types with diversified plant species. The vast majority of these plant species are vulnerable to diseases caused by fungal pathogens. Among these pathogens rust fungi (Uredinales) are the most important. The present paper deals with some interesting rust fungi infecting a wide range of forest trees belonging to various Angiosperm families: Anacardiaceae, Araliaceae, Bignoniaceae, Burseraceae, Euphorbiaceae, Fabaceae, Fagaceae, Gramineae, Rubiaceae, Rutaceae, Salicaceae, Sapotaceae, etc. Some of the genera parasitizing members of these plants are *Chaconia, Cronartium, Didymopsorella, Dasturella, Hapalophragmium, Kernkampella, Kuehneola, Maravalia, Masseella, Mehtamyces, Melampsora, Phakopsora, Ravenelia, Sphaerophragmium, Ramakrishnania, Nyssopsora,* etc. A detailed account of the morphological characters, host range and distribution is presented.

Observations on a foliar rust pathogen of grey alder in Trentino (North Italy). G. Maresi[1], M. Benelli[2], E. Bruno[2], B. Ginetti[2] and S. Moricca[2]. *[1]FEM-IASMA Fondazione Edmund Mach - Istituto Agrario San Michele all'Adige, Via E. Mach, 1 – 38010 San Michele all'Adige (TN) - Italy. [2]Dipartimento di Biotecnologie agrarie, Sezione di Protezione delle Piante, Piazzale delle Cascine 28, 50144 Firenze, Italy.*
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A severe defoliation of *Alnus incana* was first observed in the Campelle valley, a small valley running perpendicular to the Valsugana valley in Trentino, northern Italy, in the summer of 2008. The defoliation was observed and recorded by the Forest Tree Damage Monitoring (FTDM) Service of Trentino. Our surveys a year later in 2009 revealed that the epidemic occurred again in the same area in the summer of that year, with the severity of infection peaking in September. The disease was not seen in adjacent areas and valleys of the Province, where grey alder woods also sometimes grow, mainly along river banks. Similar rust symptoms have so far not been reported on other alder species (*A. glutinosa* or *A. viridis*). Alder leaves in the field exhibited a rust fungus on their lower surface, as clearly shown by the occurrence of uredinial sori producing the typical orange-coloured urediniospores in great profusion. Inspection under the light microscope and in the SEM indicated that the rust agent was a *Melampsoridium* species. Spore size and shape, spine distribution, and other micro-morphological characters positively identified this fungus as *Melampsoridium hiratsukanum*. This rust agent was clearly differentiated from the related fungi *M. alni* and *M. betulinum*, two other known pathogens of alder. *M. betulinum*, was also ruled out by field observation, which revealed that *Betula pendula* individuals growing intermixed with alder trees at some locations were not infected. Surveys of rust incidence and severity in alder are reported and the risk of epidemic spread of the disease in the Italian Alpine region is discussed. It is supposed that the pathogen, which has already been reported in other European countries, has been favoured by the heavier than usual rainy seasons in recent years.

A new *Eucalyptus* rust from Mozambique and South Africa. W. Maier[1], J. Roux[2], B.D. Wingfield[2], M.P.A. Coetzee[2] and M.J. Wingfield[2]. *[1]Ruhr-Universität Bochum, Biodiversität und Evolution der Pflanzen, Geobotanik, Universitätsstraße 150, 44801 Bochum, Germany. [2]DST/NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.*
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During surveys of *Eucalyptus* plantations in Mozambique in May and July 2009, typical rust disease symptoms were observed on eucalypt trees in several localities in the Maputo Province, as well as in the Niassa Province. Subsequently, the rust disease has also been found in KwaZulu-Natal in South Africa. These were disturbing findings given the importance of the eucalypt or guava rust fungus, *Puccinia psidii*. Thus far, *P. psidii* has been the only known rust fungus associated with *Eucalyptus* species, and it is one of the greatest threats to *Eucalyptus* plantation forestry and to *Myrtaceae* in natural forest ecosystems. Detailed examinations of the newly encountered rust fungus, of which only urediniospores have been found, have shown that it is distinct from *P. psidii*. Additionally, molecular phylogenetic analyses have been performed to develop a rapid identification tool for future studies, and also to better understand the evolutionary background of the rust fungus from southern Africa. Preliminary phylogenetic results showed that *P. psidii* and the newly found rust fungus from southern Africa do not share a recent common ancestor. They must therefore have developed independently within different phylogenetic lineages of the rust fungi.

***Uromycladium tepperianum*, the gall rust fungus from *Falcataria moluccana* in Indonesia.** S. Rahayu. *Department of Silviculture, Faculty of Forestry, Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia.*
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Batai (*Falcataria moluccana*) is a valuable tree species in forest plantations in Indonesia. Since 2001, gall rust has caused severe damage to all growth stages of batai, from seedlings in the nursery to mature trees in the field. The objectives of this study were to identify the fungus causing gall rust on *F. moluccana* in Java, Indonesia, and to ascertain the mode of infection and changes in the anatomy of infected cells. It was found that the disease is caused by *Uromycladium tepperianum*. This fungus produces three longitudinally ridged teliospores on each head, with spores that are 13–20 μm wide and 17–28 μm long. The fungus completes its entire life cycle on *F. moluccana*. The study confirmed that the teliospores themselves do not infect the host: under favorable conditions, about 10 hours after inoculation, they germinate and produce basidiospores, which form penetration pegs about 6 hours later, and it is this peg that penetrates the host cells directly through the epidermis. Pycnia, recognized as small brown pustules that break through the epidermis, form seven days after inoculation.

Occurrence of rust in teak (*Tectona grandis*) plantations in Brazil. E.L. Furtado[1]; C. De Pieri[1]; M.M. Passador[1,2]; W.B. Moraes[1]; A.A. Carvalho Junior[3]. *[1]Faculdade de Ciências Agronômicas – UNESP, CEP-18.610-307, Botucatu-SP. [2]Bolsistas CNPq. [3] Instituto de Botânica do Rio de Janeiro, Brasil.*
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The teak (*Tectona grandis*) is a specie of humid zones

of India (southeastern Asia). Today the culture of this tree has expanded in almost all the tropical zones of the world. This species has importance in the lumber industry and it is used in reforestation. Increasing attention is being payed nowadays to the rust fungus *Olivea tectonae*, the causal agent of teak rust, in several Countries where teak grows in natural or commercial plantations. The fungus causes intense crown defoliation, strongly reducing the photosynthetic area and, as a consequence, the timber production. Disease severity is higher in plants having less than 10 years. The infection first appears with brown spots, within which orange-yellow urediniospores soon develop. These symptoms and signs were observed on leaves of teak received for diagnosis by the Forest Pathology Laboratory, FCA, UNESP-Botucatu, from the northern part of the state of Mato Grosso. This is the first report of this rust in Brazil.

