United States
Department of
Agriculture

Forest Service

Pacific Southwest
Research Station

Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry

Meeting Abstracts

July 31 – August 5, 2011, Eugene, Oregon, USA

The Forest Service of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives—as directed by Congress—to provide increasingly greater service to a growing Nation.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.

Disclaimer

Papers were provided by the authors in camera-ready form for printing. Authors are responsible for the content and accuracy. Opinions expressed may not necessarily reflect the position of the U.S. Department of Agriculture.

The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Pesticide Precautionary Statement

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate state or federal agencies, or both, before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry

July 30 – August 5, 2011
Eugene, Oregon, USA

Meeting Abstracts

Welcome

Welcome to the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry. Your participation is greatly appreciated. This meeting is intended to provide a forum for information sharing and thought-provoking discussions among researchers from various disciplines as well as policy makers, industry representatives, regulators, and land managers. Presentations this week will provide an overview of the most current scientific information available on forest insect and disease resistance for a range of forest pests worldwide.

Technical Committee

- Richard Sniezko, USDA Forest Service, Dorena Genetic Resource Center, USA
- Alvin Yanchuk, British Columbia Ministry of Forests and Range, Canada
- Dan Herms, Ohio State University, USA
- Everett Hansen, Oregon State University, USA
- Jeff Stone, Oregon State University, USA
- Anna Schoettle, USDA Forest Service, Rocky Mountain Research Station, USA
- Susan Frankel, USDA Forest Service, Pacific Southwest Research Station, USA
- Dana Nelson, USDA Forest Service, Southern Research Station, USA
- Fred Hebard, The American Chestnut Foundation, USA
- Acelino Couto Alfenas, Universidade Federal de Viçosa, Brazil
- Arnaud Dowkiw, French National Institute for Agricultural Research (INRA Orléans), France
- Luis Sampedro, Centro de Investigación Forestal de Lourizán at Galicia, Spain
- Alberto Santini, Istituto per le Protezione delle Piante—National Research Council (CNR), Italy
- Charles G. “Terry” Shaw, USDA Forest Service, Western Wildland Environmental Threat Assessment Center, USA
- Dan Quiring, University of New Brunswick, Canada
- Fred Hain, North Carolina State University, USA
- Brian Stanton, GreenWood Resources, Inc., USA
- Bohun B. Kinloch, USDA Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics (retired), USA
- Fikret Isik, Research Associate Professor of Quantitative Genetics, NCSU Cooperative Tree Improvement Program, North Carolina State University, Raleigh, North Carolina, USA

Local Organizing Committee

- Richard Sniezko, USDA Forest Service, Dorena Genetic Resource Center, Oregon
    - Workshop Chair
- Katie Palmieri, University of California, Berkeley, California Oak Mortality Task Force
- Janice Alexander, University of California, Cooperative Extension, Marin County, California Oak Mortality Task Force
- Susan Frankel, USDA Forest Service, Pacific Southwest Research Station, California
- Angelia Kegley, USDA Forest Service, Dorena Genetic Resource Center, Oregon
- Sunny Lucas USDA Forest Service, Dorena Genetic Resource Center, Oregon
- Michael Crawford, USDI Bureau of Land Management, Oregon
- Larry Johnston, USDI Bureau of Land Management, Oregon

Conference Sponsors

IUFRO Working Parties: 7.03.11 (Resistance to Insects) and 2.02.15 (Breeding and Genetic Resources of Five-Needle Pines)

USDA Forest Service:
- Western Wildland Environmental Threat Assessment Center (WWETAC)
- Eastern Wildland Environmental Threat Assessment Center (EWETAC)
- Pacific Northwest Region (Forest Health Protection & Genetic Resource Programs)
- Pacific Southwest Research Station (PSW)

King Estate Winery

University of California Cooperative Extension

California Oak Mortality Task Force

Futuragene

Starker Forests

Program Overview

Sunday, July 31

Early registration at Valley River Inn
Welcome Reception

Monday, August 1

Registration
Symposium/Indoor Presentations
Evening 2-Hour Statistics Workshop

Tuesday, August 2

Registration
Symposium/Indoor Presentations
Evening Poster Session and Reception

Wednesday, August 3

Field Trip

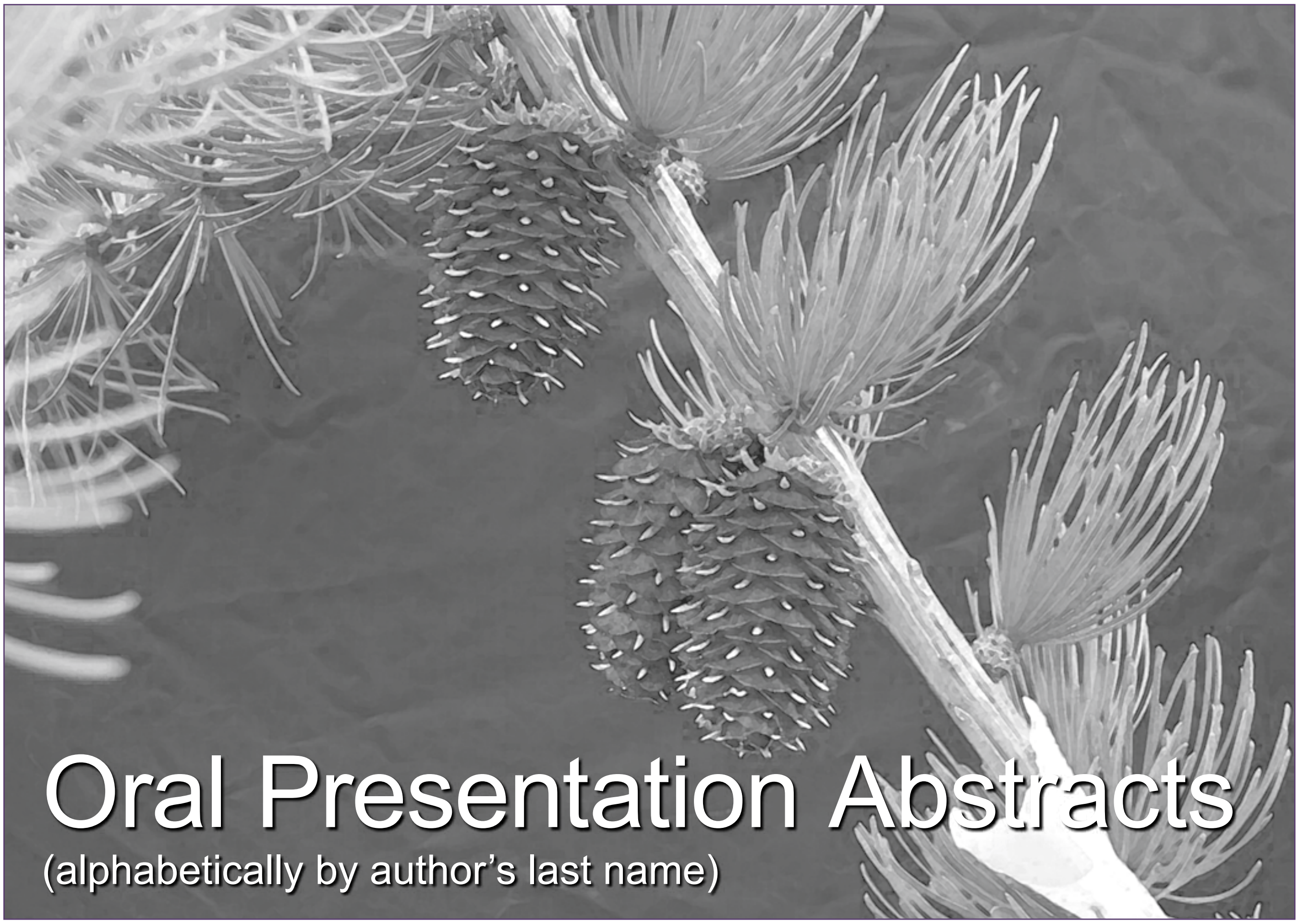
- Dorena Genetic Resource Center
- Plum Creek Timber Company
- Bureau of Land Management, Tyrrell Orchard Complex
- King Estate Winery, Reception

Thursday, August 4

Symposium/Indoor Presentations

Friday, August 5

Symposium/Indoor Presentations
Meeting Adjourned



Oral Presentation Abstracts

(alphabetically by author's last name)

An Overview of Ecological and Evolutionary Research on Disease in Natural Systems

Helen M. Alexander, Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Ave., Lawrence, KS 66045; halexander@ku.edu

Plant diseases have obvious and serious ramifications for agriculture and forestry; as a consequence, most research on plant pathogens has logically focused on economically important diseases. However, pathogens are associated with nearly all plant species. In recent decades, there has been increasing interest in the role of pathogens interacting with a diverse range of natural plant populations, communities, and ecosystems. Truly "natural" plant populations of course no longer exist; human activity has influenced all corners of the world. One can consider, however, characteristics of more natural systems in contrast to row crops of western agriculture or plantation forestry. For example, one can define natural systems as those in which plant species are genetically diverse and occur in multispecies communities with often complex spatial patterns. Further, in contrast to many human-managed plant populations, natural systems have linkage between generations: hence, the seeds produced by plants at a particular site are the likely source of future plant generations.

Research on disease in natural systems has a long history; for example, there has always been interest in searching for disease resistance genes in wild relatives of crop plants or considering wild plants as reservoirs for economically important pathogens. However, most researchers associated with ecology and evolutionary biology disciplines have historically ignored host-pathogen interactions. Starting in the 1980's, this situation began to change and there are now rigorous research programs on host-pathogen interactions in diverse natural systems. Researchers, for example, have examined the factors determining spatial patterns of disease and the effect of disease on plant population growth. Others study patterns and roles of variation in plant resistance and pathogen virulence at spatial scales ranging from populations to continents. Community ecologists have addressed the importance of disease in altering the composition of communities. In particular, there has been interest in understanding the degree to which disease affects the spread of exotic plants. There has also been increasing focus on pathogens that are not easily observed, such as work on viruses in natural systems or studies exploring the complex interactions between the soil biotic community (both pathogens and mutualists) and plant communities. Researchers have also focused on how variables such as temperature, CO_2 levels, and nutrient levels may alter the prevalence of pathogens and be important in ecosystem properties and functions.

Clearly, much research on diseases of trees fits within the above broad definition of natural systems and thus there should be strong connections with forest plant pathology and the above research programs, many of which focus on herbaceous plants.

Genetic Basis of Resistance in *Eucalyptus* spp. Pathosystems

Acelino Couto Alfenas and **Lúcio Mauro da Silva Guimarães**, Departamento de Fitopatologia/BIOAGRO, Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, MG, Brazil; aalfenas@ufv.br; and **Marcos Deon Vilela d Resende**, Embrapa Florestas, Colombo, PR, Brazil

Eucalyptus is the most widely planted hardwood crop in worldwide tropical and subtropical regions because of its high growth rate, coppicing capacity, broad adaptability and multipurpose wood properties. Until the 1970s, the *Eucalyptus* plantations in Brazil were practically disease-free. However, plantations have continued to expand into warmer and more humid areas that are more favorable to pathogen infection. Furthermore, the use of high-yielding genotypes with unknown disease resistance, the implementation of clonal forestry, and the introduction of new management techniques have favored the emergence of disease epidemics in recent decades. Presently, prominent diseases such as rust (*Puccinia psidii*), ceratocystis wilt (*Ceratocystis fimbriata*), and canker (*Chrysoporthe cubensis*) are the most damaging diseases in *Eucalyptus* plantations. During recent years, we have conducted a series of inoculation experiments with controlled-crossed progenies to better understand the genetic basis of resistance for the prominent *Eucalyptus* pathosystems.

Resistance to rust occurs naturally in many *Eucalyptus* species and it is predominantly expressed by a hypersensitive reaction (HR). In 2003, we identified and mapped the first rust-resistance major locus (*Pp*r-1) in a controlled-pollinated family of *E. grandis*. The locus *Pp*r-1 was positioned on the reference genetic map for *Eucalyptus* on the linkage group 3. Additionally, its position was validated by association genetics in one related and two unrelated background families. These results are consistent with the hypothesis that *Pp*r-1 controls a large proportion of the variation for rust resistance in *E. grandis*. In recent studies, analyses of several inter-specific families, displaying different segregation patterns for rust resistance, provided additional evidence that the genetic control of rust resistance in *Eucalyptus* is more complex and includes quantitative resistance traits.

In other disease screening tests, estimates of resistance heritability to ceratocystis wilt were based on data from 10 inoculation experiments with hybrid families derived from crosses of *E. grandis* × *E. urophylla* genotypes. The statistical analyses indicated high genetic variability for resistance to ceratocystis wilt in both species. Estimates of individual narrow (50%) and broad (59%) sense heritability suggest a high degree of additive genetic control and low allelic dominance influencing the trait. High genetic variation among and within families appeared associated with contributions to high heritability and genetic gains for resistance to ceratocystis wilt.

For the *C. cubensis-eucalyptus* pathosystem, we evaluated resistance in *E. grandis* and *E. urophylla* parents, as well as in individuals from their crossed progenies. Six-month-old plants were inoculated, and xylem and bark lesions were measured at 8 months post-inoculation. The results demonstrated that xylem lesions should be used for selection of resistant clones. The phenotypic analyses indicated the existence of a high genetic variability for resistance in both species. Individual narrow and broad sense heritability estimates were 17% and 81%, respectively, suggesting that canker resistance is a quantitative trait and highly dependent on dominance and epistasis.

Studies have also been conducted to understand the genetic structure of the pathogen populations to provide a controlled basis for selecting resistant genotypes.

Selection of Loblolly Pine Varieties Resistant to Fusiform Rust for Commercial Deployment

Andy Benowicz, CellFor, 4-6772 Oldfield Road, Victoria, BC, V8M 2A3; abenowicz@cellfor.com; and **Robert J. Weir**, CellFor, Portland, Maine

Commercial production of loblolly pine (*Pinus taeda* L) varieties through somatic embryogenesis has been increasing significantly over the last several years. Large-scale operational plantations have been established since 2004 across the Southeastern United States while the oldest field tests are now at mid-rotation. Extensive surveys of fusiform rust (*Cronartium quercuum f.sp. fusiforme)* infection rates in over 80 operational varietal plantations and demonstration plots were conducted in eight Southeastern US states. The surveyed stands were three- to nine-year old. The operational data confirm that the selection program for rust resistance in loblolly pine varieties has been very successful. Genotypes characterized by fast growth rate and tentative resistance to fusiform rust are identified at young age in several field test sites. Cryogenic tissue from the selected genotypes is used to produce seedlings for tests involving artificial inoculations. Artificial inoculation tests of CellFor-produced loblolly pine clones have been performed by the USDA Resistance Screening Center in Asheville, NC, over the last eight years. A number of loblolly pine clones of Atlantic Coastal Plains and Western Gulf origin were exposed to high concentrations of fusiform rust inocula. The inocula were developed from aeciospores collected in three regions representing the eastern, central and western distribution range of loblolly pine. The inocula from the three regions were used in separate tests. Commercial varieties ranked as resistant or very resistant based on field data and artificial inoculation tests show less than 0.5% stem infection rates based all 56 surveyed operational plantations and demonstration plots they were present in.

Rapid Evolution of Tree Pathogens via Episodic Selection and Horizontal Gene Transfer with Special Reference to Dutch Elm Disease

Clive Brasier, Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK; clive.brasier@forestry.gsi.gov.uk

Episodic selection is defined as a “Sudden ecological disturbance likely to lead to a significant alteration in a species’ population structure”. For tree pathogens, such disturbance conditions include sudden exposure to crop monoculture or to a new fungicide, sudden access to a new host or vector, sudden climate change, and transposition to a new biogeographic zone.

Transposition of a fungal pathogen to a new biogeographic zone is a major episodic selection event for the pathogen, resulting in sudden release from many previous selection constraints (such as host resistance and natural enemies) and exposure to a wide range of new constraints. The latter may include novel contact with closely related organisms. This in turn can bring a potential for rapid evolution, via interspecific hybridization (or horizontal gene transfer), between the introduced and the resident organisms. Today, introduction of pathogens into new biogeographic zones by man is increasingly frequent. It is resulting in rapid emergence of genetically modified tree pathogens and even in new pathogen species. This process therefore presents a threat to products of tree resistance breeding programs. The ecological and genetic conditions needed for pathogen hybridization and hybrid survival will be outlined.

Examples of newly emerging hybrid tree pathogens will be presented with special reference to the interaction between the introduced Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. With these pathogens, horizontal gene transfer is occurring at two different levels: selective transfer of major genes from *O. ulmi* to *O. novo-ulmi*; and full hybridization between the two subspecies of *O. novo-ulmi*. Without horizontal gene transfer the current Dutch elm disease pandemic, arguably the most destructive tree disease event in the northern hemisphere to date, might never have occurred.

The Impact of Life-History Features on Host-Pathogen Co-Evolutionary Dynamics

Jeremy J. Burdon, **Peter H. Thrall**, and **Adnane Nemri**, CSIRO Plant Industry,
Canberra ACT, Australia; Jeremy.Burdon@csiro.au

The ecological and co-evolutionary dynamics of host-pathogen systems are greatly affected by spatial scale and time, particularly with regard to how spatio-temporal heterogeneity affects the likelihood and predictability of encounters between pathogens and their hosts. Much of current thinking regarding the dynamics of wild host-pathogen interactions has focused on host species that are dispersed in small, somewhat isolated and often ephemeral populations. These demes are distributed across heterogeneous landscapes where local selective pressures over the whole metapopulation or for any local population can differ over space and time. Isolation and interaction of specific host and pathogen life-history features emphasize the importance of genetic drift, migration, extinction and recolonization in these situations. As a consequence, the evolutionary trajectory of the whole metapopulation may be quite distinct from that of its individual component demes. Strong evidence for local adaptation in a number of associations provides a clear signature of such evolutionary outcomes. While many annual and perennial herbaceous species comfortably fit into this model, they lay at one end of a continuum of spatial distribution, at the other end of which are many long-lived tree species that show more or less continuous distribution over large geographic scales. How relevant then are concepts developed in a traditional metapopulation context to an understanding of the dynamics of host–pathogen interactions in apparently more predictable continuous systems? Here we consider how variation in life-history attributes between different hosts, and different pathogens can generate discontinuities that lead to qualitatively different evolutionary trajectories for different host-pathogen associations that superficially may appear quite similar.