Conifer rusts in Friuli Venezia Giulia (north–eastern Italy). G. Frigimelica[1]. [1]*Dipartimento di Biologia e Protezione delle Piante, Via delle Scienze, 208 -33100 Udine, Italy.*
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Since 1994 a forest health monitoring programme (BAUSINVE) has been in operation in the Friuli Venezia Giulia region to record yearly any damage occurring in the forests of that region. *Chrysomyxa rhododendri* was the main needle rust agent reported in spruce stands. The intensity of *C. rhododendri* infection and the extent of overall defoliated area varied between years. *Melampsorella caryophyllacearum* occurred commonly in silver fir woods and its incidence varied greatly between forests. Its incidence was evaluated based on the percentage of trees with witches' broom and/or stem swellings. Attacks of the two-needle pine stem rust *Cronartium flaccidum* were reported on young Austrian pine stands and a small disease focus was also observed on Dwarf mountain pine. Several species of *Gymnosporangium* caused some die-backs on Common juniper. Other widespread rust agents, such as *Coleosporium* spp., did not usually cause any damage.

Biology and Epidemiology

Historical overview of the epidemics of *Cronartium flaccidum* in Italy. A. Ragazzi[1], I. Dellavalle[2], F. Moriondo[1], S. Moricca[1]. [1]*Dipartimento di Biotecnologie agrarie, Sezione di Protezione delle Piante, Piazzale delle Cascine 28, 50144 Firenze, Italy.* [2] *Istituto per la Protezione delle Piante CNR Via Madonna del Piano, 50019 Sesto Fiorentino (Firenze), Italy.*
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The heteroecious rust fungus *Cronartium flaccidum*, the causal agent of blister rust of two-needled pines, has occurred in Italy at least since 1830, as is shown by the occurrence of the uredial and telial stages of this fungus on leaf specimens of the main intermediate host, *Vincetoxicum hirundinaria*, stored as *exiccata* in the herbaria of two Institute in Florence. The fungus attacks various species of the genus *Pinus*, and occurs in Italy from the north to the south. The first recorded epidemics occurred on young plantations of *Pinus nigra* subsp. *austriaca* and *P. nigra* subsp. *nigra* var. *laricio* in the northern Appennines in 1948. Devastating outbreaks continued, in young and also in old plantations, until the seventies, on the same two hosts and also on other pine species: *P. halepensis*, *P. pinea*, *P. pinaster*, *P. nigra* subsp. *nigra* var. *italica* and, more rarely, *P. sylvestris*, a less susceptible species. Optimal conditions for propagule production, dispersal and germination have been ascertained for most spore types. Most recently, the disease gradient (DG) on the intermediate host was investigated by determining the average number of uredinia per plant. It was found that that an increase in the distance of the inoculum source from 10 to 1,000 m reduced the number of uredinia by 90.8%. This means however that even at a distance of 1,000 m all local inoculum is a potential source of infection, since at that distance the biomass of aeciospores remains abundant and highly vital. Starting from the seventies, the incidence of *C. flaccidum* has decreased remarkably, to such an extent that the fungus is currently thought to survive at an endemic state. The main causes for this reduction were: 1) a strong reduction of pine plantations; 2) a drastic decrease in the production of aecia; 3) abnormal high temperatures occurring during the aecial, uredinial and telial phases of the fungus in recent decades. This last point is obviously related to global climate change. Research has since continued in new directions. The fungus was cultured axenically to investigate aspects of the pathosystem; to study the host-parasite interaction *in vitro*; to test for the possibility of rapid selection of resistant host germplasm; to obtain mycelium for molecular approaches, etc. DNA-based investigations have enabled the structure of pathogen populations to be determined, as well as its relatedness to the non-host alternating form *Peridermium pini*.

Evolutionary trends of rust pathogens on Salicales in connection with cultivation methods and climate change. M. Gennaro[1], A. Giorcelli[1] and G.P. Cellerino[2]. [1]*Agricultural Research Council (C.R.A.), Unit for Wood Production outside Forests (P.L.F.), I-15033 Casale Monferrato (AL), Italy.* [2]*University of Torino, Department of Exploitation and Protection of Agricultural and Forestry Resources (Di.Va.P.R.A.), I-10095 Grugliasco (TO), Italy*
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Melampsora species infecting *Populus* and *Salix* are a good example of pathogens whose original populations have been strongly affected by variations in cultivation features. Several decades of spreading in monoclonal stands, especially poplar stands, have originated selection pressure on pre-existing genotypes, which initially had only few and old virulences, and then gave rise to pathotypes with new virulences that overcame the resistance of common clones. SRC willow plantations, established for biomass or wastewater filtration, for many years have often consisted of mixtures of different genotypes with similar production power; thus here the risk is that "super-races" will arise, mainly of the *M. epitea* complex, having several co-existing virulences and adapted to such mixed stands. SRC plantations build up a suitable microclimate for a high number of urediniospore generations in a vegetative season, owing to both high moisture under the closed canopy and host aggregation. Moreover, the lower temperature fluctuations under the dense canopy allow rust mycelium to overwinter inside buds, enabling leaves of the same individual to be infected directly through sprouting shoots. A stem-infecting form (SIF) of *M. larici-epitea* currently occurs on *S. viminalis* plantations of Great Britain, inducing stem cankers. Similar forms could also soon arise in poplar SRC attacked by *M. larici-populina*. These dynamic pathosystem conditions may be further destabilized by climate change. High CO_2 concentrations are connected with stronger rust infections, but however promote enhanced wood productions. Since high CO_2 is often associated with high levels of tropospheric ozone, experimental trials performed with *M. medusae* on *P. tremuloides* have shown that high CO_2 plus O_3 enhance infections mainly by altering the leaf surface integrity. Among the other parameters, higher temperatures during the growing season can be expected to induce a spread of the more thermophilic *M. allii-populina* northwards into poplar stands of central Europe, with new and possibly dangerous host-pathogen interactions, since *Melampsora* species have evolved many different types of virulence and many pathotypes.