White Pine Blister Rust Resistance Research in Minnesota and Wisconsin

Andrew David, Department of Forest Resources, University of Minnesota North Central Research and Outreach Center, 1861 Highway 169 East, Grand Rapids, Minnesota 55744; adavid@umn.edu; **Paul Berrang**, USDA Forest Service, 626 E. Wisconsin Avenue, Milwaukee, Wisconsin 53202; and **Carrie Pike**, Department of Forest Resources, University of Minnesota, Cloquet Forestry Center, 175 University Avenue, Cloquet, Minnesota 55720

Eastern white pine is the only five-needled pine species in the eastern North American forest. Relatively fast growing, long-lived, and adaptable to a wide variety of site conditions it provides great economic and ecological value where it occurs on the landscape. However, in the upper Great Lakes region, the combination of overharvesting in the late 1800s and the emergence of white pine blister rust in the early 1900s decimated the species. In Minnesota and Wisconsin research to increase blister rust resistance by Riker, Patton, and Ahlgren paralleled efforts made by other researchers in western North America - selection of putative resistant individuals followed by field screening and breeding. The research presented here chronicles some of this earliest research stressing the importance of this material to today's breeding and gene conservation efforts while describing the results of more modern techniques such as electron microscopy and green house screening to identify mechanisms of resistance and validate field trials. The combined results suggest that, due to the lack of a major gene for resistance in eastern white pine, breeding for the coexistence of host and disease may be preferable to the goal of a completely resistant eastern white pine seedling.

Identification of Pathogen Avirulence Genes in the Fusiform Rust Pathosystem

John M. Davis[1,2], **Katherine E. Smith**[2,3], **Amanda Pendleton**[2], **Jason A. Smith**[1], and **C. Dana Nelson**[3]

[1] School of Forest Resources and Conservation, [2] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611; jmdavis@ufl.edu; [3] Southern Institute of Forest Genetics, U.S. Forest Service, Saucier, MS 39574

The *Cronartium quercuum* f.sp. *fusiforme* (*Cqf*) whole genome sequencing project will enable identification of avirulence genes in the most devastating pine fungal pathogen in the southeastern USA. Amerson and colleagues (manuscript in prep) have mapped 9 fusiform rust resistance genes in loblolly pine, suggesting that at least 9 corresponding avirulence genes likely exist in the fungus. Identification of these avirulence genes would greatly facilitate selection of resistant pine genotypes for deployment to forest plantations. Based on work in other rusts, we anticipate avirulence genes may encode secreted effector proteins that interact directly or indirectly with host resistance proteins. As a step toward testing this hypothesis, we genetically mapped the *Cqf* avirulence gene *Avr1*, which specifically interacts with the *Fr1* resistance gene, with the goal of integrating this map with the *Cqf* genome sequence assembly so that *Avr1* can be identified. Once *Avr1* is identified, we want to determine its allele frequency across geographically defined hazard maps for fusiform rust, to help guide genotype deployment by growers. We view identification of *Avr1* as our initial case study, guiding our strategy to efficiently identify additional avirulence genes in *Cqf* that in turn can be used to guide plantation deployment of all genotypes currently represented in southern pine breeding programs.

Breeding Poplars with Durable Resistance to *Melampsora larici-populina* Leaf Rust: A Multidisciplinary Approach to Understand and Delay Pathogen Adaptation

Arnaud Dowkiw, **Catherine Bastien**, **Véronique Jorge**, **Marc Villar**, **Elodie Voisin**, and **Vanina Guérin**, INRA, UAGPF, 2163 avenue de la Pomme de Pin, BP20619 ARDON, 45166 Olivet Cedex, France; arnaud.dowkiw@orleans.inra.fr; **Patricia Faivre-Rampant**, **Aloïs Bresson**, and **Frédérique Bitton**, INRA-URGV, 2 rue Gaston Crémieux, CP5708, 91057 Evry Cedex, France; **Sébastien Duplessis**, **Pascal Frey**, **Annegret Kohler**, **Cécile Rinaldi**, and **Bénédicte Fabre**, INRA UMR IAM, 54280 Champenoux, France; and **Christophe Plomion** and **Céline Lalanne**, INRA UMR BIOGECO, 69 route d'Arcachon, 33612 CESTAS Cedex, France

During the last decades, European poplar breeders learned the hard way that *Melampsora larici-populina* has an impressive adaptive potential. This pathogen defeats qualitative (*i.e.* complete) resistances in less time than needed to grow a poplar tree. After several resistances inherited from *Populus deltoides* had been overcome, breeding for quantitative resistance (QR) was considered a more durable strategy.

At least three recent results raise doubts on this optimistic point of view. First, elucidating the genetic determinism of quantitative resistance in *P. detloides x P. trichocarpa* hybrid progenies did not reveal as complex a genetic determinism as expected. Loci with major effects on one or several QR components were identified, such as R_{US} inherited from *P. trichocarpa.* Second, quantitative and qualitative resistances did not appear to be completely independent. Most identified defeated qualitative resistances inherited from *P. deltoides* (e.g. R_1), had statistical effects on QR. Third, strains of the pathogen able to defeat the R_{US}-mediated QR have been identified, possibly due to pre-adaptation in the pathogen's populations.

Being both "easy" to map and defeated, R_{US} is used as a model locus to understand host-pathogen quantitative interactions. Fine mapping of R_{US} is being conducted using a 1,410 genotype F_1 progeny, and a BAC library was constructed to generate a physical map of 2,000 kb around R_{US}. The first results support the idea that qualitative and quantitative resistances share not only functional but also structural similarities.

Urged by poplar growers to deliver new cultivars, breeders explore multiple (possibly combinable) very pragmatic strategies to delay pathogen adaptation. Sources of resistance are being diversified. In particular, the genetic variability available in the co-evolved European species *P. nigra* is being explored more attentively. A collection of 1,100 *P. nigra* genotypes collected in different European populations has recently been screened for rust resistance. The available variability for additional traits like avoidance and tolerance is also being tested in fungicide-treated *vs.* untreated experiments while new deployment strategies, such as using clonal mixtures, are also evaluated.

The New Zealand Douglas-fir Breeding Program: Proposed Adjustments for a Changing Climate

Heidi Dungey, **Charlie Low**, **Mark Miller**, **Kane Fleet**, and **Alvin Yanchuk**, Scion, Te Papa Tipu Innovation Park, 49 Sala Street, Private Bag 3020, Rotorua, 3046, New Zealand; Heidi.Dungey@scionresearch.com

Genetic improvement of Douglas-fir in New Zealand was initiated in 1955 with large provenance trials established in the late 1950s. These trials showed that material of Oregon and Californian origin was growing faster than other provenances. Further collections were made to further evaluate provenance performance from these two areas, and in 1996 additional trials were established at four low-altitude sites across New Zealand.

Genotype ×environment (GxE) interaction between these sites was found to be important for diameter at breast height (DBH), less important for stem straightness and malformation, and not important for outerwood acoustic velocity (a surrogate for wood stiffness). Heritabilities were low to moderate for all growth traits, and very low for malformation. Heritability for needle cast due to Swiss needle cast, measured as needle retention, was moderate at one site (0.37) with a high infection rate, and was likely a major factor creating genotype x environment interactions for growth among sites. The heritability of wood acoustic velocity was moderate to high at individual sites (0.26-0.74) and across sites (0.49). Individual-trait selection revealed the potential for good genetic gains to be made when selecting the top 20 families for diameter growth (an average of 10.7%), straightness (an average of 11.5%), and acoustic velocity (an average of 7.0%).

When we examined how gains were distributed when selecting for needle retention and/or DBH, we found that selecting for needle cast at the affected site did not compromise DBH gains at that site. Selecting for genotypes with low needle cast at the affected site did, however, effect gains for DBH estimated across all sites and would not be an ideal scenario. In order to maximize gains across the current Douglas-fir growing estate, a division of growing sites between those affected by needle cast and those not affected would seem sensible, particularly given recent work into climate change scenarios indicating that Swiss needle cast will become more important in the South Island and even more destructive in the North Island of New Zealand. We therefore suggest addressing differences in site through the development of separate deployment populations.

Plant Compensatory Growth in Aspen Seedlings: The Role of Frequency and Intensity of Herbivory and Resource Availability

Nadir Erbilgin, University of Alberta, Department of Renewable Resources, 4-42 ESB, Edmonton, Alberta, T6G2E3, Canada; erbilgin@ualberta.ca; **David A. Galvez**, University of Alberta, Edmonton, Alberta, Canada; and **Bin Zhang**, Southwest University, Chongqing, P.R. China

Plant ecologists have debated the mechanisms used by plants to cope with the impact of herbivore damage for more than a century. During this time plant resistance mechanisms, which reduce the amount of herbivore damage before and during herbivory, have received most of the attention, while plant tolerance mechanisms, which may minimize the impacts of damage after herbivory, have been less studied. The aim of this presentation is to bring the topic of plant compensatory responses, especially compensatory growth, to the front of research in plant ecology and plant-herbivore interactions. We conducted a greenhouse experiment to evaluate how carbon sink-source relationships and compensatory plant growth operate under different intensities and frequencies of simulated defoliation of aspen seedlings, with or without N enriched media. We found large variations in plant responses, ranging from undercompensatory to overcompensatory growth depending on the resource availability and defoliation intensity and frequency. We developed a new predictive model, the Frequency and Intensity of Herbivory and Resource Availability (FIRA), based on carbon sink-source relationships. Our model incorporates the interactions between frequency and intensity of herbivory and resource availability as modulators of plant compensatory responses. We concluded our work discussing the results of this and earlier studies in the context of the FIRA model and elaborated the intricate relationship between resource availability and compensatory growth following herbivory.

Resistance to *Phytophthora cinnamomi* in the Genus *Abies*

John Frampton and **Fikret Isik**, Department of Forestry and Environmental Resources, N.C. State University, Raleigh, NC 27695; frampton@ncsu.edu; **Mike Benson**, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; and **Jaroslav Kobliha** and **Jan Stejskal**, Department. of Dendrology and Forest Tree Breeding, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Kamýcká 1176, 165 21 Praha 6 – Suchdol, Czech Republic

A major limiting factor for the culture of true firs as Christmas trees is their susceptibility to water molds of the *Phytophthora* genus. In North Carolina alone, the Fraser fir (*Abies fraseri* [Pursh] Poir.) Christmas tree industry loses $6-7 million annually to root rot primarily caused by *Phytophthora cinnamomi* Rands. Because no resistance has been found in Fraser fir, in 2003, the NCSU Christmas Tree Genetics (CTG) Program conducted an extensive resistance screening of 32 *Abies* species (52 unique taxa) from around the world. Species were classified into resistant (2), intermediate (9), and susceptible (21) groups based on mortality 16 weeks after inoculation. All North American *Abies* species were classified as susceptible with the exception of white fir (*A. concolor* (Gord. & Glend.) Lindl. *ex* Hildebr.), which was classified as the most susceptible intermediate species. Species classified as resistant and intermediate are native to Eastern Europe and Central Asia plus Japan (*A. firma* Sieb. et Zucc.).

In the species screening trial, Turkish fir (*A. bornmuelleriana* Mattf.) and closely related Trojan fir (*A. equi-trojani* Coode et Cullen) ranked third and tenth for resistance, but mortality in these species was relatively high. A systematic approach to understand and better use *Phytophthora* resistance within Turkish and Trojan fir was undertaken. Using seeds from a 2005 cone collection expedition to Turkey, greenhouse-grown seedlings from 105 open-pollinated families were inoculated with *P. cinnamomi*. Sixteen weeks after inoculation, overall seedling mortality was 56% for Trojan fir and 35% for Turkish fir. As a comparison, 97% of inoculated Fraser fir seedlings but only 3% of inoculated momi fir seedlings died. For Turkish and Trojan fir, there was a distinct relationship between mortality and geographic origin; mortality percentage decreased from west to east. Additionally, estimates of family mean heritabilities were extremely high for both Turkish (0.96+0.010) and Trojan (0.97+0.011) fir.

In another approach, hybrid firs are being developed through a collaborative effort between the NCSU CTG Program and researchers at the Czech University of Life Sciences (CULS) Prague. CULS researchers have utilized Toros fir (*Abies cilicica* Carr.) from southern Turkey and Greek fir (*A. cephalonica* Loud.) in a long-term hybrid breeding effort aimed at developing a faster growing fir that is hardier to changing ecological conditions than the native European silver fir (*A. alba* Mill.). Toros and Greek fir were ranked fourth and eighth, respectively, in the species screening trial. Seedlings of F1, F2, and complex hybrids with Fraser fir are being assessed for *Phytophthora* resistance. Preliminary data from these studies will also be presented.

Host Preference of Vector Beetle, Host Resistance, and Expanding Pattern of Japanese Oak Wilt in a Stand

Kazuyoshi Futai, **Hiroaki Kiku**, **Hong-ye Qi**, and **Hagus Tarno**, Laboratory of Terrestrial Microbial Ecology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, 606-8502, Kyoto, Japan; futai@kais.kyoto-u.ac.jp; and **Yuko Takeuchi** and **Michimasa Yamasaki**, Laboratory of Forest Biology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, 606-8502, Kyoto, Japan

Japanese Oak Wilt is caused by a fungus, *Raffaelea quercivora,* and is spread by the vector beetle, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Since the early 1980s, this epidemic disease has spread inland of Honshu Island from coastal areas along the Sea of Japan to the interior, and reached Kyoto city area in 2004, and then has killed an enormous number of oak trees in urban fringe mountains, gardens, and parks.

To develop effective control methods, we studied the spreading pattern of this disease at a nearby low elevation mountain (109 m, 35°01′N, 135°47′E) in Kyoto city. The experimental site settled at the mountain in spring of 2009 is 1.36 ha and involved 288 living oak trees (*Quercus serrata* Murray) with DBH ranging from 8.9 to 91.6 cm. Based on the quality of frass produced from beetle tunnel, the density of beetle attacks (= number of entrance holes per given area of trunk surface), exudates from the trunks, and symptoms (alive or killed, and if killed which year it was killed), we classified these trees into seven categories. Survival rates of trees after the beetle attack in 2010 was higher in the trees that had survived in preceding year attack and then were attacked again in 2010 (Category II) than that of the trees newly attacked in 2010 (Category V, VI). We also examined the spatial relationships between two categories of trees using ωindex of m-m* method (Iwao, 1977). A group of trees that had survived beetle attack in 2009 and escaped from the attack of 2010 (Category III) showed exclusive distribution to the group of trees that were newly attacked in 2010 (Category V, VI). On the other hand, the distribution of another group of trees that had survived in preceding year attack and then were attacked again in 2010 (Category II) overlapped the trees that were newly attacked in 2010.

Thus, the trees of Category II and III seem to influence the host finding behavior of *P. quercivorus*, and thereby enhance and exclude new attacks in their surrounding area, respectively. To confirm this we examined the density of beetle attack in relation to the distance from the trees of Category III, and found that the density of beetle attack was lower within a 5 m radius than that of the outer the area, suggesting the lower flight frequency in the vicinity of the Category III trees. The trees that exuded sap were mainly found among the trees that survived the beetle attack in 2009, such as those in Category I and III.

From these findings we hypothesize that there are some differences in resistance against the attack of *P. quercivorus* beetles among oak trees in a stand, and the resistant trees may survive the first attack of the beetle and then release some repellent from the trunk and thereby escape the following attack.

Needle Terpenoid Composition of *Pinus halepensis* (Mill.) Trees Infested by Scale Insect *Marchalina hellenica* (Genn.) in Southern Attica Region, Greece

Athanasios Gallis, Ministry of Education LLL and Religious Affairs/Public Institute for Vocational Education and Training, Terma Leonardopoulou, Kilkis, GR-61100, Central Macedonia region, Greece; tgallis@hotmail.com; **Carlos Arrabal**, Departamento de Ingenieria Forestal, ETSI Montes, Universidad Polytechnica de Madrid, Cuidad Universitaria, Madrid, Spain; **Aristotelis Papageorgiou**, Department of Forestry, Environment and Natural Resources, Democritus University of Thrace, N. Orestiada, Greece; and **Maria C. Garcia-Vallejo**, Departamento de Industrias Forestales, INIA-CIFOR, Apartado 8111, 28080, Madrid, Spain

Needle terpenoid composition was determined by using GLC-MS in *Pinus halepensis* (*Mill*.) trees infested as well as not infested by scale insect *Marchalina hellenica* (*Genn*.). *M. hellenica* is an important honeydew producing scale insect which attacks most pine species. It is spread all over the eastern area of the Mediterranean Sea and it is widely distributed in most Aleppo pine forests in Greece. The study area is the Forest National Park of the Cape Sounion, southern Attica region, Greece. The vegetation of the Park is typical Meditterenean and characterized by *Pinus halepensis* (Mill.) forests, which dominate the vegetation of the Park. Besides the Aleppo pine forests, the vegetation of the area comprises several dry brushwood species (phrygana) and some herbaceous species. During the middle of 1990s, *Marchalina hellenica* (*Genn*.) was introduced artificially into the pinewoods of Attica region, Greece in order to increase the total honey yield. The artificial infestations caused the loss of ecological balance in the ecosystems of Aleppo pine of the region. Nowadays, field observations show the overpopulated occurrence of the insect in the pinewoods of the area.

All trees investigated were sampled from two Aleppo pine natural stands in the area of the National Park of Cape Sounion. A total of 22 trees (including 11 trees infected by *Marchalina hellenica* (*Genn.)* and 11 healthy ones, over 30 years old) were sampled in late March 2006. From each tree, one-year-old needles were collected, put in plastic bags, and stored in a refrigerator at -20^o C until analysis with GLC-MS. A total of 43 compounds were detected, including monoterpenes, sesquiterpenes, and neutral diterpenes, 32 of which were identified. The healthy trees showed higher mean concentration for the monoterpene fraction as well as for diterpene fraction than the mean concentration of infested ones, whereas the concentration for sesquiterpene fraction was more or less similar. The statistical analysis of terpene data showed the existence of quantitative differences between healthy and infested trees mainly for the components β-caryophyllene, neoabietal, α- humulene, cembrene, and neoabietol. A Ward cluster analysis based on selected major compounds classified all trees in two chemotypes with the majority of healthy trees belonging to one chemotype and most of the infested ones belonging to the other one.

Relative Rates of Infection by and Mortality due to White Pine Blister Rust in Genetically Improved and Unimproved Western White Pine and Sugar Pine: Results from Permanent Plots in Oregon and Washington

Ellen Goheen, USDA Forest Service, FHP, Southwest Oregon Forest Insect and Disease Service Center, 2606 Old Stage Road, Central Point, OR 97502; egoheen@fs.fed.us

Comparative trials in genetically improved and unimproved sugar pine (*Pinus lambertiana*) and western white pine (*P. monticola*) were established in Oregon and Washington between 1994 and 1997. The purpose of these plantings was to: 1) determine the level of infection and mortality for stock of differing levels of genetic resistance; 2) acquire a data set with which to develop mortality functions for western white pine and sugar pine as a function of site hazard (as expressed by rust index), and the level of genetic resistance; 3) determine whether similar levels of infection (as expressed by rust index) result in the same level of mortality in western white pine and sugar pine; and 4) provide a potential data set for calibrating the White Pine Blister Rust extension to the Forest Vegetation Simulator model for the Pacific Northwest Region. Based on availability and seed zone, three or four stock types from among four potential sources (Wild (non-resistant), SB (GCOP-resistant general combiner open pollinated), F1 (from a clonal orchard of resistant parents), and F2 (from second generation seed orchards)) were planted in 49 (7 × 7) or 64 tree (8 × 8) plots which were replicated four times at each site. Families within a plot were randomly distributed in the plot. Trees were planted on a 10 x10 foot spacing.