Epidemiology and population genetics of *Melampsora larici-populina* on poplars. P. Frey, C. Xhaard, B. Barrès, F. Halkett. *INRA, Nancy Université, Interactions Arbres Microorganismes, F-54280 Champenoux, France.*
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Poplar cultivation in Europe is highly intensive and there have been many efforts by breeders during the past decades for developing cultivars with a good level of resistance to diseases, especially to rust. However, the Eurasian poplar rust fungus, *Melampsora larici-populina*, successively broke down all the complete resistances released so far, causing severe economic losses. Alternative strategies based on spatial management of available resistances and on breeding for partial resistance are on-going, but they need a good knowledge of the adaptation potential of the pathogen. Biological characteristics, such as the dispersal distances or the relative importance of sexual *vs.* asexual reproduction, are often difficult to measure for such plant pathogens. A way to infer these characteristics is the use of molecular markers. Completion of the *M. larici-populina* genome sequence provided an opportunity for developing a large number of microsatellite markers, useful for population genetics studies of *M. larici-populina* at different spatial scales. At a worldwide scale, the long distance migration capacity of *M. larici-populina* was assessed by studying eight European populations and two populations recently founded in Iceland and Canada. European populations exhibited an isolation by distance pattern, suggesting a gradual dispersal, whereas non-European populations appeared to result from strong founder effects with a long distance dispersal of a limited number of individuals. At a regional scale, we focused on the spread of a rust epidemic in a natural system, the Durance River valley, in the French Alps. This site is particularly well suited for the study of recurrent epidemics: the need of the alternate host plant (larch) to perform its sexual reproduction restricts the resident pathogen population upstream the valley, in a poplar-larch sympatry area. Then a clonal epidemic phase spreads downstream the valley during summer along a 200 km natural riparian stand of black poplar, *Populus nigra*. This region also includes a few cultivated stands with poplars carrying a qualitative resistance gene, thus exerting a selection pressure on pathogen populations. We used epidemiology and population genetics tools to: (*i*) sort *M. larici-populina* individuals according to their wild or cultivated origin; (*ii*) describe the spread of the epidemic on the wild stands; and (*iii*) assess the evolution of the genetic composition of the pathogen populations along the epidemic wave.

Current knowledge of alternate hosts of *Cronartium* spp. in Finland. J. Kaitera[1] and R. Hiltunen[2]. *[1]Finnish Forest Research Institute, Muhos Research Unit, Kirkkosaarentie 7, FI-91500 Muhos, Finland. [2]Botanical Gardens, University of Oulu, P.O. Box 3000, FI-90014 Oulu, Finland.*
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Cronartium flaccidum is causing a serious rust epidemic on *Pinus sylvestris* in northern Fennoscandia currently. The epidemic is connected to frequency of *Melampyrum* spp. in diseased stands. The rust has been found on leaves of four *Melampyrum* species, but it is rare on the most common species, *M. pratense*,

in Finland. The main alternate host of the rust is *M. sylvaticum* in northern Finland. The rust occurs also commonly on *Vincetoxicum hirundinaria* in a limited coastal area of southern Finland, and it has been found on several *Pedicularis* spp. and *Paeonia* spp. in natural forests in Finland. In artificial inoculations, the rust has been shown to infect several species in the former plant genera. *Cronartium ribicola* has killed most of the old five-needle pine plantations in Finland established in the early 1900s. The rust is most common in arboretums and botanical gardens in southern Finland, where it has been found only on a high number of cultivars of *Ribes* spp. European *C. ribicola* has been suggested to be very host-specific earlier, but in recent artificial inoculations, the rust has been shown to infect also species of plant genera other than *Ribes*. This suggests that the virulence of European *C. ribicola* is much wider than earlier reported.

***Cronartium flaccidum* in axenic culture: new insights and perspectives.** S. Moricca. *Dipartimento di Biotecnologie agrarie, Sezione di Protezione delle Piante, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy.*
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The axenic culture of microbes has always represented a significant step in investigating these organisms. Since Pasteur, Kohn and Koch, just to cite some giants in microbiology, getting a microbe – whether of clinical, microbiological or pathological interest – to grow in the lab has always been a momentous achievement which often opened up new and unexpected avenues in subsequent research. The study found that the cultivation of *Cronartium flaccidum* on suitable media for an extended period of time without disturbance improved the growth of the fungus and permitted multiphasic sporulation. Like other rust fungi, *C. flaccidum* is far from normal in many of its characteristics in culture, producing sporophore- and spore-like bodies, which sometimes appear abnormal or unusual. Fruiting bodies and propagules closely resembling four of the five sporogenous stages of the full-cyclic rust were grown in two-year-old cultures. This enabled us to better explore some aspects regarding the nutrition, saprobic habit, culture stability, growth kinetics, spore ontogeny and breeding system of this rust. The examination demonstrated that even in the third millennium rust cultures are still a valuable experimental tool that can shed light on many aspects of the biology of these biotrophic micro-organisms. *In vitro* axenic cultivation can refine our knowledge of rust metabolism, genetics, the relationship of the rust with the host plant (infection biology, metabolite production, cell-to-cell signalling, etc). Having pure rust mycelium to work with is also expected to give a boost to "omic" investigations. Such investigations were until now hampered by the unavailability of this source material.

Evaluation system to monitor eucalypt rust in the field. W. Bucker Moraes[1], W. Bucker Moraes[1], W. Cintra De Jesus Junior[*2], R. Gonçalves Mafia[3] and E. L. Furtado[*1]. *[1]Faculdade de Ciências Agronômicas de Botucatu / Universidade Estadual Paulista. [2]Center for Agrarian Sciences, Federal University of Espírito Santo, Alegre, Espírito Santo, Brazil. [3]Research and Technology Center - Aracruz Celulose S.A., 29197-900, Aracruz, ES, Brasil *CNPq Researcher.*
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The eucalypt rust is the most serious diseases of eucalypt plantations in Brazil where it causes damage and yield losses. The pathogen threatens eucalypt plantations in many parts of the world, particularly in Australia, where the eucalypt originated. This study was carried out in Teixeira de Freitas-Bahia Brazil, in a plot consisting of 6 rows of 10 plants each (3×1.5m), always kept in regrowth. Three eucalypt lines were evaluated for disease development and three other lines were kept as a source of inoculum, using the susceptible clone 3918, from September 2008 to January 2009. It was found that the middle and lower third portions of the crown provide the most accurate indications for the assessment of eucalyptus rust in plants and for monitoring the disease for possible control in the field.

A gall rust epidemic on *Falcataria moluccana* at Brumas estate, Tawau, Sabah, Malaysia. S. Rahayu[1] and L. Su See[2]. *[1]Faculty of Forestry, Gadjah Mada University, Yogyakarta 55281, Indonesia; [2]Forest Research Institute Malaysia, Kepong, 52109, Selangor, Malaysia.*
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Gall rust caused by *Uromycladium tepperianum* (Sacc.) is one of the most destructive diseases on *Falcataria moluccana* plantations in parts of south east Asia. It causes severe damage to all growth stages from the nursery to mature trees in the field, including chocolate brown, cauliflower-like or whip-like galls on the stems, branches, petioles, shoots and pods. Objectives of the study were: to determine the status of the gall rust; to establish the relationship between the incidence of the gall rust and environmental factors that may influence gall rust symptoms on the estate; and to predict the origin of the gall rust spores. Ten per cent of the total area at each site was sampled. Three blocks, three replicates, and three plots each with 10

trees were examined. A more open forest, a flat topography, the absence of fog, a greater tree age and lower altitudes were significant site conditions reducing gall rust incidence and severity. High relative humidity and slower wind speed promoted gall rust. Since on the Brumas Estate the winds are predominantly from the north-east, they may spread gall rust spores from the north, probably from the Philippines.

Laser capture microdissection of uredinia formed by *Melampsora larici-populina* revealed a transcriptional switch between biotrophy and sporulation. S. Hacquard, C. Delaruelle, E. Tisserant, V. Legué, A. Kohler, P. Frey, F. Martin and S. Duplessis. *UMR 1136 INRA/UHP Nancy 1 Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France.*
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The foliar rust caused by the basidiomycete *Melampsora larici-populina* is the main disease affecting poplar plantations in Northern Europe. The biotrophic status of rust fungi is a major limitation to study gene expression of cell- or tissue-types during host infection. At the uredinial stage, infected poplar leaves contain distinct rust tissues such as haustoria, infection hyphae, and uredinia with sporogenous hyphae and newly formed asexual uredospores. Laser Capture Microdissection (LCM) was used to isolate three areas corresponding to uredinia and subjacent zones in the host mesophyll for gene expression analysis with *M. larici-populina* whole genome exon oligoarrays. Optimisation of tissue preparation prior to LCM allowed isolation of RNA of good integrity for global expression profiling. Our results indicate that the poplar rust uredinial stage is marked by distinct genetic programs related to biotrophy in the host palisade mesophyll and to sporulation in the uredinia. A strong induction of transcripts encoding small secreted proteins likely containing rust effectors is observed in the mesophyll, suggesting a possible late maintenance of suppression of host defense by the rust in the tissue containing haustoria and infection hyphae. In contrast, cell cycle and cell defense rescue transcripts are strongly accumulated in the sporulation area. This combined LCM-transcriptomic approach brings new insights on the molecular mechanisms underlying uredospore formation in Pucciniales.

Population Genetics and Genomics

The genome sequence of the poplar rust pathogen *Melampsora larici-populina*. S. Duplessis[1], Y-C. Lin[2], E. Tisserant[1], B. Hilselberger[1], S. Hacquard[1], D. Joly[3], C. Veneault-Fourrey[1], N. Feau[3], P. Tangay[3], A. Kohler[1], E. Lindquist[4], B. Cantarel[5], P. Couthinho[5], B. Henrissat[5], P. Frey[1], C. Murat[1], J. Amselem[6], JGI Sequencing and Assembling Teams[4], A. Aerts[4], P. Rouzé[2], Y. Van de Peer[2], R. Hamelin[3], I. Grigoriev[4] and F. Martin[1]. *[1]UMR 1136 INRA/UHP Nancy 1 Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France. [2]Department of Plant Systems Biology, VIB, Ghent, Belgium. [3]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du PEPS, PO Box 10380, Stn Sainte-Foy, Québec, QC, G1V 4C7 Canada. [4]DOE Joint Genome Institute, Walnut Creek, CA 94598, USA. [5]UMR 6098 CNRS-Universités Aix-Marseille I, Marseille, France. [6]INRA Versailles, Equipe de Biologie Cellulaire, Route de St Cyr 78026 Versailles Cedex, France.*
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The foliar rust caused by *Melampsora larici-populina* is the main disease affecting poplar plantations in northern Europe with severe economic losses. Epidemiology of the disease and plant defense responses have been studied but very little is known about fungal molecular mechanisms during the infection process. In the wake of the *Populus* genome sequencing, the ~101 Mb genome of *M. larici-populina* have been sequenced (7× depth) and *ab-initio* annotation predicted about 17,000 gene models. The analysis of this genome is a great opportunity to identify loci coding for the arsenal developed by the rust fungus to penetrate and exploit its host. Surprisingly, more than half of these genes do not have fungal homologs, except for *Puccinia graminis* f. sp. *tritici*, the only other rust genome sequenced so far, with 25% of the genes specific to the two Pucciniales. Beside, 33% of the predicted genes are unique to the poplar rust. Expert annotation of functions likely to be involved in the infection process (e.g. secreted proteins, carbohydrate-active enzymes, transporters, cytochrome P450, lipases, proteases, transduction pathways) revealed specific genes that might be important to establish biotrophy. Several transcriptomic approaches were used to support gene expression in spores as well as in plant tissues during the infection process (e.g. 454-pyrosequencing, NimbleGen exon oligoarrays, RT-qPCR). Specific temporal patterns of gene expression associated with colonization, biotrophy or spore formation allowed to identify candidate genes that encode putative rust effector genes. Immunolocalization of selected small secreted proteins containing RxLR-like motif using confocal laser scanning microscopy indicated accumulation around haustorial structures, likely in the extra-haustorial matrix. Sequencing the poplar rust genome is a great opportunity to understand how rust fungi interact with their hosts.

Genetic and genomic resources for the fusiform rust fungus *Cronartium quercuum* f. sp. *fusiforme* (*Cqf*). T. Kubisiak[1], C. Anderson[2], H. Amerson[3], J. Smith[2], J. Davis[2] and C. D. Nelson[1]. [1] *U.S. Forest Service, Southern Research Station, Southern Institute of Forest Genetics*, [2]*School of Forest Resources and Conservation, University of Florida, FL, USA* [3]*Forest Biotechnology Group, North Carolina State University, NC, USA.*
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Recent progress on the fusiform rust pathosystem is showing the importance of specific host-pathogen gene interactions. That progress has largely depended on the use of either half-sib or full-sib host families and single-spore-derived genotypes of the pathogen. Single-aeciospore isolates (SAI's) or single-uredinial pustule (SUP) isolates are necessary to limit genetic variation in the pathogen, and thus facilitate or permit investigation of specific host resistance genes. Simple and efficient SAI or SUP oak inoculation protocols coupled with pathogen-specific microsatellite markers are now providing a means by which to develop single-genotype isolates and provide the QA/QC needed to ensure isolate identity and continued purity. The availability of single-genotype isolates will aide further gene discovery work and allow tree breeders the ability to predict the likely resistance gene composition of elite trees in their breeding programs. We developed a single dikaryotic isolate (*P2*) that is heterozygous for avirulence against fusiform rust resistance gene *Fr1*. Basidiospores derived from *P2* were used to challenge seedlings of controlled pollinated loblolly pine family known to be heterozygous at the *Fr1* resistance gene. DNA markers tightly linked to *Fr1* were used to classify progeny as either *Fr1*/*fr1* resistant or *fr1*/*fr1* susceptible and DNA extracted from pycniospores (spermatia) collected from galled trees were used to genetically map the *Avr1* locus in isolate *P2*. Given the success of this approach it will now be possible to map as many as five additional Avr genes that are potentially heterozygous in *P2*. Our long-term research goals are to genetically map all of the currently known Avr genes, clone them, and develop markers that will be predictive of avirulence. Such information would provide a reliable means by which more informed management decisions could be made regarding the deployment of host resistance genes. We recently performed a single full-plate Roche GS FLX 454 TITANIUM® sequencing run on a single haploid isolate of *Cqf* and obtained ~407 Mb of sequence. This represents approximately 4.4X coverage of the 91Mb *Cqf* genome. We are currently characterizing this sequence and will provide a brief summary of this analysis. The U.S. Department of Energy's Joint Genome Institute Community Sequencing Program is scheduled to obtain additional genome sequence for a single haploid isolate of *Cqf* during the 2010 sequencing cycle. Our hope is that this additional sequence will provide a complete assembly of the *Cqf* genome.