Ten- or 15-year remeasurement has been completed on plots. White pine blister rust infection was noted as early as three years post-planting on some sites. Infection and white pine blister rust-caused mortality levels vary widely between sites. In general, genetically improved stock exhibits lower levels of infection and mortality than unimproved stock when compared on a given site. Detailed analysis of the most up-to-date remeasurement data will be presented.

Screening Port-Orford Cedar for Resistance to *Phytophthora lateralis*

Everett Hansen, **Paul Reeser**, and **Wendy Sutton**, Botany and Plant Pathology, Oregon State University; hansene@science.oregonstate.edu; and **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon

Port-Orford cedar (POC) is an economically and ecologically valuable tree in the forests of southwest Oregon and northern California, and in the horticultural trade worldwide. *Phytophthora lateralis*, the aggressive, invasive cause of POC root disease, was introduced to the forest range of POC about 1950, and has since killed trees along roads and streams throughout the tree's range. The federal forest management agencies responded with an integrated disease management program designed to slow further spread of the pathogen in order to protect remaining POC, and restore the species with planted resistant stock in diseased areas. In this paper we describe and compare the efficacy of the various inoculation methods and screening tests we use to evaluate POC parents for resistance.

The first evidence of heritable resistance in POC to *P. lateralis* was published in 1989, and an active program of field selection and resistance screening soon followed. The USDA Forest Service and USDI BLM funded and soon led the effort to locate candidate resistant trees in the forest and to collect vegetative material and seeds for testing. Inoculations and resistance screening, carried out at Oregon State University, have evolved through time to take advantage of the unique features of POC, and to meet the changing needs of the program. Candidate parents from the field are initially challenged with a rapid stem dip zoospore inoculation test. "Winners" are then propagated as rooted cuttings, which are in turn exposed to zoospore inoculum in a root dip test. The best individuals are incorporated into containerized seed orchards at Dorena Genetic Resource Center in Cottage Grove, Oregon. Seedlings from cross or self pollination within the orchards are further challenged with the root dip test and by planting into infested soil for a two-year exposure in raised beds. Finally, the most promising materials are included in long-term field tests on infested sites and maintained in the seed orchards by breeding zone. Each step has its own advantages and disadvantages, and opportunities for improvement.

Stilbenes as Constitutive and Inducible Protection Compounds in Scots Pine (*Pinus sylvestris* L.)

Anni Harju and **Martti Venäläinen**, The Finnish Forest Research Institute, Punkaharju Unit, Finlandiantie 18, FI-58450 Punkaharju, Finland; anni.harju@metla.fi

Stilbenes are plant secondary metabolites involved in plant responses inducible by a great variety of biotic and abiotic hazards such as fungi, nematodes, herbivores, mechanical wounding, and UV radiation, for example. In Scots pine (*Pinus sylvestris* L.), stilbene pinosylvine (PS) and its methylated dervative (PSM) are also produced constitutively when sapwood turns into heartwood. Together with bark, heartwood forms a passive barrier to protect living parts of the tree stem. Due to its high content of extractives, Scots pine heartwood timber has earlier been used in constructions where high decay resistance is required. Today, after the era of super effective wood preservatives, interest in pine heartwood has been reincarnated.

The goal of our studies is to describe the natural variation in the concentration of constitutive heartwood extractives; to estimate the genetic parameters related to heartwood characteristics; to find out whether there is a genetic connection between constitutive and inducible production of stilbenes; together with technical experts to develop fast and reliable techniques to quantify stilbenes from wood samples; and to find out whether there are rapid ways to utilize the existing variation for forest management. We are also collaborating with molecular geneticists to find the markers for pine stilbene biosynthesis that could be used early in selection.

The study materials include three pedigrees of Scots pine: the 1st generation consists of grafted clones in seed orchards, their half-sib progenies growing in 40-year-old progeny trials are the 2nd generation, and the 3rd generation includes seedlings in a nursery. The first and the second generation provide heartwood samples for stilbene analysis and the third generation has been used for studies on mechanical induction of stilbenes. Moreover, after surveying a sample from a large natural stand for heartwood stilbenes (2010-2011), we will be ready to make experiments on seedlings and their biotic challengers.

We hypothesize that trees having strong inducible defense ability against biotic and abiotic stresses in their living tissues may also have high concentration of stilbenes in their heartwood when they mature. Such trees would be optimal to be cultivated as timber with natural stilbene impregnation. One vision with practical importance is the exploitation of the genetic variation in stilbene production for the improvement of Scots pine regeneration material.

For the evaluation of the possibilities and strategies to breed Scots pine trees to produce more durable heartwood and better resistance against plant pathogens and pests when young, we need to estimate the interaction between genotype and environment as well as the genetic correlation between quality and growth traits. For the moment we are analyzing material from two progeny tests and their mothers to be presented at the workshop.

Screening for Disease Resistance in *Dalbergia sissoo*, an Important Timber Species of Asia

N. S. K. Harsh, Forest Pathology Division, Forest Research Institute (Indian Council of Forestry Research & Education), P.O. New Forest, Dehradun-248006, India; nirmalharsh57@gmail.com

Dalbergia sissoo Roxb. is one of the most important timber trees in the neighboring countries of southeast Asia, including India, Nepal, Bhutan, Bangladesh, Myanmar, and Pakistan. It occurs naturally from sea level up to 1000 m sometimes even up to 1500 m, from the Kabul River in Afghanistan through northern Pakistan, northern India, Nepal, Bhutan, and Bangladesh, and is commonly planted throughout all of Pakistan, India, and the foothills of Nepal. Its timber provides important raw material for a variety of wood-based industries, especially furniture and building construction.

The species is susceptible to a number of diseases, the most important of which are vascular wilt disease caused by *Fusarium solani* f. sp. *dalbergiae*, root rot caused by *Ganoderma lucidum*, and leaf and petiole rust caused by *Maravalia achroa*. These diseases are causing total failure of plantations by mortality as well as slow growth and vigor, reduced volume production, deformed stems, reduced wood properties, and disease spread.

In the present study, 67 clones of *D. sissoo* have been evaluated against *Ganoderma lucidum* root rot in the field for five years to identify susceptible and resistant clones in a naturally occurring sick plot. Clones were ranked with scores attributed to growth form (height, girth, clear bole) and disease (percent disease/mortality).

Fifteen clones of *D. sissoo* were also assessed for resistance against rust disease by artificial inoculation with the urediniospores of *Maravalia achroa* and expression of disease afterwards. On the basis of disease scores, the clones were ranked as resistant or susceptible.

Selection and use of disease resistant plants will provide an economical and long-term measure for effective disease management in forestry. The findings can be applied directly using resistant planting material to reduce losses to these diseases in plantations, increasing yields of high-quality timber for this important species.

Developing a Disease Resistance Research Program for Tanoaks

Katherine J. Hayden, University of California, Berkeley, Environmental Science, Policy, and Management, 137 Mulford Hall, Berkeley, CA 94702; khayden@berkeley.edu; **Jessica W. Wright**, USDA Forest Service, Pacific Southwest Research Station, Davis, CA; and **Richard S. Dodd** and **Matteo Garbelotto**, University of California, Berkeley, Environmental Science, Policy, and Management, Berkeley, CA

The introduced pathogen *Phtyophthora ramorum* has had a devastating impact on populations of tanoak (*Notholithocarpus densiflorus*) in California and Oregon, where it causes the disease known as sudden oak death. Tanoaks are a keystone species ecologically, and one of the few species that is both killed by *P. ramorum* and contributes to its spread. While ecologically important, tanoaks were little studied prior to the onset of the sudden oak death epidemic, resulting in few intellectual or material resources on which to base a disease resistance research program.

Here we report on progress to date on a project funded by the USDA Forest Service, Pacific Southwest Research Station since 2006, the goal of which is to collect the information required to understand what role resistance might play in the disease dynamics and/or management of tanoak populations. A common garden population of open-pollinated tanoak seed families has been established. These trees have been evaluated for variation in neutral genetic markers, growth and morphology, and quantitative resistance to laboratory inoculations. Simultaneously, a field disease trial, using the same families of trees, was initiated to validate and expand on laboratory observations, under natural disease pressures.

The work thus far has resulted in a significant expansion of the understanding of tanoak phylogeography and population genetics (Nettel et al. 2009), the discovery of heritable quantitative resistance to disease in the lab (Hayden et al. 2010), and potential links between growth and morphology and disease resistance in the lab and field (Wright et al. 2009, Hayden et al. 2010). Here, we tie together the investigations, presenting three years of data from laboratory and field resistance trials along with their correlations to growth traits, in light of the understanding of tanoak population dynamics generated from neutral markers. We show the relevance of laboratory studies to field outcomes, and discuss the potential for disease resistance in driving the management or evolution of the species in response to this considerable threat.

The Breeding Program of the American Chestnut Foundation at Meadowview

Frederick V. Hebard, Meadowview Research Farms, The American Chestnut Foundation, 29010 Hawthorn Dr., Meadowview, VA 24361; Fred@acf.org

Charles Burnham hypothesized in 1981 that the blight resistance of *Castanea mollissima* could be backcrossed into *C. dentata*, producing a tree that would perform in the forest similarly to the formerly dominant American chestnut. The American Chestnut Foundation was formed in 1983 to test this hypothesis, using a traditional plant breeding program. The program has been guided by full-time professionals since 1989. It was jump started by using two B1 chestnut trees produced by the old breeding programs of the U.S. Department of Agriculture and the Connecticut Agricultural Experiment Station, known as "Clapper" and "Graves." The old breeding programs also largely developed the methods used in the current program, and identified Chinese chestnut as the most promising source of blight resistance.

"Clapper" and "Graves" were each crossed into approximately 30 American chestnuts from the vicinity of Sugar Grove, Virginia, backcrossed again, then intercrossed twice, with selection for blight resistance and recurrent morphological type prior to each cross. B3-F3 seedlings were planted back into the forest for testing beginning in 2009. The forest performance and blight resistance of those seedlings should start becoming evident by 2015 or 2020. Tests of their parents suggest that their blight resistance will fall somewhere between an intermediate and a high level. The first formal orchard test of the blight resistance of B3-F3 seedlings will be inoculated this year, in 2011, which should clarify this somewhat. The ultimate blight resistance of these trees in the forest will be influenced by their adaptation and may also be affected by hypoviruses and other factors; it may take decades for a clear picture to emerge.

Other sources of blight resistance include "Nanking" Chinese chestnut, which has been backcrossed into 20 American backgrounds. These will begin producing B3-F2 progeny in a few years. More than 20 other sources of blight resistance from Chinese chestnut have been backcrossed to varying degrees into American chestnut, but into only a few backgrounds, so would not by themselves constitute a viable breeding population. Other sources of blight resistance, such as *C. crenata*, have been used to lesser extents, and *C. henryi* and *C. sequinii* will be used in the near future. Using another type of blight resistance, American chestnut trees with low-levels of blight resistance have been increased into generic American chestnut and these crossed within and between sources of resistance. The best of these have intermediate levels of blight resistance.

Where possible, selection for American type was made using molecular markers, but this effort has been hampered by a lack of suitable markers, throughput, and funds. The fruits of the Fagaceae Genomics Project and the Forest Health Initiative may result in more routine use of markers in the project in the near future. It might be especially interesting to try to dissect out some of the components of forest performance, which may differ between lines within sources of blight resistance.

Strains of the blight fungus isolated from various hosts have shown no differences between host and general virulence. They have differed in general virulence among themselves. Isolates from the extremes of pathogenicity for virulent isolates are used to screen for resistance. The genome of two isolates has been sequenced and progeny of their cross evaluated for pathogenicity. We will look for qtls for pathogenicity in their genetic maps.

Bulked Fusiform Rust Inocula and Fr Gene Interactions in Loblolly Pine

Fikret Isik, **Henry Amerson**, **Saul Garcia**, **Ross Whetten**, and **Steve McKeand**, North Carolina State University, Cooperative Tree Improvement Program, Raleigh, NC 27695; fisik@ncsu.ed

Fusiform rust disease in loblolly (*Pinus taeda* L.) and slash (*Pinus elliottii* Engelm. var *elliottii*) pine plantations in the southern U.S. causes multi-million dollar annual losses. The disease is endemic to the region. Fusiform rust fungus (*Cronartium quercuum* sp. *fusiforme*) infects young pine trees and develops galls on stems and braches. Research has shown that single pathogen gene and single host (pine) gene interactions can predict phenotype (disease expression) with high accuracy. There are nine known pathotype-specific Fr genes in seven loblolly pine families.

We investigated interactions of R genotypes (seedlings bearing R allele) and r genotypes (seedling bearing r allele) of seven families with 10 bulked inocula. Our specific objectives were to (i) understand the response pine R and r genotypes to different bulked inocula and (ii) assess the level of virulence in the fungus population that are able to overcome resistance in pine families. Such results would have significant effect on the breeding and disease management in pine plantations. Half-sib progeny of seven loblolly pine families were challenged with 10 different bulked inocula of the fusiform rust fungus and assessed for disease incidence in greenhouse. Progeny of these families were genotyped to identify carriers of known alleles, “R” for resistance or “r” for lack of resistance/susceptibility. The R alleles condition resistance to specific genotypes of the fungal pathogen while r alleles do not condition for resistance. Interactions were tested among different host genotypes and different bulked inocula.

Significant differences were detected among bulked inocula with regards to their ability to incite disease at the family level. Bulked inocula were also significantly different in virulence against R and r genotypes. Across the inocula, disease levels differed significantly among half-sib families, and seedlings that carried only r alleles typically exhibited higher disease rates than did carriers of R alleles within each family. Interaction of bulked inocula with R genotypes from seven families was highly significant (<0.001), but it was not significant with r genotypes because regardless of inocula, r genotypes developed high levels of disease. However, the response of R genotypes varied across inocula. The magnitude of difference (odds ratio) between the R vs. r genotypes for disease incidence within each family varied from 1.0 times to 32 times. The results demonstrate that greenhouse assessments of pathogen virulence against known fusiform rust resistance alleles can detect virulence variation among inocula. Such virulence assessments should be effective guides for the field deployment of plants carrying specific R alleles to regions where inocula samples are observed to show low or no corresponding virulence.

Genetic Selection in Coastal Douglas-fir for Tolerance to Swiss Needle Cast Disease

Keith Jayawickrama and **Terrance Ye**, Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon; keith.jayawickrama@oregonstate.edu; and **David Shaw** and **Robin Mulvey**, Department of Forest Engineering, Resources, and Management, Oregon State University, Corvallis, Oregon

Swiss needle cast (SNC) of Douglas-fir, caused by the ascomycete fungus *Phaeocryptopus gaeumannii*, is associated with significant volume growth losses (20-50%) along the Oregon Coast. Although the pathogen is endemic, disease symptoms have intensified in coastal forests of Oregon and Washington since the early 1990s, and reached a peak of 393,923 symptomatic acres detected in the 2010 aerial survey of western Oregon. Climatic conditions near the Oregon coast are often conducive to SNC disease development. Coastal Douglas-fir is an enormously important timber species for Oregon; consequently, there has been considerable interest and investment in developing seed sources suitable for this zone.

Early research on genetic tolerance versus resistance confirmed that there are no known resistance mechanisms, as all foliage and all trees are susceptible given the right climatic conditions during spore dispersal and colonization. Fungal infection and needle colonization occur passively, through needle stomates and the needle apoplast, respectively, and neither process requires physical or enzymatic penetration of host tissue that might trigger a host defense response. Nonetheless, tolerance to the disease was documented, with some families continuing to grow well in the presence of SNC, and family-mean narrow-sense heritabilities of 0.6 to 0.8 for traits such as needle retention, crown density, and foliage color. Several studies have centered on the Nehalem progeny trial series of 400 first-generation families established on 10 sites in 1986 and subject to moderate to heavy SNC disease pressure. This series was re-measured in summer 2010, and age-26 diameter growth results from 200 families on five sites will be presented. Selections have also been made from other first-generation programs near the coast.

The Trask and South Central Coast Douglas-fir breeding co-operatives, operating within the Northwest Tree Improvement Cooperative, have established 26 new progeny test sites along the Oregon Coast between 2002 and 2006 to obtain 2nd-cycle selections. Ability to maintain height and diameter growth and needle retention scores will be the primary selection criteria. The goal is to develop seed sources capable of acceptable growth rates on sites averaging ≥ 2 years of needle retention. In areas with higher disease pressure and foliage retention ≤ 2 years, it may be prudent to use alternate timber species, such as western hemlock, red alder, western white pine, and Sitka spruce (some forest owners have been making this switch during the past 10-15 years). The Swiss Needle Cast Cooperative has collaborated with PNW tree improvement researchers and funded several tree improvement projects over the past 12 years, and recently developed an Integrated Pest Management strategy that depends on tree improvement efforts to aid in the long-term management of the disease.

Resistance to *Phytophthora cinnamomi* among Seedlings from Backcross Families of Hybrid American Chestnut

Steven N. Jeffers, Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC; sjffrs@CLEMSON.EDU; **Joseph B. James**, Chestnut Return, Seneca, SC; and **Paul H. Sisco**, The American Chestnut Foundation, Asheville, NC

American chestnut (*Castanea dentata*) once was a primary hardwood species in forests of the eastern United States. Sometime during the 18th century, it is speculated that *Phytophthora cinnamomi*, which causes *Phytophthora* root rot (PRR) on many woody plant species, was introduced to the southeast region of the U.S., and this pathogen spread as people moved inland from the coast. In the 1800s, PRR (also known as ink disease) caused extensive mortality to American chestnut trees in the southern portion of its range. Then, in the early 1900s, chestnut blight, caused by *Cryphonectria parasitica*, almost eliminated American chestnut trees from eastern forests. Since 1989, The American Chestnut Foundation (TACF) has been producing hybrid chestnut seedlings by crossing Chinese chestnut (*C. mollissima*), which is resistant to *C. parasitica*, with American chestnut and then backcrossing progeny to *C. dentata* to produce resistant American-type chestnut trees. Since 2000, hybrid seedlings planted in some locations in southeastern states have died from PRR before they could be challenged by *C. parasitica*. Therefore, we wanted to determine if any of the backcross trees selected for resistance to *C. parasitica* were resistant to *P. cinnamomi* as well because Chinese chestnut also is resistant to this pathogen.

Each year from 2004 to 2010, hybrid seeds from trees of known parentage were obtained from TACF cooperators, and seeds from *C. dentata* and *C. mollissima* were collected in the field. All seeds from a single cross were considered to be a family. Seeds were stratified and then planted outside in April in six or seven replicate 570-liter plastic tubs filled with a soilless container mix at a field site in Oconee Co., SC. Each tub contained a sub-sample of seeds from each family. Inoculum consisted of two isolates of *P. cinnamomi* originally recovered from chestnut seedlings growing at the study site. Isolates were grown on autoclaved rice grains or sterilized vermiculite moistened with V8 Juice broth. Seedlings were inoculated 12 to 14 weeks after planting. Inocula from the two isolates were combined, mixed thoroughly, and then evenly distributed in 1- to 3-cm-deep furrows between rows of seedlings. Seedlings were watered as needed throughout the study period, and the container mix in each tub was brought to saturation at least once while plants were actively growing to encourage disease development. Plants were evaluated in December when fully dormant. Each seedling was scored for mortality and PRR severity by examining the roots.