Genetic structure of Scots pine blister rust (*Cronartium flaccidum* and *Peridermium pini*). B. Samils[1], K. Ihrmark[1], J. Kaitera[2], P. Hansson[3] and P. Barklund[1]. [1]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden*, [2]*Finnish Forest Research Institute, Muhos Unit, Kirkkosaarentie 7, FIN-91500 Muhos, Finland*, [3]*Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-90183 Umeå, Sweden.*
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There is currently widespread infection by blister rust in young stands of Scots pine in Northern Sweden. Two forms of the Scots pine blister rust have been distinguished: the host-alternating form with five spore stages, *Cronartium flaccidum*, and the autoecious (pine-to-pine) form, *Peridermium pini*. Morphological and molecular characterization suggests that the two forms belong to the same species. We used SSR markers to analyze genetic variation within and between blister rust cankers in Scots pine stands in Sweden. Each sample was taken from a single blister (aecidium) in the canker. About half of the 27 investigated cankers contained multiple SSR genotypes, while the other contained only a single genotype. This indicates the occurrence of multiple mating between the resident spermogonia and fertilizing spermatia. Five of the single-genotype cankers accommodated aeciospores that were homozygous for all seven SSR markers analyzed. Homozygous aeciospores have previously been recognized as an attribute of the pine-to-pine form, *P. pini*. Genetic analysis using SSR markers was also done on 165 aeciospore samples collected over a wide area in Northern Sweden. In this collection, 36% of the samples were homozygous (i.e. presumably *P. pini*). Several of the homozygous samples showed to be clones that were found on different trees and in different locations. All the heterozygous samples (*C. flaccidum*) had unique SSR genotypes. These results are consistent with the suggested differences in life cycles between the two forms of Scots pine blister rust, where *P. pini* spreads clonally from pine to pine and *C. flaccidum* occurs as a sexual population. There was little overall genetic differentiation between the two forms of the rust.

The hunt for effectors in the secretome of *Melampsora larici-populina*. D.L. Joly[1], S. Hacquard[2], Y.C. Lin[3], E. Tisserant[2], N. Feau[1,4], P. Tanguay[1], F. Martin[2], S. Duplessis[2], and R.C. Hamelin[1,5]. [1]*Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du PEPS, PO Box 10380, Stn Sainte-Foy,*

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It is now well established that obligate biotrophs such as rust fungi deliver a battery of effector proteins inside host cells which probably act to subdue defense responses by interacting with host proteins. The recent completion of the *Melampsora larici-populina* genome sequence has revealed an arsenal of ~2,000 secreted proteins, from which only one third have identifiable homologs in the genome of the wheat stem rust. Still, similarities with effectors previously described in *Pucciniales* were uncovered, such as homologs of the Rust Transferred Protein from *Uromyces fabae* and of most of the Haustorially Expressed Secreted Proteins and avirulence proteins from *M. lini*. More than 10% of the secretome encodes highly divergent proteins belonging to different families with conserved cysteine residues, the largest family encompassing more than 100 members, mostly found in gene clusters. Interestingly, some of these families appear to be specifically expressed *in planta*. Transcriptome analysis based on 454-pyrosequencing and NimbleGen systems oligonucleotide arrays were performed to concentrate on effectors which could act as crucial determinants for host-pathogen interaction and allowed the identification of transcripts coding for Small Secreted Proteins (SSPs) specifically and/or highly expressed *in planta*. Since more than one third of the SSPs belong to gene families, intra- and inter-specific comparative genomics appeared as a complementary strategy to target candidate effector genes, which should exhibit the hallmarks of positive selection due to the coevolutionary arms races with host factors. By unravelling the expression and evolutionary dynamics of *M. larici-populina* SSPs, it appears that most of those specifically and/or highly expressed *in planta* with evidence of positive selection are rich in cysteine residues, have no homology in public databases and a high rate of tandem duplication. Further characterization of the rust candidate genes reported here is currently ongoing to precise their exact roles in pathogenicity.

Toward functional genetics in the obligate biotrophic *Melampsora* fungi. P. Tanguay[1], G. Pelletier[1], D. L. Joly[1], N. Feau[1*] and R. C. Hamelin[1]. *[1] Laurentian Forestry Centre, Canadian Forest Service, 1055 rue du PEPS, Québec, G1V 4C7, Canada. [*]Present address: UMR 1202, INRA-Université Bordeaux I, Biodiversité, Gènes et Communautés, Centre INRA de Bordeaux-Aquitaine, 33612 Cestas Cedex, France.*
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Genome sequencing has been undertaken and completed for more than 70 fungal species including the poplar leaf rust *Melampsora larici-populina*. The next major challenge is to translate this corpus of genome sequence information into biological functions. Two strategies were assessed to study the functions of genes in *Melampsora* fungi: genetic transformation, and host driven silencing of the pathogen genes. A dominant selection marker to retrieve stable transformants of *Melampsora larici-populina* was developed by introducing a point mutation in the succinate dehydrogenase gene of this fungus. The resulting gene (Cbx^R) encodes a protein that confers resistance to the systemic fungicide carboxin. Biolistics and *Agrobacterium*-mediated transformation systems were used to promote integration of a DNA vector containing the Cbx^R gene and the *GUS* gene under the control of the *M. larici-populina* actin gene. Despite several attempts, only a few transient transformants expressing the GUS gene were obtained by biolistics. To overcome the problem of stable integration and expression of transgenes in *Melampsora*, we investigated the host-induced gene silencing in which transgenic INRA 717-1B4 hybrid poplar *P.* × *canescens* (*P. tremula* × *P. alba*) lines expressing a dsRNA of the *Melampsora aecidioides* actin gene (*MaACT*) were produced. Following inoculation, poplar transgenic lines with lower level of infection were identified. The transcript and the siRNA of the *MaACT* gene are now being quantified to validate the inoculation results. The host-induced gene silencing would opens up new avenues of research in obligate biotrophic pathogens such as the poplar leaf rust fungi. This would allow the analysis of biological functions of genes in the biotrophic fungi resilient to genetic transformation, and the development of pathogen specific resistant tree.

Molecular population genetics of guava rust *Puccinia psidii*, an invasive pathogen of native Hawaiian forests and a potential threat to eucalypts world-wide. Graca R.N.[1], A.L Ross-Davis.[2], M.-S. Kim[3], A.C. Alfenas[1], T.L. Peever[4], P.G. Cannon[5] and N.B. Klopfenstein[2] *[1]Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil; [2]USDA Forest Service - Rocky Mountain Research Station, 1221 South Main Street, Moscow, ID 83843 USA; [3]Department of Forestry, Environment and Systems, Kookmin University, Seoul, Korea 136-702; [4]Department of Plant Pathology, Washington State University, Pullman, WA 99164 USA; [5]USDA Forest Service, Forest Health Protection, Vallejo, CA 94592 USA.*
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Puccinia psidii is the cause of a rust disease of many hosts in the *Myrtaceae* family, including guava, eucalypt, rose apple, and ohia. First reported in 1884 on guava in Brazil, the rust has since been detected in South America (Argentina, Brazil, Colombia, Paraguay, Uruguay, Venezuela), Central America (Costa Rica, and Panama), Caribbean (Cuba, Dominica, Dominican Republic, Jamaica, Puerto Rico, Trinidad and Tobago, Virgin Islands), Mexico, USA (Florida, California, and Hawaii), and most recently Japan. Of present concern is the recent introduction of the pathogen to Hawaii, where it infects an endemic tree species known as ohia (*Metrosideros polymorpha*), the dominant tree species in Hawaii's remnant native forests. Guava rust also poses serious threats to several hosts in the *Myrtaceae* including *Eucalyptus*, a genus native to Australia and planted extensively in numerous tropical and subtropical countries. Despite the potential threats to many forest ecosystems world-wide and the expanding geographic range of this disease, little is known about the genetic structure of pathogen populations, migratory routes and sources of introductions. To determine population genetic structure of the pathogen in the putative center of origin, approximately 150 single-pustule isolates of *P. psidii* have been collected from diverse host species and locations in Brazil and scored for variation at 12 microsatellite loci. Additional isolates have been collected from Hawaii, Puerto Rico, California, Paraguay, and Uruguay; and collaborators are currently being sought to sample isolates from other global regions. Collections from these putative introduced populations will facilitate inferences about the spread of this rust pathogen throughout the world. Preliminary results indicate that host species strongly influence population structure; distinct multilocus haplotypes are uniquely associated with specific hosts across diverse geographic locations. This information will help identify rust races that pose threats to global populations of *Myrtaceae* and help prevent their introduction into new regions.

Identification of *Hemileia vastatrix in planta* expressed genes through 454-pyrosequencing of rust-infected *Coffea arabica* leaf tissues. D. Fernandez[1], E. Tisserant[2], P. Talhinhas[3], H. Azinheira[3], A-S Petitot[1] and S. Duplessis[2]. *[1]IRD, Institut de Recherche pour le Développement, UMR 186 IRD-Cirad-UM2 Résistance des Plantes aux Bioagresseurs, 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France. [2]INRA, Institut National de la Recherche Agronomique, UMR 1136 INRA/Nancy Université Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France. [3]CIFC - Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, 2784-505 Oeiras, Portugal.*
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Coffee (*Coffea arabica* L.), one of the key export and cash crops in tropical and subtropical countries, suffers severe losses by the leaf rust fungus *Hemileia vastatrix* (Berk. and Broome). We performed a transcriptomic analysis to identify small proteins of *H. vastatrix* specifically secreted during the infectious process, and which could control the interaction with the coffee-tree. The transcriptome of *H. vastatrix* strain 178a (containing virulence genes *v2,3,4,5*) was analyzed during a compatible interaction to obtain an exhaustive repertory of the genes expressed during infection. Pyrosequencing technology (454-Titanium, Rock) of cDNA obtained from coffee infected leaves allowed to generate 352,146 sequences which were gathered in 23,125 contigs. These sequences were annotated starting from the international databases (NR, Swissprot, GO, KOG) to assign a putative function to the sequences expressed by the fungus and the plant. In order to separate the fungal transcripts from plant ones in the absence of the sequences of the reference genomes of the two organisms, we applied an approach based on the specific trinucleotide frequencies within ESTs (program EST3, MIPS), the percentage in GC and the homology scores after blastn against reference angiosperm and basidiomycete genomes and Pucciniales and *Coffea* spp. ESTs. More than 30% of the sequences could be assigned to the fungus, and 57% to the plant. In parallel, the search of *H. vastatrix* secreted proteins was carried out *in silico* by the systematic detection of signal sequences, and 388 sequences were identified as putatively secreted. By associating the data resulting from the two partners of the interaction, the results obtained enable us to target candidates genes for virulence in *H. vastatrix* and markers of the compatible interaction in the *C. arabica* plant.

Resistance

Screening for resistance to fusiform rust in southern US forest trees. J. Bronson. *Resistance Screening Center, 1579 Brevard Road, Asheville, NC, 28805 USA.*
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The Resistance Screening Center (RSC) is operated by the Forest Health Protection unit of the USDA Forest Service, Southern Region, State and Private Forestry. The Center is located at the Bent Creek Experimental Forest near Asheville, NC, USA. The Center evaluates seedlings for resistance to disease, primarily fusiform rust (caused by *Cronartium quercuum* f. sp. *fusiforme*) and pitch canker (caused by *Fusarium circinatum*) as a service to tree improvement specialists, seed orchard managers, scientists, government agencies, research institutions, universities, and private industry. Testing

enables clients to obtain information on the relative resistance of their materials in much less time than is possible in field progeny tests. The RSC has the flexibility to modify current screening procedures to accommodate specialized requests, such as unique species or inoculation procedures. This allows researchers to use the RSC as an additional experimental tool. In a research assistance capacity, the RSC has played an important role in newly developed understanding of genetic interactions in the pine-fusiform rust pathosystem and will continue to do so in the foreseeable future. By using information from the Resistance Screening Center tests, trees producing resistant progeny can be identified or questions may be answered concerning such aspects as the nature of pathogen variation or the effectiveness of fungicides. The RSC remains open to service screening work or research endeavors in an effort to improve forest health.