Each year, seedlings began to die approximately 3 weeks after inoculation and continued to die until the end of the growing season. *C. dentata* seedlings consistently were susceptible and died, and *C. mollissima* seedlings consistently were resistant and survived. In 7 years, we have tested 194 hybrid families that ranged from F1s to B4s. Families with seedlings resistance to *P. cinnamomi* occurred each year, but the number of resistant seedlings and PRR severity ratings varied considerably among families. Annual mortality for all hybrid seedling families combined averaged 74% over 7 years. The genes for resistance to *P. cinnamomi* and *C. parasitica* do not appear to be linked because most families were derived from backcross trees selected for blight resistance, but PRR resistance was infrequent. The pattern of inheritance of resistance to PRR was consistent with it being controlled by a single gene. This may facilitate introgression of PRR resistance into breeding populations of backcrossed American chestnut.

Strong Partial Resistance to White Pine Blister Rust in Sugar Pine

Bohun Kinloch (Emeritus), Institute of Forest Genetics, Pacific Southwest Research Station, Box 245, Berkeley, CA 94701; **Deems Burton**, Klamath National Forest; **Robert Westfall**, USDA Forest Service, Pacific Southwest Research Station, Albany, CA; **Detlev Vogler**, USDA, Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics; dvogler@fs.fed.us; and **Joan Dunlap**, Sugar Pine Rust Resistance Program, R5 Genetic Resources Program, Eldorado National Forest

Sugar pine, one of the most susceptible to the blister rust fungus (*Cronartium ribicola*) of all white pines, has a major gene (*Cr1*) at low frequency in populations that confers immunity to the disease. Sugar pines bearing at least one copy of *Cr1*, however, are vulnerable to specific virulence from a complementary gene (*vcr1*) in the pathogen. Partial resistance (PR; aka, slow-rusting) is a suite of traits that reduces susceptibility to blister rust in pines challenged by either the avirulent (*Avcr1*) or virulent (*vcr1*) forms of the pathogen. Combining the two types of resistance in synthetic lines could be mutually reinforcing by reducing the likelihood of infection in pines challenged by either pathotype.

In 2006, a plantation was established at Happy Camp in northwestern California to assess the degree and inheritance of PR in 135 sugar pine families having diverse pedigrees. Progenies consisted of open-pollinated seedlings from survivors of previous epidemic waves on the same site (all presumed to carry some degree of PR), control-pollinated families from parents with known or suspected PR, and bulk seed lots from wild stands, presumed to be highly susceptible. Since 2006, all progenies have been naturally exposed to blister rust at the test site, in which alternate host *Ribes* spp. have been interplanted among the rows. Progenies were evaluated in 2010 after successive wave years of *C. ribicola* caused overall infection to reach 70%. Numbers of separate infections were counted on each seedling and characterized as normal susceptible or reactive PR responses. Families varied from 10 to 100% in the percentage of progeny with normal susceptible infections. Mean infections per family ranged from 0.39 to 12.03 infections per tree, a 30-fold difference. The most susceptible families were from open-pollinated parents from wild stands, or parents with a history of yielding susceptible offspring. Families with the least infection tended to come from F1 parent survivors from prior epidemics. As expected, the presence of the *Cr1* gene in a parental genotype had no effect on PR responses of the progeny. PR was demonstrably strong in some pedigrees and presents opportunities for combining PR with major gene resistance to develop long-term, durable resistance. Future data will provide more insight into the consistency of these results among families.

Ash (*Fraxinus* spp.) Mortality and Survival in Areas Infested by Emerald Ash Borer (*Agrilus planipennis*)

Kathleen S. Knight, USDA Forest Service, Northern Research Station, 359 Main Rd, Delaware, OH 43015; ksknight@fs.fed.us; **Reid Plumb**, **Eileen Sawyer**, **Daniel Spalink**, and **Karen Menard**, Metroparks of the Toledo Area, Toledo, OH; and **Robert P. Long**, USDA Forest Service, Northern Research Station, Irvine, PA

Emerald ash borer (EAB) (*Agrilus planipennis*), an introduced insect pest, has killed millions of ash (*Fraxinus* spp.) trees in the Midwest and is spreading rapidly. We are studying the effects of EAB on ash mortality through yearly canopy health data collection at monitoring plots across the region. Our results show that healthy ash stands typically experience nearly complete mortality within five to seven years.

An intensive survey of ash trees >10 cm DBH (diameter at breast height, 1.4 m) along seven miles of Swan Creek floodplain forest in northwest Ohio was conducted in 2010 to understand the characteristics and dynamics of the few surviving trees. Recently, ash was the most abundant tree species in the Swan Creek floodplain. Now, nearly all of these ash trees have died. Our monitoring plots at Swan Creek showed nearly complete mortality by 2008.

The 2010 survey found 302 live ash trees, representing 2.8% of the estimated total number of ash trees present when EAB arrived. Spatially, these trees were mixed with dead ash trees that had clearly died from EAB several years ago. Of the live trees, 111 had healthy canopies, while the others had some thinning and dieback. The trees with thinning and dieback exhibited more EAB symptoms, including EAB exit holes, woodpecker holes, splitting bark, and sprouting, than the healthy trees.

The diameter distribution of the live ash trees differed from that of the dead ash trees. While dead ash diameters ranged from 10 cm DBH to 92 cm DBH, almost all of the live ash was less than 40 cm DBH. It is too early to determine the fate of these ash trees as EAB populations are persisting at low densities; however, it is possible that the healthy live trees are tolerant to EAB. Perhaps rare genetic tolerance to EAB may allow smaller, vigorously-growing trees to survive EAB, while genetically tolerant over-mature trees may still succumb to EAB pressure. We will continue to monitor the surviving trees to understand the dynamics of potential EAB tolerance in this natural system. We are also working to obtain funding to collaborate with Jennifer Koch (USFS NRS) to collect scion and graft these trees for EAB tolerance testing.

Breeding Strategies for the Development of Emerald Ash Borer-Resistant North American Ash

Jennifer L. Koch, **Kathleen Knight**, and **David W. Carey**, US Forest Service, Northern Research Station, 359 Main Road, Delaware, OH 43015; jkoch@fs.fed.us; **Therese Poland**, US Forest Service, East Lansing, MI.; and **Daniel A. Herms** and **Mary E. Mason**, Department of Entomology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH

Emerald Ash Borer (EAB) is an invasive insect pest originating in Asia. Initially identified in the Detroit area in 2002, it has now been confirmed in 15 states. Native ash species are highly susceptible with mortality of over 99% typically occurring within five years of infestation. We are currently employing two different breeding strategies for the development of EAB-resistant North American ash species. The first approach is to look for EAB-resistance in ash species that are native to the regions of origin of the insect and to utilize these species as parents in hybrid crosses with North American ash species. Should the resulting F1 hybrids be EAB-resistant, they will likely have immediate value to the nursery industry. However, for restoration purposes, the long-term goal is the introgression of the resistance genes from the exotic species into North American ash species through subsequent rounds of backcrossing. Such an approach would allow the retention of all of the traits of the North American species along with EAB-resistance, similar to the American Chestnut Foundation’s hybrid breeding program.

To initiate the identification of potential EAB-resistant exotic parents, we have been accessioning many different exotic ash species from across a wide taxonomic, geographic, and ecological range. Each accession is confirmed for the proper species identity using ITS sequencing and AFLP markers. This step is important because there is no comprehensive global taxonomic key, and few traits are diagnostic between closely related, but geographically isolated species. The EAB phenotype for each accession will be determined through bioassays and/or natural infestation of field plantings and progeny will be tested when large enough.

The second approach is to look for rare EAB resistance or tolerance in native North American ash species. We are identifying surviving North American ash in monitoring plots that have been established in natural stands where over 99% of ash trees have been killed by EAB. These “lingering ash” that have a healthy crown, are in the dominant or co-dominant crown class (or open grown), and are large enough to have been exposed to EAB in the main infestation, are selected for propagation and further study. Currently we have identified and grafted 33 lingering ash trees (both green and white ash). Initial bioassays have been conducted on a few of the genotypes. Analysis of adult EAB choice feeding bioassays has demonstrated that two of the lingering ash selections were less preferred than unselected seedling controls. These data were further supported by a natural EAB infestation in the nursery growing area at the Delaware, OH Forest Service laboratory in which some lingering ash selections remained uninfested while known susceptible selections in close proximity were infested. Controlled cross-pollinations between lingering ash have been performed and the resulting seedlings will be tested. Select lingering ash will also be used as parents in hybrid breeding.

Exploiting Native Resistance: A Breeding Strategy for Beech Bark Disease Management

Jennifer L. Koch, USDA Forest Service, Northern Research Station, 359 Main Rd, Delaware, OH 43015; jkoch@fs.fed.us; **Mary E. Mason**, Ohio State University, Ohio Agricultural Research & Development Center, Wooster, OH; and **David W. Carey**, USDA Forest Service, Delaware, OH

Beech bark disease (BBD) is an insect disease complex that has been killing American beech (*Fagus grandifolia* Ehrh.) trees since the accidental introduction of the beech scale insect (*Cryptococcus fagisuga* Lind.) to Canada around 1890. Insect infestation is followed by infection with *Neonectria ditissima* Samuels & Rossman or *Neonectria faginata* Castlebury. Mortality levels in the first wave of the disease can be as high as 50%, with consequent loss to stand health, merchantable timber, and many wildlife and ecosystem services. It is currently estimated that between 1 and 5 percent of the native American beech are resistant to beech bark disease, and resistance has been shown to be to the insect part of the complex.

Recent work has shown that artificial infestation techniques can be used to screen seedlings for scale resistance. Using this screen, both superior families and superior individuals within families were identified. When full-sibling families using parents of known scale phenotype were screened, an enriched proportion of resistant progeny was observed only in families with two resistant parents. Repeatability (used to estimate upper limit for heritability) is high enough to suggest enough genetic control of scale resistance to support improvement by breeding. Trees selected in the field can be grafted and the assay is useful to confirm the scale resistant phenotype. We are currently identifying, grafting, and testing scale resistant beech trees as part of a multi-state, multi-agency cooperative effort. Confirmed resistant genotypes will be used to establish seed orchards to supply regionally adapted disease-resistant beechnuts for use in restoration plantings and BBD management. The use of only resistant parents is necessary to produce significant improvement over unselected seed lots. Between 20 and 25 unrelated trees with confirmed resistant phenotypes will be included in the seed orchards, allowing collection of a genetically diverse nut crop with sufficient percentage of R nuts for genetic improvement. Our work has established a regional beech genetic resource that is being used to pursue linkage mapping, biomarker prospecting and development, and support of future genetics and genomics research.

Here we present results from additional beech insect resistance screening experiments, including additional seedling families, grafted parental ramets of seedlings screened in parallel, wide-cross families, and non-native beech species. Parents screened in parallel with progeny allow estimates of parent-progeny correlations. Results further confirm the utility of the screen to allow selection of better performing individuals, even within families that perform poorly overall.

Molecular and Genetic Basis for Partial Resistance of Western White Pine against *Cronartium ribicola*

Jun-Jun Liu and **Arezoo Zamany**, Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, British Columbia, V8Z 1M5, Canada; Jun-Jun.Liu@NRCan-RNCan.gc.ca; and **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR 97424

Western white pine (*Pinus monticola*) is an important forest species in North America, and forest genetics programs have been breeding for durable genetic resistance against white pine blister rust (WPBR) caused by *Cronartium ribicola* J.C. Fisch in the past few decades. As various genetic resistance resources are screened and available from breeding programs, we are interested in understanding the genetic mechanisms at the molecular level. This will facilitate a breeding strategy of pyramiding several resistance mechanisms in white pine elite stocks, which would make durable resistance possible for effective and long-term WPBR management.

Both major gene resistance and quantitative partial resistance have been identified in *P. monticola* populations. In contrast to the *Cr2*-mediated major gene resistance that occurs on pine needles by the hypersensitive response (HR), quantitative partial resistance was observed in stems after *C. ribicola* infection as slow canker growth, or bark reaction. Quantitative stem resistance was displayed with various phenotypes in different seed families, ranging from complete susceptibility (rust dead) to complete stem resistance (stem symptom-free), including normal canker development, partial bark reaction with incomplete inhibition of fungal growth, complete bark reaction without fungal activity in the bark, delayed stem infection, fewer stem infections, or longer time to mortality as compared to susceptible controls. Partial resistance is believed to be a heritable and polygenic resistance trait that allows greater survivability of infected white pine trees. Based on phenotypic data collected yearly from western white pine seed families sowed in 2003 and inoculated by *C. ribicola* in 2004, partial resistance levels were analyzed and ranked for each seedling.

Using these plant materials, we used a candidate gene-based association study to reveal what genomic DNA variants underlie phenotypic variation of white pine partial resistance. A set of pathogenesis-related (PR) genes was selected as candidates, including defense-related genes encoding for ß-1,3-glucanases (PR2), chitinases (PR3), thaumatin-like proteins (PR5), cytosolic nuclease-like proteins (PR10), and anti-microbial proteins (AMPs). Genomic DNA sequences of these genes were determined in white pine trees. DNA sequence data of selected candidate genes showed rich single nucleotide polymorphisms (SNPs) in white pine seed families. We will report on the genetic variations of these genes. Development of DNA markers will be discussed for marker-assisted selection of partial resistance to speed up of white pine breeding programs.

Defense responses of white pine trees with partial resistance were also investigated by measuring PR protein accumulation post *C. ribicola* infection. Using Pm-AMP1 as a biochemical indicator, a few patterns were documented for protein regulation in white pine seed families. Our results revealed that different molecular mechanisms may be responsible for various phenotypes of partial resistance in white pine populations. Cross-pollination between seed families with different resistance mechanisms would produce progeny with more durable resistance against *C. ribicola*.

Genetic Variation in Resistance to Pine Pitch Canker and Western Gall Rust in Monterey Pine (*Pinus radiata* D. Don)? Results from a Three-Country Collaborative Field Trial

A. C. Matheson, CSIRO Plant Industry; **W. R. Mark**, California Polytechnic State University; wmark@calpoly.edu; **G. Stovold**, SCION, Rotorua; and **C. Balocchi**, Bioforest S.A.

In 1998, Australia, Chile, and New Zealand agreed to work together in a program designed to test their elite breeding lines and to test for the genetics of resistance to Pitch Canker (causative organism *Fusarium circinatum* Nirenberg & O'Donnel). The first step was a conference, (called IMPACT Monterey) held in Monterey, California to bring together present knowledge of the disease, its epidemiology, and known vectors as well as host responses. Proceedings were published by CSIRO (Devey et al. 1999). A greenhouse trial was conducted at Pebble Beach, California, comparing the responses of about 500 open- or control-pollinated families to inoculation by *Fusarium circinatum*. Results showed there were significant differences between families for the length of the lesion developed 6 weeks following inoculation and that there were significant differences between male parents, but apparently not between female parents (Matheson et al. 2006). The next step was to test the same families in the field to see if the family rankings would match those obtained in the greenhouse.

In 2005, a field trial was set up at Davenport, California at the Swanton Pacific Ranch, owned by California Polytechnic State University, to test disease scores for 264 families in the same environment as a disease-affected natural stand of the species. The trial was designed to be removed before pollen from the trial would materially affect the gene pool of the native stand nearby.

The field trial was assessed annually for disease symptoms until the final assessment in February 2011 after which the trial was removed. Apparent quite early in the life of the trial were galls produced by the Western Gall Rust (causative organism *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka), acquired both at Institute of Forest Genetics at Placerville where many of the trial seedlings overwintered as well as at Swanton following planting. Other disease symptoms included *Diplodia pinea* (Desmaz.) J. Kickx, particularly in the 2009 assessment. Pitch canker was confirmed present in the trial in 2009, and became more severe in 2010 and 2011. This paper presents some results from this field trial and discusses their significance for the plantation industries in Australia, Chile, and New Zealand.

References

Devey, M.E, Matheson, A.C. and Gordon, T.R. eds (1999) "Current and Potential Impacts of Pitch canker in Radiata pine." Proc IMPACT Monterey Workshop, Monterey, California 30/11-3/12 1998. CSIRO Forestry and Forest Products Technical Report No 112, 120 pp.

Matheson, A.C., Devey, M.E. Gordon, T. R., Balocchi, C., Carson, M.J. and Werner, W. (2006) The genetics of response to inoculation by pine pitch canker (*Fusarium circinatum* Nirenberg and O'Donnell) infection by seedlings of radiata pine (*Pinus radiata* D. Don). *Australian Forestry* 69(2), 101-6.

Tandem Selection for Fusiform Rust Disease Resistance to Develop a Clonal Elite Breeding Population of Loblolly Pine

Steve McKeand, **Saul Garcia**, **Josh Steiger**, **Jim Grissom**, **Ross Whetten**, and **Fikret Isik**, North Carolina State University Cooperative Tree Improvement Program, Raleigh, NC 27695-8002; Steve_McKeand@ncsu.edu

The elite breeding populations in the NC State University Cooperative Tree Improvement Program are intensively managed for short-term genetic gain. Fusiform rust disease caused by the fungus *Cronartium quercuum* sp. *fusiforme* is the most economically important disease in pine plantations across the southeastern U.S., causing multi-million dollar annual losses. In order to cull susceptible crosses and progeny prior to field testing, seedling progeny of 51 crosses were challenged with bulked inocula of the fusiform rust fungus at the USDA Forest Service Resistance Screening Center in Ashville, NC. We used a high spore load (50,000 spores/ml) of a broad-base field inoculum that covered the entire range of deployment for these pine genotypes. Through this culling, we eliminated genotypes that may be susceptible in the field to rust, and we are able to focus on selection for growth and quality traits in the remaining population. Overall mean disease incidence was 48% which was ideal for assessing genetic variation for a binary trait. Family disease means ranged from 5% to 81%. We fit a generalized linear mixed model and logit link function to partition observed variance in the response variable (1=gall, 0=no gall) into causal (additive and dominance) components. The genetic control for disease incidence was very high with family mean heritability of 0.95, and the individual tree narrow-sense heritability was 0.5. The results showed that substantial genetic gains can be achieved for fusiform rust disease incidence in loblolly pine in a relatively short time (six months) using greenhouse screening protocols. Subsequent to the greenhouse screening, we cloned 2,442 full-sib progeny that were non-galled for field testing to carry out selection for growth and stem quality traits. Cloned progeny have been established in various locations in 2009 and 2010 using a resolvable alpha incomplete block design.

Breeding Resistance to Butternut Canker Disease

James McKenna, USDA Forest Service, Northern Research Station Hardwood Tree Improvement and Regeneration Center at Purdue University, 715 West State St., Department of Forestry and Natural Resources, West Lafayette, IN 47907; jrmckenn@purdue.edu; **Keith Woeste**, USDA Forest Service, Northern Research Station Hardwood Tree Improvement and Regeneration Center at Purdue University, 715 West State St., Department of Forestry and Natural Resources, West Lafayette, IN 47907; and **Michael Ostry**, USDA Forest Service, Northern Research Station, 1561 Lindig Ave, St. Paul, MN 55108

Butternut (*Juglans cinerea*) is being killed throughout its native range by an exotic fungus *Ophiognomonia clavigignenti-juglandacearum* (*Ocj*). In recent years, many disease-free trees have been determined to be complex hybrids with an admixture of Japanese walnut (*J. ailantifolia)*. Recently developed molecular and morphological characterizations allow us to accurately identify and separate hybrid and pure butternut progeny. Disease-free-trees, from across butternuts native range, are the basis of our breeding program in the Central Hardwood Region of the eastern U.S. Our first clone banks and seed orchards were grafted and established in the 1990s and 2000s and are now producing seed for resistance screening. In 2008 we challenged five-year-old trees from our first two field progeny tests with *Ocj*.

The first test, planted in 2003, had 37 diverse families (n=319). Thirty-two of these seedling families were derived from a grafted orchard of putatively resistant selections. Five additional families were collected from healthy hybrid trees. In early fall of 2008, trees were inoculated with two isolates of *Ocj* obtained from branch cankers on trees in two locations in Indiana. The trees were scored 8, 12, 20, and 24 months after inoculation for canker incidence and severity. Native butternuts in the adjacent woods provided a source of inoculum whereby natural infections from *Ocj* began to occur in the third year. Cumulative natural canker incidence and severity were recorded at 5 and 7 years.

The second test, planted in 2004, had 12 pure butternut half-sib families collected from a woodlot with: four resistant, four moderately resistant, four susceptible, and one resistant hybrid families (n=213). Resistance ratings were based on the disease status of the mother trees in the stand when the seed was harvested in the fall of 2002. In early fall of 2008, trees were inoculated with the same two isolates of *Ocj* used in the first test. The trees were scored 8, 12, 20, and 24 months after inoculation for canker incidence and severity. There was no natural infection in the second test.

Hybrid butternut families were more resistant to natural infection than the pure butternut families. Eight months after inoculation, canker incidence and severity varied significantly among butternut hybrid families and *Ocj* isolate, but not among pure butternut families. After 12, 20, and 24 months, canker incidence and severity of pure butternut families changed. By 24 months, hybrid families in general have shown reduced canker expansion and a high level of resistance. Pure butternut families exhibit more variation from highly susceptible to resistant. Year-to-year variation in canker growth suggests that it may take several years to determine the resistance status of butternut with artificial stem inoculations.

Coast Live Oak Resistance to *Phytophthora ramorum*

Brice McPherson and **David L. Wood**, University of California, Berkeley, CA 94720; bmcpherson@berkeley.edu; **Sylvia R. Mori**, USDA Forest Service, Albany, CA 94710; and **Pierluigi Bonello**, Ohio State University, Columbus, OH 43210

Sudden oak death (SOD), caused by the introduced pathogen *Phytophthora ramorum*, is changing the composition of forests in California and threatens forests worldwide. Coast live oak (*Quercus agrifolia*) has been heavily impacted by the epidemic, with infection and mortality rates of 5% y^{-1} and 3% y^{-1}, respectively, in northern California forests. Since this species is one of the dominant mast-producing trees in much of northern and central California, the future of many of these forested ecosystems is in doubt. Long-term disease progression plots maintained in Marin County since 2000 show that, while coast live oak mortality has steadily increased, the infection rate in these sites is declining. In heavily affected areas, coast live oaks persist that have never exhibited the bleeding symptom of infection or that are in apparent remission from previously recorded bleeding.

Resistance to canker pathogens in trees can be assessed by measuring canker sizes induced by inoculation of the pathogen into mechanical wounds, which in resistant trees are not significantly different from wounds alone or are below a critical threshold. We inoculated a total of 80 asymptomatic mature coast live oaks with *P. ramorum* and mock-inoculated (wounded without inoculation) 40 trees in two natural populations in Marin County in July 2002 and followed disease development to July 2009. By April 2003, 70% of the trees exhibited bleeding. We estimated the probability of survival to 2009 using a Logit model to regress on maximum external canker length measured 9 months after inoculation. Trees with external cankers < 21.2 cm (50% of the trees) had $\geq 80\%$ probability of survival, while cankers < 6.6 cm (35% of the trees) predicted $\geq 90\%$ probability of survival. The trees that died by 2009 all had external cankers ≥ 40 cm in 2003. Because the study started in 2002, after the most susceptible individuals in the stands had already been infected or killed by *P. ramorum* infection, our data likely overestimate the proportion of resistant trees in the pre-epidemic stands. Even so, our study shows that coast live oaks in Northern California exhibit substantial quantitative resistance to *P. ramorum*. The distribution of canker lengths is consistent with quantitative multi-gene resistance to the pathogen.

Integrating Molecular Tools and Conventional Approaches in the OSU Hazelnut Breeding Program

Shawn A. Mehlenbacher, Department of Horticulture, Oregon State University, 4017 ALS Building, Corvallis, OR 97331; mehlenbs@hort.oregonstate.edu

The Oregon State University hazelnut breeding program, initiated in 1969, continues to develop new cultivars for the Oregon hazelnut industry that combine suitability to the blanched kernel market with resistance to eastern filbert blight (EFB). An average of 4,000 seedlings (100 seedlings from each of 40 controlled pollinations) has been planted each year in the past decade. Ten cultivars and 10 pollinizers have been released to date. Hazelnut cultivars are highly heterozygous and clonally propagated. In addition to hazelnut (*Corylus*), the family Betulaceae includes the economically important forest tree genera *Betula* (birch) and *Alnus* (alder). Hazelnut shows amazing genetic diversity. We have identified many simply inherited traits and estimated heritability of quantitative traits. The USDA and OSU hazelnut collections in Corvallis, assembled over the past decades, include representatives of all 11 *Corylus* species and >450 clones of *Corylus avellana*. The most interesting accessions have been used as parents in breeding. The response of many accessions to EFB inoculation has been assessed. Greenhouse inoculation revealed a very high level of resistance in eight cultivars and 15 numbered selections, which represent about 2% of the tested accessions. Several resistant accessions have been used as parents in breeding. Quantitative resistance, expressed as fewer and smaller cankers, is present in OSU releases “Lewis,” “Clark,” and “Sacajawea.” The very high level of resistance in “Gasaway” is conferred by a dominant allele at a single locus. The recently released “Yamhill” and “Jefferson” carry “Gasaway” resistance. At OSU, DNA marker-assisted selection (MAS) has been practiced for ‘Gasaway’ resistance for a dozen years. MAS was initiated because of the pathogen’s two-year life cycle and a desire to select for resistance in the absence of the pathogen. RAPD markers 152-800 and 268-580 flank the “Gasaway” resistance locus, are robust and easy to score, and are useful in multiple populations. Only seedlings that carry one or both RAPD markers are planted in the field. Resistance from three other sources is also simply inherited, and linked DNA markers have been identified. A linkage map for hazelnut was constructed using the double pseudo-testcross strategy and RAPD markers for a population created by crossing two highly heterozygous parent clones. We developed 230 microsatellite marker loci, and most have been placed on the linkage map where they serve as anchors. Microsatellite markers show transferability across genera in the Betulaceae. Twenty-one microsatellite loci, used to fingerprint 270 hazelnut accessions and study genetic diversity in the collection, revealed that 72 were duplicates. *C. avellana* accessions in the Black Sea group, *C. americana* accessions, and *C. americana* x *C. avellana* hybrids were recently characterized. Based on co-segregation with microsatellite markers, EFB resistance from “Gasaway” was assigned to linkage group 6 (LG6), from OSU 408.040 to LG 3, from “Ratoli” to LG 7, and from Georgian OSU 759.010 to LG 2. We will use DNA markers to pyramid different resistance genes. We have a BAC library for hazelnut, and used it for map-based cloning of the “Gasaway” EFB resistance. OSU has partnered with Rutgers University, the University of Nebraska-Lincoln, and the National Arbor Day Foundation to form the Hybrid Hazelnut Research Consortium. The Consortium’s goal is to develop *C. americana* x *C. avellana* hybrid selections with EFB resistance and adaptation to the climate of the eastern and Great Plains states. Tests at Rutgers University, where EFB disease pressure is very high, have shown that quantitative resistance is insufficient. “Gasaway” and its seedling VR 20-11 develop a few small cankers on small twigs. The cankers have few stroma and expand slowly. To date, “Ratoli” and OSU 495.072 (from southern Russia) have remained free of

EFB in New Jersey, while Georgian OSU 759.010 becomes infected but shows less disease that "Tonda di Giffoni." The latter has a high level of quantitative resistance. Selections with resistance from *C. americana* "Rush" and *C. heterophylla* "Ogyoo" remain free of disease in New Jersey. New York hybrids with resistance from *C. americana* "Rush" have remained free of EFB at several locations in the eastern U.S. for many decades. The European hazelnut, *Corylus avellana*, is a diploid (2n=2x=22). Its small genome (0.48 pg/1C nucleus, 413 Mb) is slightly smaller than rice. With Todd Mockler and Illumina, we have sequenced the genome and transcriptome of "Jefferson" hazelnut. We are currently mining the assembled sequence information for microsatellites and single nucleotide polymorphisms. We will search for marker-trait associations, with a special interest in quickly developing PCR-based DNA markers for newly identified dominant EFB resistance genes.

Developing Hazelnuts (*Corylus* spp.) with Durable Resistance to Eastern Filbert Blight Caused by *Anisogramma anomala*

Thomas J. Molnar, **John Capik**, **Clayton W. Leadbetter**, **Ning Zhang**, **Guohong Cai**, and **Bradley I. Hillman**, Plant Biology and Pathology Department, Rutgers University, 164 Foran Hall, 59 Dudley Road, New Brunswick, NJ 08901; molnar@AESOP.Rutgers.edu

Eastern filbert blight (EFB) is a devastating fungal disease of European hazelnut, *Corylus avellana* L., and is considered to be the primary reason hazelnuts have not been developed as a commercial crop in the eastern U.S. The pathogen, *Anisogramma anomala* (Peck) E. Müller, is native to a wide area east of the Rocky Mountains, where it is harbored by the wild hazelnut *C. americana* Marshall. While the shrubby, tiny-nut producing *C. americana* is largely tolerant of the disease, EFB causes stem cankers, dieback, and death of *C. avellana,* the hazelnut of commerce. The absence of EFB, along with a mild climate amenable to hazelnut culture, allowed for the development of a commercial hazelnut industry in the Pacific Northwest (PNW), which thrived for nearly a century. However, despite quarantine efforts, EFB was discovered in WA in the late 1960s and has subsequently spread throughout the Willamette Valley of Oregon, where 99% of the U.S. crop is produced, causing significant economic losses. To combat this challenging disease, a major investment in hazelnut research and breeding was made by Oregon State University (OSU), the USDA, and the Oregon hazelnut industry. Over the last 30 years, these efforts have resulted in the development of a better understanding of *A. anomala*, as well as effective disease management protocols and improved resistant cultivars, leading to a recent revival and expansion of the industry.

Capitalizing on the advances made in Oregon, as well as by early breeding in the eastern U.S., a hazelnut research and genetic improvement program was initiated at Rutgers University in New Jersey in 1996. Realizing the major limiting factor to production in this region was EFB, the initial focus of the program was to evaluate known sources of genetic resistance to the disease (in both *C. avellana* and other *Corylus* species) along with investigating the pathogenic variation of the fungus, which was mostly unknown at the time. In support of this work, close collaboration was developed with the OSU hazelnut breeding program to evaluate their EFB-resistant plant material (clonal and seedling) when exposed to *A. anomala* isolates originating from across its native range; it is believed that only a limited diversity of the fungus is present in the PNW due to its occurrence likely having arisen from a single point introduction. In addition, wide germplasm collections have been made of *C. avellana* in Europe to search for novel sources of resistance to EFB and to identify plants expressing other traits important to developing an industry in the eastern U.S., such as cold hardiness. Concurrent with investigations of the pathogen and evaluation of new germplasm, controlled intra- and interspecific hybridizations have been made annually at Rutgers to incorporate genes for EFB-resistance and cold hardiness from a diversity of backgrounds with the superior nut quality and yield expressed by leading *C. avellana* cultivars and breeding selections developed at OSU. Today, over 20,000 hazelnut seedlings are under evaluation in greenhouses and field nurseries at Rutgers.

An overview of the objectives and progress to date of the Rutgers hazelnut program will be provided, including breeding efforts, resistance screening protocols, findings on the pathogenic variation of *A. anomala*, the identification of novel sources of resistance, and the development of molecular biology tools (draft genome sequence, SSR markers, and real-time PCR) to assess genetic diversity and population structure of the pathogen and to assist in its early detection for disease management and more efficient breeding.

Using Survival Analysis for Assessing Resistance to *Phytophthora lateralis* in Port-Orford-Cedar Families

Sylvia Mori, USDA Forest Service, Pacific Southwest Research Station, 800 Buchanan, Albany, CA 94710; smori@fs.fed.us; **Richard A. Sniezko** and **Angelia Kegley**, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, Oregon 97424; and **Jim Hamlin**, USDA, Umpqua National Forest, Roseburg, Oregon

In a greenhouse trial to examine genetic resistance among seedling families (half-sib, full-sib, and selfed) of Port-Orford-cedar to the root pathogen *Phytophthora lateralis*, the root tips of seedlings were inoculated, and the subsequent ensuing mortality was followed over a three-year period. Mortality levels among families varied from 0 to 100 percent, with some mortality occurring throughout the trial period. A preliminary examination of mortality curves for the families indicated that some families were highly susceptible (100 percent mortality in less than 290 days), some appeared to have high survival putatively from a single major gene (0 to 50 percent mortality with little or no additional mortality after 180 days), and the majority of families appeared to show varying degrees of partial resistance, with different final levels of mortality and with the within-family mortality occurring at different rates. Here we explore the use of survival analysis to examine the different patterns of mortality among the 125 families and place them into different resistance groups. A statistical survival analysis was used to predict resistance to *P. lateralis* of 98 selected POC families using their survival rates. We fitted three survival curves to the survival responses of seedlings from the same families: (1) Cox Proportional Hazard regression (semi-parametric approach), (2) Weibull, and (3) Gamma survival regression models (parametric approach). Because, overall, the Gamma survival curve fitted the data best, we used this curve in most of the cases (in a few cases we used the Weibull curve and some other interpolation from Cox survival output) for determining the 25, 50, and 75 quantiles (Q25, Q50, and Q75) for assigning each family into a resistance group. Four resistance groups were defined for a given threshold day (say, THR=840 days): (1) G1, more than 75% dead at THR days, (2) G2,between 50% and 75% dead at THR days, (3) G3, between 25% and 50% dead at THR days, and (4) G4, less than 25% dead at the THR day. The width of these groups, 25%, would hypothetically allow for mortality variability. We started the groups' assignment with seedlings from the self-pollinated parents (selfing). These seedlings could only fall into three groups G1 (rr*rr), G2 (Rr*Rr), and G4 (RR*RR). The few that did not fall into any of the 3 groups were left for later examination. Therefore, almost every parent from the self-pollinated trees was assigned with a genetic pattern (RR, Rr, rr, or some other resistance pattern). Then we selected a half-sib or full-sib family from one parent with just assigned genetic pattern rr whose partner has not yet been assigned, and applied similar rules; these half-sib or full-sib families could only fall into the three groups: G1 (rr*rr), G3 (rr*Rr), and G4 (rr*RR). Next we selected half-sib or full-sib families from one parent with prior or current assigned genetic pattern Rr whose partner was not yet assigned, and applied similar rules; these families could only fall into the three groups: G2 (Rr*Rr), G3 (Rr*rr), and G4 (Rr*RR). This process was applied iteratively until no non-assigned selfed, half-sib, or full-sib families were left, with exception of those parents not falling readily into these groups (for later examination). We used the Logrank (with Sidak adjustment) test to compare the groups and families within the groups; families assigned to different groups should be statistically significant from each other, and families within a group should be, in their majority, not significantly different from each other. The 35 families not used in the iterations, that we obtained their genetic pattern indirectly, were used as a validation group. The survival of control families (with known putative patterns) were statistically compared to the survival of other families.

Resistance Gene Management: Concepts and Practice

Christopher C. Mundt, Dept. of Botany and Plant Pathology, 2082 Cordley Hall, Oregon State University, Corvallis, OR 97331-2902; mundtc@science.oregonstate.edu

There is now a very long history of genetics/breeding for disease resistance in annual crops. These efforts have resulted in conceptual advances and frustrations, as well as practical successes and failures. This talk will review this history and its relevance to the genetics of resistance in forest species. All plant breeders and pathologists are familiar with boom-and-bust cycles of single major resistance genes. Though there may be occasional situations where a single-gene approach to resistance is reasonable, most interest is in finding alternatives to this approach. This is especially true of long-lived species, for which durability of resistance becomes essential.

Combinations or “pyramids” of major genes can greatly increase durability of resistance and have provided some major success stories in annual crops. The mechanisms by which pyramids contribute durability, however, still are not entirely clear, and this is of importance to potential for success of this approach. Accumulating multiple resistances in a single genotype is complicated by epistatic effects of major genes, though both phenotypic and marker approaches can reduce this difficulty to some degree. Deploying major genes in mixed populations has been highly successful in some cases, including for some woody perennials. There is considerable variation in the epidemiological effectiveness of mixtures, however, and much is yet to be learned about the mechanisms behind this variability. Concern about selection for “superraces” in host mixtures has likely been greatly overstated, and the mechanisms by which selection for multiple virulences occur are much more complex than a simplistic evaluation of “virulence costs.”

Once firmly established in a breeding program, the value of quantitative resistance (QR) is difficult to argue against. Though erosion due to pathogen adaptation may be possible, QR has a solid history of durability. Some have avoided QR because of its perceived complexity of inheritance. Research has shown, however, that QR is much less complex than initially thought. In recent years, many QTL studies have been conducted, and this information may be useful in both augmenting field selection for QR and in avoiding “Vertifolia effects” in the presence of effective major genes. A significant challenge, however, will be in identifying QTL that express in multiple genetic backgrounds of the host.

Theoretical considerations aside, the best approach to breeding for resistance also will depend strongly on availability of resistance sources and on other traits being addressed in the breeding program. Flexibility and forethought must also be used in preparing for changes in silvicultural practices, introduction of new pathogen populations, and climate change that may influence disease. Combining different approaches to resistance should increase both the epidemiological effectiveness and durability of resistance.