No resistance is sacrosanct: unveiling the genes controlling quantitative resistance to *Melampsora larici-populina* in interspecific hybrid poplars and quantifying potential adaptation of the pathogen. A. Dowkiw[1], V. Jorge[1], E. Voisin[1], P. Faivre-Rampant[2], A. Bresson[2], F. Bitton[2], S. Duplessis[3], P. Frey[3], A. Kohler[3], C. Rinaldi[3], C. Plomion[4], C. Lalanne[4] and C. Bastien[1]. *[1]INRA, UAGPF, 2163 avenue de la Pomme de Pin, BP20619 ARDON, 45166 Olivet Cedex, France. [2]INRA-URGV, 2 rue Gaston Crémieux, CP5708, 91057 Evry Cedex, France. [3]INRA UMR IAM, 54280 Champenoux, France. [4]INRA UMR BIOGECO, 69 route d'Arcachon, 33612 CESTAS Cedex, France.*
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Because of its mixed mating system and high potential gene flow, *Melampsora larici-populina* belongs to the group of pathogens with the highest evolutionary risk. Indeed, all qualitative resistances that have been selected by breeders during the last decades have been defeated. Although quantitative resistance (QR) is often considered more durable, a multidisciplinary approach is being conducted at INRA not only to identify the genes controlling QR but also to predict potential adaptation of the pathogen. Starting with a single *Populus deltoides* × *P. trichocarpa* F_1 pedigree, this work led to the identification of several QTLs with contrasted levels of effect and strain-specificity. Much attention is being paid to two major loci, R_1 and R_{US}, that both map on LG XIX. Dominant allele R_1 is inherited from *P. deltoides*. It controls a qualitative resistance to *M. larici-populina*, and it was found to be statistically associated with QR levels in several *P. deltoides* × *P. trichocarpa* F_1 progenies. Dominant allele R_{US} is inherited from *P. trichocarpa*. It has major effect of uredinia size and other QR components in both intraspecific and interspecific F_1 progenies. Fine mapping of R_{US} is being conducted using a 1300 genotype F_1 host progeny, and a BAC library was constructed to generate a physical map of 2000 kb around R_{US}. A promising BAC clone has been identified and sequenced. In parallel, an expressional approach based on the analysis of R_{US} and r_{US} infected material generated transcriptional and translational candidates. On the pathogen side, one strain was found to be able to circumvent R_{US}, thus offering the opportunity to study the frequency and the mechanisms of adaptation to QR. Several populations of *M. larici-populina* that underwent contrasted selection pressures by R_{US} are being characterized for their phenotypes and for their genotypes at several microsatellite loci. The first results demonstrate the existence of pre-adaptation in "wild" pathogen populations and a clear selective pressure by the host.

Constitutive monoterpenes and rust susceptibility in jack pine. M. Michelozzi[1] and K. Raffa[2 1]. *Istituto di Genetica Vegetale, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italy, [2]Department of Entomology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA.*
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Terpenoids play numerous relevant roles in the ecosystem and their defensive role against insects and fungi has been the subject of the most extensive research in the multiple terpene interactions with different organisms. The relative content (percentages) of the constitutive monoterpenes is under strong genetic control and little affected by abiotic factors. Chemosystematic studies have shown the variability in monoterpene composition between species, populations and provenances, hybrids, families and clones and these variations in the constitutive mixture of monoterpenoids may have important consequences in chemical defence of plants. Fusiform rust caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is the most destructive disease of pine in the southern United States. The aim of this study was to investigate the relationships among cortical monoterpenes and rust resistance in jack pine. Cortical samples were collected from 13 open-pollinated families of *Pinus banksiana* Lamb. with known breeding values for rust resistance growing in an experimental plantation at Beyer Christmas Tree Farm at Hancock, Wisconsin (USA). Monoterpene composition was determined by means of headspace gas chromatography (HS-GC). Based on these data trees having relatively high content of an unknown compound, β-pinene, 3-carene, limonene, and γ-terpinene, tended to be less susceptible than trees characterized by low amount of these terpenes. These data are in agreement with previous work on slash and loblolly pines and confirm that characterization of diffe-

rent terpene profiles can be used as an aid in selecting pines less susceptible to fusiform rust.

Selection of eucalypt genotypes resistant to the rust *Puccinia psidii* using monocyclic parameters. C.L. Marino[1]; K.C.Z. Ferreira[1]; E.L. Furtado[2], C.A. Bonine[3] and D.D. Costa[3]. *[1]Depto. de Genética/IB/UNESP, Botucatu 186018-970-SP, Brazil, [2]Depto. Produção Vegetal/FCA/UNESP, Botucatu 18603-970 SP. Brazil.CNPq, [3]Votorantin Celulose e Papel VCP Jacareí 14210-000 SP. Brasil.*
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Genetic variability and physiological factors are successful for disease resistance in the genus *Eucalyptus*. This is especially true for resistance to eucalypt rust (*Puccinia psidii* Wint.). A first control measure is to prevent the commercial planting of susceptible plants. The search for quantitative resistance traits can lead to superior genotypes with better durability. This study aimed at identifying parameters for evaluating monocyclic rust in eucalypt plantations, most closely related to a major resistance gene and to establish genotypes ranking for better resistance to corrosion in a breeding program. Host responses to disease were also evaluated. A comparative assessment of rust development in the field was therefore carried out, with the support of a qualitative disease severity scale, with quantitative monocyclic parameters in a controlled environment. The prevalence of susceptible individuals suggested distortions of segregation in the crosses, in agreement with previous studies. The results showed, therefore, that the resistance gene in eucalypt population depends on the action of others genes, that have been indirectly related to resistance and to defense metabolic pathways.