Relationship Between Field Resistance to *Phytophthora ramorum* and Constitutive Phenolic Chemistry of Coast Live Oak

Annemarie Nagle and **Pierluigi (Enrico) Bonello**, Ohio State University, Columbus, OH; bonello.2@osu.edu; and **Brice A. McPherson**, **David L. Wood**, and **Matteo Garbelotto**, University of California, Berkeley, CA

Sudden oak death, caused by *Phytophthora ramorum*, has resulted in high levels of coast live oak (CLO) mortality. However, some CLO survive in areas with high disease pressure and may thus be resistant. We tested the hypothesis that such field resistant trees contain constitutively higher levels of phenolics than susceptible trees. Phloem was sampled from the trunks of two groups of trees (one previously inoculated, one naturally infected with *P. ramorum*) categorized over the course of several years as putatively resistant (PR, no symptoms), in remission (IR, showed symptoms but then recovered), and symptomatic (S). Individual and total soluble phenolics from these trees were quantified. There were no significant differences in individual or total soluble phenolics between groups of naturally infected trees. However, inoculated PR and IR trees were characterized by higher constitutive levels of total phenolics as well as ellagic acid, a tyrosol derivative, and an unidentified phenolic than S trees. Ellagic acid and tyrosol-like compounds in CLO phloem are promising resistance biomarker candidates that could be developed into tools for identification of resistant trees in advance of the epidemic front and thus enhance management of this disease for this tree species.

Breeding for Disease Resistance in *Hevea* spp.—Status, Potential Threats, and Possible Strategies

Chaendaekattu Narayanan, **Kavitha K. Mydin**, and **James Jacob**, Rubber Research Institute of India, Kottayam 686 009 Kerala, India; cnarayanan@rubberboard.org.in

Hevea brasiliensis, a forest tree native to the tropical rain forests of Central and South America, has only been recently domesticated outside its natural range of distribution. The tree is the major source of natural rubber, and hence, has great commercial significance in many countries. Almost all of the commercially cultivated clones of *H. brasiliensis* represent a very narrow genetic base since they originated through hybridization or selection from a few seedlings of so called Wickham germplasm. Hence, the commercial rubber cultivation, due to their genetic vulnerability, is under a constant threat of attack by native as well as exotic diseases and insect pests. The change in weather parameters due to the increasing trend in climate change has further complicated the above issue. Climate change, which is clearly felt in the traditional rubber growing regions of India, may possibly alter the host-pathogen interactions. This will lead to emergence of otherwise minor disease. Besides, there is every possibility that hitherto unreported exotic pathogens may be favored by the altered weather situations. *Corynespora* leaf disease represents one such scenario. This pathogen is rapidly progressing into new areas, thus highlighting the need for stronger and advanced resistance breeding approaches.

Pathogenic fungal diseases including *Phytophthora* abnormal leaf fall (ALF) and shoot rot, *Corticium* pink disease, *Corynespora* leaf disease, and *Oidium* powdery-mildew are challenging diseases posing epidemic threats to rubber cultivation. South American Leaf Blight (SALB) is a devastating disease caused by *Microcyclus ulei* (=*Dothidella ulei*) which has prevented large-scale planting of rubber in Brazil due to epidemic outbreaks. SALB is a looming threat to other rubber growing areas. Hence, it is essential that a global SALB-resistance breeding program has to be implemented to tackle such future threats of epidemics.

Hevea clones clearly exhibit variable levels of susceptibility to pathogenic diseases. Field evaluation through visual observation and laboratory assays through excised leaf inoculation at RRII and elsewhere have led to screening of putatively resistant Wickham clones and wild germplasm. However, laboratory bioassays are preliminary and may not ensure actual field level resistance as observed for diseases of other forest trees. *Hevea* clones have also been tested for their capacity to produce phytoalexins and a strong correlation was observed between phytoalexin accumulation and clone resistance. More lignin accumulation was also often associated with clone resistance. These two physiological reactions could interfere by limiting fungal development in several clones. However, neither phytoalexin nor lignin accumulation could individually explain the behavior of all clones. A preliminary attempt was made at RRII to identify possible disease resistance gene analogues in rubber. Although none of the clones obtained had high homology to resistance gene sequences, the putative protein encoded by one sequence had some homology to a resistance gene. The role of *M13-1bn* marker (a putative quantitative trait locus) in screening for resistance to SALB had been investigated through genome mapping.

Earlier selection and breeding of *Hevea* clones resistant to *Microcyclus ulei* (=*Dothidella ulei*) and *Phytophthora sp.* in Brazil led to screening of resistant clones. The list included interspecifc selections from *H. brasiliensis* and *H. pauciflora*, but most of the resistant material had been derived from *H. benthamiana* “F4542,” a selection of upper Rio Negro origin. Few other attempts for inter-specific hybridization have been made, particularly for SALB resistance (*H. camargoona* x FX 4098), but they did not follow large-scale evaluations for field resistance.

Several man hours of labor and enormous quantities of fungicidal chemicals are required every year for management of above diseases in vast areas of rubber plantations in India and other rubber growing countries. The cost of fungicides and their long-term effect on environment justify the need for breeding disease resistant trees. There are several theories for genetic basis for disease resistance (horizontal/vertical) in *Hevea*. Nevertheless, there is every possibility for breakdown of resistance due to ever-evolving pathogenic races coupled with climate change, which is exemplified by evolving SALB races. Multidisciplinary breeding program for development of disease resistant clones would have to continuously utilize Wickham resource, as well as wild germplasm in addition to other *Hevea* spp., in order to have sustainable rubber production.

Screening for Host Resistance to the Balsam Woolly Adelgid

Leslie Newton, **John Frampton**, and **Fred Hain**, North Carolina State University, NC; Fred_Hain@ncsu.edu

Nearly all Fraser fir Christmas trees need to be treated one or more times during a rotation to prevent or lessen damage by the balsam woolly adelgid (BWA). Pesticide applications result in an annual cost estimated at $1.53 million not including direct losses due to BWA (*Adelges piceae*) damage or increased miticide control costs. Our long-term objective is to develop BWA-resistant Fraser fir trees for native stand restoration and the Christmas tree industry. Our short-term objective is to screen for resistance across multiple fir species (of equal age, grown under the same conditions, with insects from the same source) and to observe the reactions of both host and insect on the various species. A BWA resistance screening trial was established at the Upper Mountain Research Station in Ashe County, NC. The study included 13 fir species (4-year-old seedlings), some representing the range of known susceptibility and some of unknown susceptibility. After one season (summer/fall), one half of the study was dismantled and seedlings brought back to the lab for assessment of first instar settlement. The following spring, the remainder of the study was dismantled and the seedlings brought back for assessment of BWA development and egg production. Can BWA successfully insert its stylet into the bark tissue of the various fir species? Can BWA successfully develop and reproduce on the various fir species? The species tested were: *Abies fraseri* (3 seed sources), *A. balsamea* (Balsam fir), *A. balsamea* var. *phanerolepis* (Canaan fir), *A. lasiocarpa* var. *arizonica* (var. Subalpine fir), *A. koreana* (Korean fir), *A. procera* (Noble fir), *A. concolor* (White fir), *A. alba* (Silver fir), *A. firma* (Momi fir), and *A. veitchii* (Veitches fir). Species representing unknown susceptibilities include: *A. bornmuellariana* (Turkish fir), *A. equitrojani* (Trojan fir), and *A. pindrow* (Himalayan Silver fir). BWA can successfully insert its stylet into the bark tissue of Fraser fir, and successfully develop and reproduce. However, in some species BWA has difficulty finding suitable feeding sites, but once settled, successfully develops and reproduces. On other species, BWA can successfully insert its stylet into the bark tissue, but growth and reproduction is poor.

Selection of Eucalypt and Acacia Clones for Disease Resistance and High Productivity

Nguyen Hoang Nghia and **Pham Quang Thu**, Forest Science Institute of Vietnam, Dong Ngac, Tu Liem, Hanoi, Vietnam; nhnghia@netnam.vn

Eucalypts and acacias, mainly *Eucalyptus camaldulensis*, *E. urophylla*, eucalypt hybrids, *Acacia mangium*, *A. auriculiformis*, and acacia hybrids, are the main species planted in Vietnam for pulp, chips, MDF board, and saw log production. Eucalypt and acacia plantations have been widely established from the north to the south of Vietnam in recent decades, and now occupy about one million ha of the 3 million ha of forest plantations.

Since the late 1980s, severe diseases, especially leaf spots and shoot blight followed by crown dieback and stem canker have been observed in eucalypt plantations in many locations. The main identified pathogens are as follows: *Cryptosporiopsis eucalypti*, *Cylindrocladium reteaudii* (*Cylindrocladium quinqueseptatum*), *Mycosphaerella* spp., *Teratosphaeria destructans* (*Kirramyces destructans*), *Ralstonia solanacearum*, and *Chrysoporthe cubensis*. Severe diseases to acacia plantations, especially leaf diseases and stem diseases, have been found since the 1990s. The main identified pathogens for acacias are: *Colletotrichum gloeoporioides*, *Meliola* spp., *Oidium* spp., *Botryosphaeria* spp., *Ganoderma* spp., and *Corticium salmonicolor*.

The tree improvement program for disease resistance in Vietnam began in 1996 and has been implemented from 2000 to 2010. Disease scores have been conducted in progeny/provenance trials based on 5 levels of disease index from nil (healthy tree, no disease) to level 4 (severe damage, dead tree caused by disease). Selection of plus trees with superior growth and without disease damage has been conducted in trials and plantations in diseased areas. The selected plus trees are propagated by cuttings. About 65 ha of eucalypt and acacia clone trials were established in areas of high risk for diseases. Disease scores and growth measurements are implemented every year at the beginning of the dry season in November or December. Clones in the trials with very low disease index, good stem form, and high mean annual increment (MAI) are selected as technical varieties for large-scale planting. Eleven clones of two eucalyptus species with MAI greater than 20 m^3/ha/yr have so far been selected: SM7 (*E. brassiana*), SM16, SM23, EF24, EF39, EF55, SM51, SM52, B28, B32, and B34 (*E. camaldulensis*). Clone SM16 gave a MAI for volume of 40 m^3/ha/yr at about 6 years of age. Seven clones of acacia hybrids with MAI of more than 20 m^3/ha/yr have been selected, namely AH1, AH7, AH4, M8, MA1, AM3, and AM2. Notably, clone AH7 gave a MAI for volume of 35 m^3/ha/yr at about 4 years of age. There are currently 7 clones of *Acacia auriculiformis* with MAIs greater than 20 m^3/ha/yr: AA1, AA6, AA7, AA9, AA10, AA12, and AA15, and clone AA15 had a MAI for volume of 33 m^3/ha/yr after about 5 years in trials. One clone of *Acacia mangium* with MAI more than 35 m^3/ha/yr has been selected in an 8-year trial.

Oak and Beech Disease Resistance Assessed with DNA Markers

Justyna A. Nowakowska, Forest Research Institute, Department of Silviculture and Genetics, Sekocin Stary, Braci Lesnej 3 Street, 05 090 Raszyn, Poland; j.nowakowska@ibles.waw.pl; and **Tomasz K. Oszako**, Forest Research Institute, Department of Forest Protection, Sekocin Stary, Braci Lesnej 3 Street, 05 090 Raszyn, Poland

The oak and beech decline phenomenon has been observed in Poland since the 1980s. The reasons are still unknown, but the discovery of pathogens in the genus *Phytophthora* shed a new light on it. On the other hand, genetic diversity ensures the survival of forest tree species which can adapt and evolve under changing environmental conditions. In order to compare the health of forest stands, chloroplast microsatellites (SSR) and PCR-RFLP markers have been investigated in the genetic differentiation study among healthy and damaged oak and beech trees in different Forest Districts in Poland. Oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) populations were analyzed in order to determine genetic variation in relation to health and resistance to stress factors. Pathogen identification was performed using sequencing of the ribosomal DNA fragments. Based on the study of the chloroplast DNA variability combined with the defoliation and disease symptoms present on the trees, we found a higher genetic diversity level in healthy trees compared to damaged ones. Healthy trees were nearly 50% more genetically diverse than declining ones, which can lead to the conclusion of better adaptability to the harmful environmental conditions.

Genomics Assisted Breeding for Resistance to *Phytophthora cinnamomi* in Chestnut (*Castanea* sp.)

Bode A. Olukolu, Genetics and Biochemistry Department, 100 Jordan Hall, Clemson University, Clemson, SC 29634; bolukol@clemson.edu; **C. Dana Nelson**, U.S. Forest Service, Southern Research Station, Southern Institute of Forest Genetics, 23332 Success Road, Saucier, MS 39574; and **Albert G. Abbott**, Genetics and Biochemistry Department, 100 Jordan Hall, Clemson University, Clemson, SC 29634

Phytophthora cinnamomi (*Phytophthora* crown and root rot or ink disease) is now known to infect several hundreds of species in the world and is especially linked to the widespread death of mature chestnut and evergreen oak trees in the southeastern U.S. With an expanding geographical distribution of *P. cinnamomi* in North America, and coupled with the chestnut blight disease (*Cryphonectria parasitica*) that initially decimated the 4 billion-strong American chestnut population (about 30% of trees in the Appalachian mountains), *P. cinnamomi* is becoming a crucial limiting factor in natural regeneration and reforestation efforts due to the high susceptibility of both young seedlings and mature trees. In this study, we show the use of genomic tools for the increased efficiency and precision towards breeding *Phytophthora*-resistant chestnut trees. The strategy involves the use of an interspecies hybrid population, segregating for *Phytophthora* resistance, for mapping a major QTL. In conjunction, transcriptome data, the chestnut physical map, high-throughput sequencing of the QTL genomic region, high density SNP chestnut linkage maps, and comparative genomics were used for map-based identification of candidate genes which explained possible underlying genetic resistance mechanisms. Currently, we are validating candidate genes by: structural and functional genomics studies of infected and uninfected trees; haplotype analysis in backcross progenies and a wild population; as well as, ongoing transformation studies of candidate genes in American chestnut. We expect to provide genomic tools for functional molecular marker-assisted selection in breeding programs for introgression of resistance traits.

Insertion Site Selection and Feeding by the Hemlock Woolly Adelgid: Implications for Host-Plant Resistance

Kelly Oten, North Carolina State University, B-1104 Grinnells Lab, CB 7626, Raleigh, NC 27695; klfelder@ncsu.edu; and **Allen Cohen** and **Fred Hain**, North Carolina State University, Raleigh, NC

The hemlock woolly adelgid (HWA; *Adelges tsugae*) is an invasive sucking insect that has caused extensive mortality to two species of hemlocks in the eastern U.S.: eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*). In its native range (Asia and the Pacific Northwest), HWA does not kill its host. This has been attributed to a complex of natural enemies, the intermittent distribution of hemlocks, and innate host-plant resistance. Despite their documented susceptibility, some stands and individuals of hemlocks in the eastern U.S. are surviving in light of pressure from HWA. This suggests the presence of naturally occurring host-plant resistance in eastern and Carolina hemlocks. This research investigates the mechanisms of such resistance by observing the feeding behavior of HWA through behavioral assays, scanning electron microscopy, and analyses of hemlock foliar waxes. Understanding sources of resistance will assist selection of HWA-resistant trees, ultimately advancing the efforts to save the hemlock through resistance breeding programs, gene conservation, and replantings.

Factors Influencing *Leptocybe invasa* Infestation of *Eucalyptus* Species in Kenya

B. O. Otieno, **K. E. Mutitu**, and **K. N. Mwangi**, Kenya Forestry Research Institute,
P.O. Box 20412-00200, Nairobi; bnyambune@yahoo.com

Eucalyptus have been grown for about 100 years in Kenya and are highly favored for production of poles, fuelwood, and timber due to their rapid growth, ease of cultivation, and their adaptation to a wide variety of growing conditions. Eucalyptus trees in Kenya have been under attack from a constant stream of specific insect pests mostly originating in the center of origin of the tree.

Leptocybe invasa Fisher & La Salle, sp. n (Hymenoptera: Eulophidae), a gall-forming wasp which causes extensive damage to Eucalyptus, was first reported in Kenya in 2003 and positively identified in 2004. It primarily attacks seedlings and field saplings. It causes damage to its host by forming massive typical bump-shaped galls on the tree canopy, specifically on the leaf midribs, petioles, and stems of new growth.

The objective of this study was to determine the factors responsible for infestation and damage to Eucalyptus trees by the pest in Kenya. Selected Eucalyptus stands from major growing regions were assessed. Three randomly established plots per stand were assessed for *L. invasa* population and damage severity on a 4-point scale. A damage severity index was calculated from the damage severity and damage incidence data collected per sample plot.

The pest population and damage is highest in western and Nyanza regions where the infestation was first reported. *Eucalyptus grandis, E. camaldulensis*, and Hybrid GC14 were very susceptible to the pest while GC581 and *E. henrii* were not attacked. No pest damage was observed over 2,000 m above sea level. The insect annual population in Western Province was highest from May to June, while the highest damage occurred in April when there was production of more new shoots. These factors could guide plantation managers in designing an integrated management strategy for this pest.

What Do We Know about Mechanisms for Tolerating Pathogens, and Can Tolerance Be Applied to Managing Tree Diseases?

Bitty A. Roy, Center for Ecology and Evolutionary Biology, University of Oregon,
Eugene, OR; bit@uoregon.edu

The terms ‘‘resistance’’ and ‘‘tolerance’’ have been used by different scientists to refer to different things, and they have often been measured (and thus operationally defined) in ways that confuse the two concepts with each other. In keeping with the emerging consensus on resistance and tolerance, the following conceptual distinction is useful: resistance refers to traits that prevent infection or limit its extent, and tolerance refers to traits that do not reduce or eliminate infection, but instead reduce or offset its fitness consequences. Thus, resistance and tolerance can both improve host fitness; resistance does so by reducing infection, whereas tolerance does so by reducing the fitness loss under infection. In this review, I will briefly set up the differences between resistance and tolerance, then discuss what we know about mechanisms for tolerance and what is known about tolerance in relation to tree diseases.

Developing Durable Western Redcedar Populations for a Changing Climate

John H. Russell and **Alvin Yanchuk**, Forest Genetics, British Columbia Forest Service; John.Russell@gov.bc.ca

Western redcedar (*Thuja plicata*), a member of the Cupressaceae, is an important commercial species in British Columbia (BC) and the Pacific Northwest (PNW), and is prized for its naturally durable outdoor products. A breeding program has been ongoing for only 15 years in BC, but tremendous progress has been made owing in part to its unique genetic and biological properties. Initial population improvement focused on growth as well as resistance to heartwood pathogens through selection for secondary extractives. However, as climates change and pests, known and unknown, become more abundant, breeding strategies need to adapt to better accommodate these shifts in environmental stresses. Increases in ungulate populations and occurrence of cedar leaf blight (*Didymascella thujina*) have caused western redcedar plantation failures, reduced growth, and longer times to reach free-to-grow status. Developing durable populations that are resilient to multiple pests through breeding for enhanced resistance mechanisms including secondary extractives, as well as maintaining adaptability and growth, becomes important for future forest health. This presentation will outline the progression of the western redcedar tree improvement program in BC, and challenges that we currently face in breeding, production, and operational deployment.

Endophyte Mediated Plant-Herbivore Interactions or Cross Resistance to Fungi and Insect Herbivores

Kari Saikkonen, MTT Agrifood Research Finland, Plant Production Research, 31600 Jokioinen, Finland; kari.saikkonen@mtt.fi; and **Marjo Helander**, Section of Ecology, Department of Biology, University of Turku, 20014 Turku, Finland

Endophytic fungi are generally considered to be plant mutualists, which protect the host plant from pathogens and herbivores. Defensive mutualism appears to hold true particularly for seed-transmitted, alkaloid producing grass-endophytes. However, we propose that the mutualistic nature of plant-endophyte interactions via enhanced plant resistance to pathogens and herbivores should be reconsidered when focusing on woody plants.

We compared phenotypic and genetic frequency correlations for two endophytic fungal genera (*Fusicladium* and *Melanconium*) and birch rust (*Melampsoridium betulinum*) with the performance of six invertebrate herbivores growing on the same half sib progenies of mountain birch (*Betula pubescens* ssp. *czerepanovii*) in two environments over a 3-year period. We found little support for causal association between fungal frequencies and performance of herbivore species. Instead, genetic correlations, particularly between autumnal moth (*Epirrita autumnata*) and birch rust, suggest that herbivore performance may be affected by (1) genetic differences in birch quality for fungi and herbivores, or (2) genetic differences in responses to environmental conditions. Genetic analysis of *Venturia ditricha* (anamorph *Fusicladium betulae*) revealed that (1) birch genotypes and environment influence the probability of infection by particular endophyte genotypes, (2) genetic variation correlated negatively with infection frequencies of the fungus, and (3) the susceptibility of the birch to a particular endophyte genotype may change when environmental conditions are changed (environment-host genotype interaction).

Furthermore, statistical perusal of endophyte literature revealed clear contrasts between grass and tree endophytes. Grass endophytes appear to provide support for the hypothesis of defensive mutualism, whereas variability appears to be the nature of the tree endophyte mediated plant herbivore interactions.

We propose that (1) the performance of endophytes, pathogens, and herbivores may be responses to genetically determined plant qualities rather than interconnected associations. (2) The observed genetic correlation structure may have importance for evolution of birch resistance to fungi and herbivores (e.g. negative correlation between birch resistance to rust fungus and autumnal moth may constrain birch population from reaching optimal species-specific resistance). This should be considered also in forest tree improvement attempting to overcome pests and pathogens. (3) More knowledge about multispecies coevolution is necessary to fully understand bilateral interactions between plants and the organisms living on them.

References

Ahlholm, J., M. L. Helander, P. Elamo, I. Saloniemi, S. Neuvonen, and S. Hanhimäki and K. Saikkonen (2002): Micro-fungi and invertebrate herbivores on birch trees: fungal mediated plant-herbivore interactions or responses to host quality? Ecology Letters 5: 648-655.

Ahlholm, J.U., M. Helander, J. Henriksson, M. Metzler and K. Saikkonen (2002): Environmental conditions and host genotype direct genetic diversity of *Venturia ditricha*, a fungal endophyte of birch trees. Evolution 56: 1566-1573.

Saikkonen K., Wäli P., Helander, M. and S.H. Faeth (2004) Evolution of endophyte-plant symbioses. Trends in Plant Science 9: 275-280.

Saikkonen, K., P. Lehtonen, M. Helander, J. Koricheva and S. H. Faeth (2006) Model systems in ecology: dissecting the endophyte-grass literature. Trends in Plant Science 1 1: 428-433.

Saikkonen, K. (2007) Forest structure and fungal endophytes. Fungal Biology Reviews 21/2-3: 67-74.

Saikkonen, K., S. Saari and M. Helander (2010) Defensive mutualism between plants and endophytic fungi? Fungal Diversity 41: 101-113.

Trade-Offs Between Induced and Constitutive Resistance in Two Pine Species: Secondary Chemistry, Effective Antiherbivore-Resistance and Effect of Nutrient Availability

Luis Sampedro and **Xoaquín Moreira**, Centro de Investigación Forestal de Lourizán – UA MBG-CSIC, Apdo 127, 36080 Pontevedra, Galicia, Spain; lsampe@uvigo.es; and **Rafael Zas**, Misión Biológica de Galicia, MBG-CSIC, Apdo. 28, 36080 Pontevedra, Galicia, Spain

During millions of years of co-existence with herbivores, conifer trees have evolved effective traits and strategies for defending themselves. Morphological and chemical defenses present in trees have been traditionally classified as either constitutive, the preformed defensive levels always expressed in the plants, and inducible, plastic defenses, synthesized or mobilized in response to plant injury or other cues or herbivory risk. Preformed defenses and plastic defensive responses differ in their benefits in terms of fitness for long-lived plants. Induced defenses are considered to be less expensive than constitutive preformed defenses since the cost is realized only when required. But induced chemical resistance also has disadvantages derived from the time required for their synthesis or re-allocation. Plant defense theory predicts that, as secondary metabolism is costly for the plant, presenting effective levels of constitutive defenses and the ability of expressing efficient inducible defenses by a plant are two resource-related attributes that are not likely to be maximized at the time. Furthermore, selective pressure favoring the expression of induced responses is likely to be lower in those lineages well defended constitutively, as they would be less subjected to herbivore attacks. Genotypes constitutively well-defended are expected to gain little boosting to their defenses after damage to be protected against subsequent attacks; conversely, genotypes with low constitutive defenses are likely to be under the pressure of being able to express effective inducible responses. A negative, non spurious, genetic correlation between constitutive and inducible defenses illustrates this classical trade-off. The existence of these evolutionary conflicts has been many times suggested in the literature and sometimes reported for angiosperms, but rarely in conifers and not yet in pine trees. However, the emergence of this genetic constraint could be hidden by environmental factors affecting growth potential, such as nutrient availability.

In this paper we present the results from three independent experiments aimed to explore the existence of this trade-off in two-year-old seedlings of Maritime pine (*Pinus pinaster*) and Monterey pine (*P. radiata*), native from similar climate regions in Europe and North America. We used 22 mM methyl-jasmonate (MJ), a phytohormone involved in the biosynthetic pathways of chemical defenses, to induce pine defensive responses. In the first experiment we analyzed the secondary chemistry of constitutive (control) and MJ-induced pine seedlings belonging to 18 half-sib families of *P. pinaster* from the Atlantic population of Galicia (NW Spain). One month after induction, we performed an *in vivo* feeding bioassay with a generalist insect herbivore (the large pine weevil, *Hylobius abietis*) to check how the expressed defenses reflected the ability to resist the attack. In the second experiment we reproduced the same design with 34 half-sib families belonging to *P. radiata*. And in the third experiment we analyzed this trade-off in a wider collection of *P. pinaster* genetic entries (33 half-sib families) grown either in low or in high phosphorus availability, to test if this trade-off could be dependent on environmental context. We explored the existence of trade-offs regressing the difference in mean resistance levels between experimentally MJ induced individuals and control individuals from a given family (induced -control), against the family means of control treatment. Correlations were checked not to be spurious by means of Monte-Carlo analysis.

We identified, in both species, strong negative genetic correlations between induced and constitutive concentrations of total polyphenolics, resin content, and also for the effective resistance against weevil damage, with R^2 ranging from 0.38 to 0.80 (all relationships significant at $P < 0.001$), which constitute strong experimental evidences that this genetic trade-off exists in this pine tree. All three experiments confirmed the existence of this classical trade-off in *P. pinaster*, measurable in the physiological defensive traits and in the effectiveness of the defensive compounds against the insect herbivore. Besides, in the third experiment, Monte-Carlo only confirmed as non-spurious those negative genetic correlations observed under phosphorus limitation, while those under complete fertilization appeared to be likely spurious. Thus, in *P. pinaster*, this trade-off only emerged in circumstances of nutrient limitation, but it is not measurable when plants have plenty of nutrients. We found, within the studied populations, a range of strategies from families with reduced expression of constitutive defenses which exhibited the ability to increase dramatically their defenses after induction signals, and families with a strong expression of constitutive defenses which are poorly capable of increasing their defenses after attack.

Modeling Elm Growth and DED Susceptibility

Alberto Santini, Institute of Plant Protection, CNR, Via Madonna del Piano, 10, 50019 Sesto fiorentino, Firenze, Italy; a.santini@ipp.cnr.it; and **Luisa Ghelardini**, Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080, S-750 07 Uppsala, Sweden

The susceptibility to Dutch elm disease (DED) displays strong seasonal variation. The period during which elms can become infected and express DED symptoms is generally restricted to several weeks after growth resumption in spring, although it can vary among species, provenances, and environmental conditions. The reason for this phenomenon is not understood, but the few studies correlating DED susceptibility with the host's rhythm of seasonal morphogenesis, suggest that the seasonal variation in disease development depends on the pattern of growth. Susceptibility to DED is correlated to the date of bud burst in the European field elm, suggesting that a differentiation in spring phenology might cause an asynchrony between the period of susceptibility of the host and the phase of disease transmission by the insect vector allowing the tree to escape the disease.

In order to verify which conditions are determinant to express a susceptible response in elm, a thorough study of the time course of longitudinal growth, including reactivation of cambial activity and timing of bud burst, of leaf area development, radial growth, wood ring anatomy, and their relations with DED susceptibility, assessed through repeated artificial inoculations from late winter to early summer, was conducted in an intermediately susceptible elm clone. Growth and seasonal variation in the response to DED infection were modeled in two consecutive years.

Three different patterns of shoot growth were observed at the same time in elm, i.e. predetermined growth, free growth, and growth for successive flushes. Each of the observed types of elongation growth had a specific spatial and temporal distribution, which corresponded to different anatomical patterns in the wood and different response to DED inoculation.

The period of maximum susceptibility to DED coincided with a phase of initial and slow growth, during which reserves were rapidly exhausted, expanding leaves were behaving as energy sinks, and early wood was formed. The first decrement in DED susceptibility coincided with the transition to a subsequent phase of fast growth, when hefty energy was supplied by an increasing number of mature leaves able to efficiently photosynthesize, and a more compact late wood was formed.

The results fit the "Growth-Differentiation Balance Hypothesis" (Herms & Mattson 1992), which provides a framework for predicting possible allocation trade-offs in between differentiation-related and growth-related processes over a range of environmental conditions (Lorio 1986). Similarly to all other plants, elms are challenged by the highly unpleasant dilemma: to grow or to defend.

Genotype × Environment Interaction and Growth Stability of Several Elm Clones Resistant to Dutch Elm Disease

Alberto Santini, **Francesco Pecori**, and **Alessia L. Pepori**, Istituto per la Protezione delle Piante—C.N.R Via Madonna del Piano, 10 50019 Sesto fiorentino, Italy; a.santini@ipp.cnr.it; and **Luisa Ghelardini**, Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080, S-750 07 Uppsala, Sweden

The elm-breeding program carried out in Italy at the Institute of Plant Protection - CNR during the last 40 years aimed to develop Dutch elm disease (DED) resistant elm selections specific to the Mediterranean environment. The need for genotypes adapted to Mediterranean conditions was evident from the poor performance of the Dutch elm clones in the hot and dry areas of Central Italy, and the opportunity to breed better adapted hybrids was offered by the favorable adaptation in Italy of the Siberian elm (*Ulmus pumila* L.), a species that does not thrive in Central Europe and the Netherlands. For this reason a base of valuable individuals of native elms was bred with several accessions of Asian elm species that proved to easily acclimate to different conditions in the Mediterranean region. As a result, four resistant hybrid elms have been patented and released to the market and many experimentally tested DED resistant elm hybrids of different parentage are under evaluation to be released in the next future.

Conventionally hybrid elm clones obtained within breeding programs DED resistance were selected to meet requirements for use as ornamentals. However, it has been long and commonly observed that these clones may show hybrid vigour and enhanced growth.

Nowadays DED resistant hybrid elm clones, which have been released to the market or are under evaluation for an upcoming release, are numerous enough to be considered for timber production or short rotation coppice (SRC). But experimental testing of the growth performances of these clones in different environments is still lacking. Here, growth and stability of performance of several DED resistant hybrid elm clones planted at three experimental sites with contrasting environmental conditions in Italy were studied. Height and diameter were measured yearly from 2001 to 2009, and the mean yearly increments after plant establishment were calculated. The study revealed a general good growth performance of the majority of the clones with mean height increments above 1 m per year, and an excellent growth performance of some genotypes. Analysis of variance showed significant effects of clone, site, and clone site interaction, for both height and diameter increments. Stability analysis of diameter and height increments was performed by using two parametric (CV% and W^2) and two non-parametric (Hühn's $S_i^{(1)}$ and $S_i^{(2)}$) indexes. According to all indexes, two clones showed superior and stable growth. These clones may be suitable for planting in a range of environments. In addition, several other clones had high growth in general or at a particular site. The results support our belief that these elm clones could be successfully used for timber and biomass production, and provide new knowledge for an informed choice of the most suitable genotypes.

High-Resolution Genetic and Physical Mapping of Eastern Filbert Blight Resistance in Hazelnut

Vidyasagar Sathuvalli and **Shawn A. Mehlenbacher**, Department of Horticulture, 4017 ALS Building, Oregon State University, Corvallis, OR 97331; vidyasas@hort.oregonstate.edu

Eastern filbert blight (EFB) caused by the pyrenomycete *Anisogramma anomala* is a serious threat to the hazelnut (*Corylus avellana*) industry in the Pacific Northwest. Host genetic resistance from the obsolete pollinizer 'Gasaway' has been extensively used in the hazelnut breeding program at Oregon State University. Map-based cloning of the EFB resistance gene from “Gasaway” was initiated by constructing a BAC library for “Jefferson” which is heterozygous for resistance. The BAC library was constructed using the cloning enzyme MboI and the vector pECBAC1 (BamHI site). The library consists of 39,936 clones arrayed in microtiter plates with an average insert size of 117 kb and estimated coverage of 12 genome-equivalents. Chromosome walking initiated with eight RAPD markers closely linked to resistance, and extended with two further rounds of walking, identified a total of 93 BACs in the resistance region. A high-resolution genetic map of the region was created with 51 markers in a mapping population of 1,488 seedlings. In parallel, a physical map was constructed. Analysis indicated that the resistance gene is located in a single contig of three BACs. Whole BACs in the resistance region (< 1cM) were sequenced using an Illumina IIx genome analyzer, with multiplexing and barcoded adapters to reduce the cost, and paired-end reads to facilitate de novo sequence assembly. Assembly was carried out using the programs Velvet and SOPRA, and the resulting contigs were further aligned using CodonCode software. Generated contig length ranged from 356 bp to 99632 bp. Estimated coverage of the BACs ranged from 64 to 100%. The gene prediction program AUGUSTUS identified 233 genes from these sequences using *Arabidopsis* as the model. Of these, RNA-Seq data supported 32 genes at 100% support. The predicted gene sequences were compared with sequences in GenBank using a BLASTP search and identified two putative genes encoding a p-loop NTPase and F-box super family. Genes in these two superfamilies have defense response properties. Future expression, complementation, and mapping studies are essential to confirm which gene confers resistance.

Proactive Use of Genetic Resistance to Pathogens to Sustain Ecological Function of Threatened Ecosystems

A. W. Schoettle, USDA Forest Service, Rocky Mountain Research Station, 240 W. Prospect Rd, Ft Collins, CO 80526; aschoettle@fs.fed.us; **R.A. Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR; **J. G. Klutsch**, Department of Agricultural & Resource Economics, Colorado State University, Fort Collins, CO, and USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO; and **A. Kegley**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR

Remote natural mountain ecosystems are not beyond the reach of non-native pests and pathogens or climate change. The ecosystem services provided by these ecosystems, such as water supply, wildlife habitat, and slope stability, depend on the long-term sustainability of species diversity and ecosystem processes. The spread of an established non-native organism may be inevitable, but the consequences of invasion by that organism on populations and ecosystems may be mitigated with early, informed intervention. The goal of proactive management in a natural ecosystem is to prepare and position the landscape to promote self-sustaining populations and sustained ecosystem function into the future in the presence of the invader. Sustaining population resiliency requires maintenance of (1) population recovery capacity after disturbances, (2) adaptive capacity, and (3) multi-generational persistence. Management in natural ecosystems therefore requires a long-term and evolutionary perspective.

The proactive approach is a shift from reactive or crisis management in response to one stressor to management for sustained resiliency to multiple stressors. In cases where a known threat continues to spread, the proactive strategy takes advantage of the option to act before populations or ecosystems are affected, thereby increasing a managers' toolbox beyond silvicultural and genetic approaches to include also a choice of when to implement management for optimal ecological efficacy. Management to sustain ecosystem function may be more successful than managing to restore function after population or ecosystem collapse.

This presentation will deliver an interdisciplinary approach to gather and utilized information to develop proactive management options to sustain populations and ecosystem function. Examples from the application of the Proactive Strategy in the Southern Rocky Mountains to mitigate impacts of white pine blister rust to bristlecone and limber pine ecosystems will be presented. We will demonstrate, using a population genetic model, examples of proactive management actions that can mitigate impacts of white pine blister rust to pine populations and ecosystems. Positioning ecosystems for greater resilience is especially important for traditionally minimally managed ecosystems where the risk of ecological impacts is high.

Operational Program to Develop *Phytophthora lateralis* Resistance in Port-Orford-Cedar (*Chamaecyparis lawsoniana*)—Progress through 2011

Richard A. Sniezko, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon; rsniezko@fs.fed.us; **Everett M. Hansen**, Botany and Plant Pathology, Oregon State University, Corvallis, Oregon; and **Jim Hamlin**, USDA Forest Service, Umpqua National Forest, Roseburg, Oregon

Port-Orford-cedar is a forest tree species of southwestern Oregon and northwestern California. It has also been grown horticulturally in many parts of the world. However, the presence of a non-native pathogen, *Phytophthora lateralis*, in the native range of Port-Orford-cedar has caused significant mortality in portions of the species' range and made landowners reluctant to use this species in reforestation. The horticultural use of Port-Orford-cedar has also declined steeply in the Pacific Northwest, and recent reports of the pathogen in western Europe have raised concerns there.

Genetic resistance to *P. lateralis* would provide a vital tool to aid restoration and reforestation use of Port-Orford-cedar. Fortunately, previous testing at Oregon State University indicated that some level of genetic resistance to *P. lateralis* existed. Subsequent joint trials with the USDA Forest Service have refined the information on the extent of the resistance and provided more information on the types of resistance. In 1996, the USDA Forest Service and USDI Bureau of Land Management began a large-scale operational program, based at Dorena Genetic Resource Center, to develop resistance populations. This program aims to utilize naturally occurring genetic resistance in Port-Orford-cedar, while maintaining genetic diversity and adaptability in the species. Oregon State University has been a key partner in this applied effort, providing seedling screening and research pathology support.

The Port-Orford-cedar program has been fast developing due to the combination of concerted work of numerous individuals and the biological advantages of working with Port-Orford-cedar. Over 12,500 parent trees have been selected from throughout the range of the species, and have undergone the first level of screening, a stem dip test. Rooted cuttings have been produced from over 1,000 top stem dip parents and placed in a containerized breeding orchard. The top parents from the stem dip test have undergone (some still in progress or planned) a second level of testing: a root dip test, using rooted cuttings and/or seedling progenies. At least two patterns of resistance are apparent in the greenhouse trials. A subset of families has also been tested in field trials and raised bed trials. Initial results from field trials are encouraging, but further examination over a range of sites and longer time periods is needed. More information is also needed on the *P. lateralis* isolates recently found in Europe and Taiwan, particularly their virulence and aggressiveness on the resistance Port-Orford-cedar now in use in the Pacific Northwest U.S.