Impact of mycorrhiza formation on the infection of poplar with rust fungi. C. Pfabel[1], C. Baum[1] and C.Struck[2]. *[1]Institute for Land Use, Faculty for Agricultural and Environmental Sciences, Justus-von-Liebig-Weg 6, 18051 Rostock, German, [2]Institute for Land Use, Crop Health, Faculty of Agricultural and Environmental Sciences, Satower Str. 48, 18059 Rostock, Germany.*
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Poplar clones (*Populus* spp.) can host simultaneously mycorrhizal and rust fungi. Interactions between mycorrhizal and rust fungi sharing the same host plants seem to be probable since both consume assimilates. Mycorrhizal fungi can promote the assimilate production of their host plants directly by an improved nutrient supply and indirectly by leading to an increased photosynthetic activity of their hosts. Therefore, they could increase the attractivity but also the tolerance of poplar clones to rust attacks. Alternatively, they could decrease the attractivity of their hosts by leading to increased concentrations of secondary metabolites, which might contribute to increased resistance of their host plants against rust attacks. *Melampsora larici-populina* is one of the most important rust fungi of *Populus* species from the sections Aigeiros (e.g. *P. deltoides*) and Tacamahaca (e.g. *P. trichocarpa*) as well as their hybrids. In the present study, one of this hybrid clones (*Populus trichocarpa* × *deltoides cv*. Beaupré) was used as model plant to investigate the response of single and simultaneous colonisation of this clone by an ectomycorrhizal fungus (*Hebeloma* spp.) and a *Melampsora larici-populina* strain 98AG31 to plant growth, production of secondary metabolites and intensity of rust attacks. Possible response mechanisms in simultaneously colonised plants were defined and their consequences were discussed.

Linking nucleotide polymorphisms in candidate genes and quantitative resistance to *Melampsora larici-populina* Kleb. in *Populus nigra* L. V. Jorge[1], A. Dowkiw[1], M.Villar[1], I. Paolucci[2], F. Bitton[2], P. Faivre-Rampant[2] and C. Bastien[1]. *[1]INRA, UAGPF, 2163 avenue de la Pomme de Pin, BP20619 ARDON, 45166 Olivet Cedex, France. [2]INRA-URGV, 2 rue Gaston Crémieux, CP5708, 91057 Evry Cedex, France. [3]INRA UMR IAM, 54280 Champenoux, France. [4]INRA UMR BIOGECO, 69 route d'Arcachon, 33612 CESTAS Cedex, France.*
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Association studies aim at linking phenotypic variation with allelic variation in candidate genes and benefit from several generations of recombination in natural populations to identify causative polymorphisms. Such association studies are under development on *Populus nigra* L., with emphasis on nucleotide polymorphism associated to quantitative resistance to *Melampsora larici-populina* Kleb (*Mlp*), the causal agent of leaf rust. *Populus nigra*, the natural host of *Mlp*, is widely represented in European riparian ecosystems, where the occurrence and impact of the fungus are highly variable. Geographical patterns of nucleotide diversity in genes involved in the host-pathogen interaction is expected from local co-evolution. As a first step, Single Nucleotide Polymorphisms (SNPs) have been identified in a discovery panel of twenty-one *P. nigra* genotypes covering French natural range. Sequencing was done on both functional and positional candidate resistance genes (closeness to mapped QTLs and resistance genes). An automated procedure for primer design allowed us to obtain successful amplification in *P. nigra* for 90% of gene fragments. Average nucleotide variation in *P. nigra* is high (1 SNP/97 pb). A collection of more than 1100 *P. nigra* genotypes collected in different natural

populations mainly in France but also in Germany, Netherlands, Italy and Spain was evaluated for rust resistance under natural inoculation in a clonal test in France and under artificial inoculations with different strains in leaf-disk bioassays. Even if most of the genetic variation for rust resistant could be observed at the individual (genotype) level, significant differentiation between natural populations was detected. The high level of genetic diversity observed at both gene and phenotypic levels, the rapid linkage disequilibrium decay between SNP is offering a favourable situation to identify interesting resistance alleles and to avoid false positive associations.

Management and Control

The multiple weapons of the plurivorous rust hyperparasite *Cladosporium tenuissimum*. G. Assante and S. Moricca. *Dipartimento di Biotecnologie agrarie, Sezione di Protezione delle Piante, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy*.
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The rust hyperparasite *Cladosporium tenuissimum* is a cosmopolitan, plurivorous fungus that has a number of parasitization strategies. It is often misidentified and confused with more common contaminants or saprophytes such as *Cladosporium cladosporiodes* and *Cladosporium herbarum,* and this has led to an understimation of the role of *C. tenuissimum* in both natural and artificial forests, and in agricultural systems. Here we demonstrate that the fungus is morphologically clearly distinct from its congeneric species, and that it possesses multiple aggression mechanisms, which make it a promising biocontrol agent of many rust fungi. It exerts a mechanical force, with which it displaces spore ornamentations, disintegrates the fungal cell wall, and penetrates the rust spores and empties them of their contents. Besides having a mechanical force, *C. tenuissimum* also possesses enzymatic activity, since it is endowed with a biochemical arsenal consisting of metabolites with antifungal properties. These antifungal compounds – the cladosporols – are a family of biologically active molecules that to varying degrees inhibit β-1,3-glucan synthetase, the enzyme involved in the synthetic pathway of β-1,3-glucan, a component of the fungal cell wall. Besides the cladosporols, which are essentially deputed to preserve the ecological niche of *C. tenuissimum,* the hyperparasite possesses other life-history traits that ensure its persistence in the environment. Its plurivorous nature enables it to survive even in the absence of its target hosts (which are a number of rust agents) and to establish itself on the plant matrices before the arrival of the rust propagules. The life cycle of *C. tenuissimum* appears to be synchronized with the life cycle of most rust fungi. Aerobiological investigations indicate that the hyperparasite reaches its peak in conidial production and dispersal in summer, precisely when the rust spores burst from the epidermis of their hosts.

***Cronartium ribicola* impacts in plantations of improved *Pinus monticola* in Northern Idaho.** J.W. Schwandt and H.S.J. Kearns. *USDA Forest Service, Forest Health Protection, Coeur d'Alene Field Office, 3815 Schreiber Way, Coeur d'Alene, Idaho, 83815, USA.*
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Since its introduction into western North America, *Cronartium ribicola* has spread throughout the range of nearly all native 5-needled pines. *C. ribicola* has played a major role in dramatically reducing the dominance of *Pinus monticola* on several million hectares and is primarily responsible for major changes in successional pathways throughout western North America. The current emphasis for blister rust control and management is to incorporate natural resistance mechanisms into regeneration. Over the past 50 years, more than 40,000 hectares of *P. monticola* plantations with improved levels of resistance have been established in the northern Rocky Mountains. A survey of 66 plantations found infection levels varied from 0% to 95% after 7-15 years, but improved stock always out-performed natural regeneration. Rust infection levels in permanent monitoring plots in 20 of these plantations increased from an average of 13.7% to 39.3% over an 11 year period. We are also finding some abnormal cankers related to canker expansion rates in plantations of improved stock. Annual monitoring of over 100 branch cankers during a five-year period found an average canker expansion rate of 4.8 cm per year. Cankers that were less than 15 cm from the stem had a 74% probability of reaching the bole within five years while cankers more than 50 cm from the bole had an 85% probability of dying before reaching the stem.