The range of Port-Orford-cedar has been divided into 13 breeding zones to help maintain adaptability, and containerized seed orchards are now established for several of these zones. The containerized orchards allow for rapid upgrading of the populations as new resistant parents are identified. Seed production in Port-Orford-cedar is easily accomplished within a few years of orchard establishment. Resistant seed is now available for several zones and is currently being used by a variety of organizations for restoration and reforestation needs. Future work will focus on completing the root dip testing of the initial stem dip 'winners,' examining field trials to validate seedling screening results and examine durability of resistance, and making control crosses to enhance resistance.

White Pine Blister Rust Resistance in *Pinus monticola* and *P. albicaulis* in the Pacific Northwest U.S—A Tale of Two Species

Richard A. Sniezko, **Angelia Kegley**, and **Robert Danchok**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon; rsniezko@fs.fed.us

Western white pine (*Pinus monticola*) and whitebark pine (*P. albicaulis*) are white pine species with similar latitudinal and longitudinal geographic ranges in Oregon and Washington. Throughout these areas whitebark pine generally occurs at higher elevations than western white pine. Both species are highly susceptible to white pine blister rust, caused by the non-native fungus *Cronartium ribicola*, and both have suffered extensive mortality in many parts of their range. The high susceptibility of these two species to blister rust has limited their use in reforestation and restoration. An operational program to find and utilize naturally occurring genetic resistance in western white pine has been ongoing for more than fifty years, while the program for whitebark pine has been in progress for only a decade. For both species, parent trees are rated for resistance based on performance of their seedling progeny in artificial inoculation trials. In the first cycle of selection and testing, using open-pollinated seed from selections in the forest, progeny of over 4,000 parent trees of western white pine have been evaluated for resistance, and progeny of 360 parent trees of whitebark pine have been evaluated or are currently in testing. Resistance screening of hundreds of additional whitebark pine seedling families is anticipated in the next 5 years. Seedlings are assessed annually for up to 5 years after artificial inoculation for a range of resistance types, including number of needle lesions (‘spots’), type of needle spots (normal or hypersensitive reaction), number of stem infections, type of stem infection (normal canker or bark reactions), severity of stem infection, and timing of stem infection and mortality.

Although western white pine and whitebark pine inhabit similar geographic distributions in the Oregon and Washington portions of their geographic ranges, there are both similarities and differences in their resistance to blister rust. Nearly 100 percent of both species develop needle spots in artificial inoculation trials at Dorena Genetic Resource Center. Ninety to 100 percent of the seedlings in the most susceptible families develop stem infections, usually within 8 to 24 months after inoculation. Progeny of a small percentage of western white pine parents from western Oregon show signs of a hypersensitive type reaction in the needles (HR), but no whitebark pine with HR have been noted to date. In western white pine, HR appears to be present only in western Oregon and parts of California. Seedling families of western white pine with moderate levels of other types of resistance such as bark reaction are relatively rare and appear to be somewhat scattered throughout the range in Oregon and Washington. Only a few non-HR families of western white pine show canker-free levels of >30 percent in the seedlings testing at DGRC. The early work in whitebark pine indicates that the highest levels of resistant families occur in the Cascade Range from central Oregon to central Washington, with a much lower frequency of resistance in eastern Oregon. In some of these areas, many of the families have 20 to 50 percent or more of the seedlings canker-free, and often the cankered seedlings in these same families live longer than cankered seedlings from southern or eastern Oregon. The frequency of families with moderate levels of resistance, notably a higher percentage of canker-free seedlings, is higher in whitebark pine that in western white pine.

It is encouraging that both western white pine and whitebark pine show family variation in resistance to *C. ribicola*. For western white pine, seed orchards have been established, breeding to increase resistance is underway, and a large number of field trials have been established. For whitebark pine, land managers will be notified about which parent trees show resistance to facilitate additional seed collection for restoration, and a few small field trials have recently been established.

Cross-Resistance against Diseases and Insects in a Breeding Population of *Pinus pinaster*

Alejandro Solla, **María Vivas**, and **Elena Cubera**, Universidad de Extremadura, Virgen del Puerto 2, 10600 Plasencia, Spain; **Luis Sampedro** and **Xoaquín Moreira**, Centro de Investigación Forestal de Lourizán, Apdo 127, 36080 Pontevedra, Spain; **Esther Merlo**, CIS Madeira, Ourense; and **Raúl de la Mata** and **Rafael Zas**, Misión Biológica de Galicia, CSIC, Carballeira 8, 36143 Pontevedra, Spain; rzas@cesga.es

The different plant defenses existing within a given taxon have been commonly assumed to trade-off among each other because of both evolutionary and physiological reasons. On one hand, the higher the efficiency of a single defensive trait, the lower the selective pressure for other redundant defenses. Besides, production of multiple defenses might be constrained by the resources required for maintaining, at a time, high growth and reproductive rates. However, different defensive traits do not need to be always redundant, and plants may require the combination of multiple defenses to contest all possible enemies. This is particularly the case in forest trees, which due to their large size and long-life span, are apparent targets for a wide range of enemies. Theory predicts that these apparent species will tend to evolve generalized defenses that are effective against a wide array of herbivores, and that will function by means of quantitatively reducing the edibility or nutritional quality of the plant, and thus the herbivore performance. Empirical evidences for this expected cross resistance in forest trees are, however, lacking.

Here, we have put together data from several independent experiments on the same genetic material to explore whether resistance to different pests and diseases are genetically correlated in *Pinus pinaster*, an emblematic forest species of SW Europe. We also aimed to determine at what extent resistance is based on quantitative defensive traits, and may be genetically related to other fitness correlates such as growth and cone production. We compiled data of several field and greenhouse experiments designed for screening resistant genotypes to insects and diseases and for exploring the role of different phenotypic traits in tree resistance. Plant material consisted of open-pollinated families of 39 maternal plus trees selected for superior growth and form in mature plantations of *P. pinaster* in NW Spain. The selected trees have been established in several clonal seed orchards that provide genetically superior seeds for reforestation in the region. The pathogens tested included *Fusarium circinatum*, *F. oxysporum*, and *Armillaria ostoyae*, and the insects tested included *Thaumetopoea pithyocampa*, *Hylobious abietis*, and *Dioryctria sylvestrella*, all of them causing important damage to *P. pinaster* forests in the area. Quantitative resistance traits examined were the constitutive levels and inducibility potential of diterpene content in the stem, leaf total phenolics, and leaf condensed tannins. Seed weight, early growth, cone production, and root morphology were also considered as fitness related traits. The relationships between parameters were examined by Pearson's correlation between parental breeding values estimated independently from each experiment. Principal Component Analysis was also applied in order to reduce the dimensionality of the data, and to enable clustering and identification of putative defence syndromes.

Results do not support the initial hypothesis, and lack of cross-resistance appears to be the norm in this pine species. Although some significant correlations revealed a relevant role of some defensive traits protecting the plant against specific pests or pathogens, the quantitative defenses studied cannot be classified as generalized defenses against all enemies. Rather, pines appear to be jacks of-all-trades, masters of all, and may need to combine multiple defensive traits to fight against the wide range of potential enemies.

Breeding for Resistance in Norway Spruce to the Root and Butt Rot Fungi *Heterobasidion* spp.

Gunilla Swedjemark and **Bo Karlsson**, The Forestry Research Institute of Sweden,
Ekebo 2250,SE-26890 Svalöv, Sweden; gunilla.swedjemark@skogforsk.se

The root pathogen *Heterobasidion spp.* is one of the very few fungal pathogens that are able to infect living conifer roots of all ages. It causes serious damage to coniferous forests throughout the Northern Hemisphere and can rapidly destroy a stand under optimal conditions. The fungus spreads both by spores and by vegetative growth: spores land on recently exposed wood, such as freshly cut stumps, germinate and form a mycelium, which colonizes the stump and its roots. It then infects healthy trees by growing via root contacts and grafts between stumps and trees and then extends through the roots and up into the stem

Results from our previous studies (inoculation of differently aged clonal material and screening of naturally infected clone stands) of resistance in Norway spruce to *Heterobasidion spp.* are promising: There is significant genetic variation in resistance to fungal growth and in spore susceptibility in Norway spruce. The results show the same pattern in inoculation experiments and in naturally infected Norway spruce clone stands. The genetic variation and the heritability are large enough for practical breeding purposes and there is no adverse correlation between *Heterobasidion* infection and growth traits. The economic calculations show that losses due to root rot are significant; there is an economic incentive to use plant materials which are more resistant than those used today.

A significant problem when studying resistance in forest ecosystems is the relevance of results from artificial trials in young material versus the natural situation. Are we really measuring actual resistance to the pathogen and what will happen during a rotation period? Recent research on developing the testing methods have demonstrated that we need to identify new markers and early traits for screening young material before we can include resistance to the pathogen in existing breeding programs. In an ongoing project we are analyzing the differences in the chemical defense and the transcriptome of *Heterobasidion spp* infected *Picea abies* clones with different susceptibility to *Heterobasidion spp* infection. The selected clones have previously shown high or low susceptibility to natural infections of *Heterobasidion spp*, thus providing an interesting material to elucidate the role of transcriptional changes and secondary metabolite in *Picea abies* defense responses. Preliminary results show that the quantity of certain substances involved in defense mechanisms are specific for resistant clones. For example, astringin may be a constitutive chemical marker for resistance.

Latent Infection by *Fusarium circinatum* Influences Susceptibility of Monterey Pine Seedlings to Pitch Canker

Cassandra L. Swett and **Thomas R. Gordon**, Department of Plant Pathology, University of California, Davis, California; clswett@ucdavis.edu

Pitch canker, caused by *Fusarium circinatum*, is a serious disease affecting *Pinus radiata* (Monterey pines) in nurseries, landscapes, and native forests. A typical symptom of pitch canker is canopy dieback resulting from girdling lesions on terminal branches. More extensive dieback can result from coalescing lesions on large branches or on the main stem of the tree. The severity of disease depends, in part, on susceptibility of the individual tree. Some will suffer no more than a few infected branch tips, whereas others sustain extensive damage and may ultimately die from the disease, often in conjunction with other forms of stress. However, some trees that become severely diseased eventually recover and the absence of new infections has been attributed to systemic induced resistance. To date, induced resistance in Monterey pine has been examined only in mature trees but the disease can also affect seedlings, with potentially significant impacts on regeneration. Although the pitch canker pathogen can be a cause of mortality in seedlings, those that are not killed may remain infected without showing symptoms. The present study was undertaken to determine if seedlings with symptomless infections manifest systemic induced resistance to pitch canker.

To establish symptomless-infected seedlings, seed was grown in sand infested with either 100 or 1,000 propagules per gram, referred as the low and high inoculum treatments, respectively. Control seedlings were grown in non-infested sand. Six months after sowing, symptomless seedlings representative of each treatment were challenge inoculated by depositing a suspension of $1.25\text{x}10^4$ spores per ml into a 1.0 mm diameter wound on the main stem. Susceptibility to pitch canker was quantified as the length of the lesion developing at the site of inoculation.

The results showed that resistance was elevated in seedlings previously exposed to the pathogen. Lesions were 32-54% shorter than controls in the low inoculum treatment, and 63% shorter in the high inoculum treatment. Similar results were obtained in experiments using 18-month-old seedlings, suggesting that systemic induced resistance can persist as seedlings mature. If subsequent studies confirm these findings, we aim to determine if similar effects can be documented to occur under natural conditions. If so, it will be of interest to know what factors determine whether infections at the seedling stage result in death or a longer-lasting association that may enhance resistance to subsequent challenge by the pitch canker pathogen.

Genetic Variation of Lodgepole Pine Physical and Chemical Defenses Associated with Each Step in the Host Selection Behavior Sequence by Mountain Pine Beetles

Kimberly Wallin, The University of Vermont and State Agricultural College, The Rubenstein School Environment and Natural Resources, Burlington, VT and USDA Forest Service, Northern Forest Research, South Burlington, VT; kwallin@uvm.edu; **Dan Ott**, The University of Vermont and State Agricultural College, The Rubenstein School Environment and Natural Resources, Burlington, VT; and **Alvin Yanchuk**, British Columbia Ministry of Forest and Range, Victoria, B.C. Canada and Scion, Private Bag 3020, Rotorua, New Zealand

Host selection behavior by insects is a series of direct and indirect behavioral responses to hosts. The sequence of steps in host selection behavior includes habitat location, host location within the habitat, host acceptance, and host use. In addition, there is a well-known hierarchy in host tree defensive responses to each sequential step. However, there is less known about the heritibility of the traits involved in hieratical interactions and their ecological function.

The objective of the study is to provide a framework to observe the multiple steps in host selection behavior of mountain pine beetle (MPB) *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) and its associated fungus *Grosmannia clavigera* (formerly known as *Ophiostoma clavigerum*) and how they interact with the heritable physical and chemical defense characteristics of lodgepole pine *Pinus contorta* Douglas ex Louden var. latifolia Engelm. ex S. Watson (Pinales: Pinaceae). We empirically tested these interactions using provenance plantings of lodgepole pines. Many tree variables associated with the sequence of steps in host selection behavior were heritable and correlated with the subsequent step in the sequence. These results suggest that lodgepole pine and MPB have co-evolved resulting in specific families of lodgepole pine possessing more optimal levels of specific physical and/or chemical traits that are deterrent or toxic to MPB.

Recent Advances in the Selection Breeding for Resistance to Pine Wilt Disease in China

Xiaoqin Wu, **Jianren Ye**, **Fengmao Chen**, and **Xiaolei Ding**, Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing Forestry University, Nanjing 210037, China; jrye@njfu.com.cn

The pine wilt disease caused by *Bursaphelenchus xylophilus* was first found in Nanjing, China in 1982. Up to now, the epidemic has spread to more than 10 provinces such as Jiangsu, Anhui, Guangdong, Zhejiang, Shandong, Fujian, and Jiangxi, etc. Annually 100,000 ha are affected, which may lead to the death of 50 million pine trees and result in a $2.5 billion direct financial loss, as well as a $5 billion indirect financial loss. Recently, the spread situation has also been severe, with 34 million ha of south pine trees, natural landscape, and eco-environment facing a serious threat from the disease. The disease has become the most dangerous forest problem in China. Some southern pine trees like: Masson pine (*Pinus massoniana*), Japanese red pine (*P. densiflora*), and black pine (*P. thunbergii*) are moderately or highly susceptible to pine wilt disease. Because of that, various conventional approaches cannot successfully control the spread situation. That is to say, it is of great importance to select and breed pine wood nematode (PWN)-resistant trees. The major work undertaken in China includes:

1. Provenance selection of resistant pine trees. Masson pine (*P. massoniana)* from 19 provinces in south China with a total number of 40 provenances were selected as inoculation materials in 1995-1998. After artificial inoculation, the disease-sensitive index and death rate of three provenances were zero, and no symptoms of pine wilt disease were observed after another 4 years of continuous inoculation, so those pine trees from three provenances (Guangxi 2, Guangxi 3 and Guangdong 5) were predicted to be PWN-resistant. Analysis of the resistant trees indicated that they all had a higher amount of phenol relative to moderately or highly susceptible trees. Phenol compounds extracted from pine trees were added into nematode culture substrate. After several days of incubation, an obvious inhibition on nematode was found among resistant pine trees compared to that of moderately or highly susceptible pine trees.

2. Individual resistant pine selection. The “Resistance Breeding Plan for Pine Wood Nematode in Anhui Province” was carried out by Japan-China technical cooperation from 2001 to 2006. Mother trees of Masson pine were chosen from survivors in 10 heavily damaged stands in Anhui Province. The "GZ3B" PWN isolate was used for the inoculation test since it has the strongest pathogenicity. Sixty thousand seedlings from 334 families were evaluated for their resistance in the first inoculation test. The second inoculation test was carried out on the seedlings which survived the first inoculation. Of those tested, 1,209 seedlings from 251 families were finally selected to be PWN-resistant candidates. According to inoculation results, some of the surviving trees were used to establish resistant stands in Quanjiao (0.7ha) and Chuzhou (0.8ha), Anhui. These resistant pine stands manifested a better growth index and pine wilt disease resistance. Grafting propagation was undertaken among 146 PWN-resistant candidate families in 2008; 5,315 individuals were successful. The resistance of the grafted trees was tested. Sixty percent of them surpassed the resistance of *P. taeda*. In addition, DNA atlas and database of all those 251 resistant candidate families were established to distinguish the individual pine trees from the whole families. Further inoculation analysis is going to be done soon to determine the resistant clones, and a clonal seed orchard of resistant *P. massoniana* will be established.

3. Introduction of resistant pine tree families. Since 2003 we introduced 27 resistant Japanese red pine (*P. densiflora*) and 13 resistant black pine (*P. thunbergii*) families from Japan. A 3 ha seed orchard of resistant pine trees was established. It contained 923 resistant Japanese red pine (*P. densiflora*) and 959 resistant black pine (*P. thunbergii*). Six-year-old pine trees from 40 introduced *P. densiflora* and *P. thunbergii* families were tested for their disease resistance in 2010. The results showed that the introduced pine trees exhibited satisfactory PWN-resistance. Furthermore, there were seven highly resistant families in *P. densiflora* compared to nine in *P. thunbergii*, and 19 moderately resistant families in *P. densiflora* compared to three in *P. thunbergii*.

4. Research on resistant Japanese red pine, black pine, and Masson pine tissue culture technique. From 2005 we started micropropagation of resistant *P. densiflora*, *P. thunbergii*, and *P. massoniana*. After several years of extensive research, we have primarily established a resistant *P. densiflora*, *P. thunbergii*, and *P. massoniana* rapid propagation technique and succeeded in acquiring *P. densiflora* and *P. thunbergii* tissue culture plantlets. Plantlets of the resistant *P. densiflora* were utilized to establish pine plantations in Jurong, Jiangsu in 2009. The growth situation and resistance of the pines in the plantations will be monitored.

Tree Breeding for Pest Resistance for the Next 50 Years: the Search for Cross Resistance?

Alvin Yanchuk, British Columbia Ministry of Forests, Lands and Mines, Tree Improvement Branch, 727 Fisgard Street, Victoria, BC CANADA V8W 1R8; alvin.yanchuk@gov.bc.ca

Research activities aimed at developing resistances to pests (insect, pathogens, mammals) in forest trees can be documented back now over 5 decades. While a substantial body of research has been published on resistances in forest trees, not much of this work has made its way into applied tree improvement programs. There are several reasons for this, e.g.; (i) a new or interesting incursion is noticed, and studies are developed that work on materials not related to a breeding program; (ii) adequate infection or artificial inoculation techniques are too expensive and a large enough population cannot be screened, and (iii) the genetic gain in resistance may not be ‘silviculturally’ useful or effective. However, a few notable exceptions are present, and important lessons should be taken from our experiences over the last 50 years.

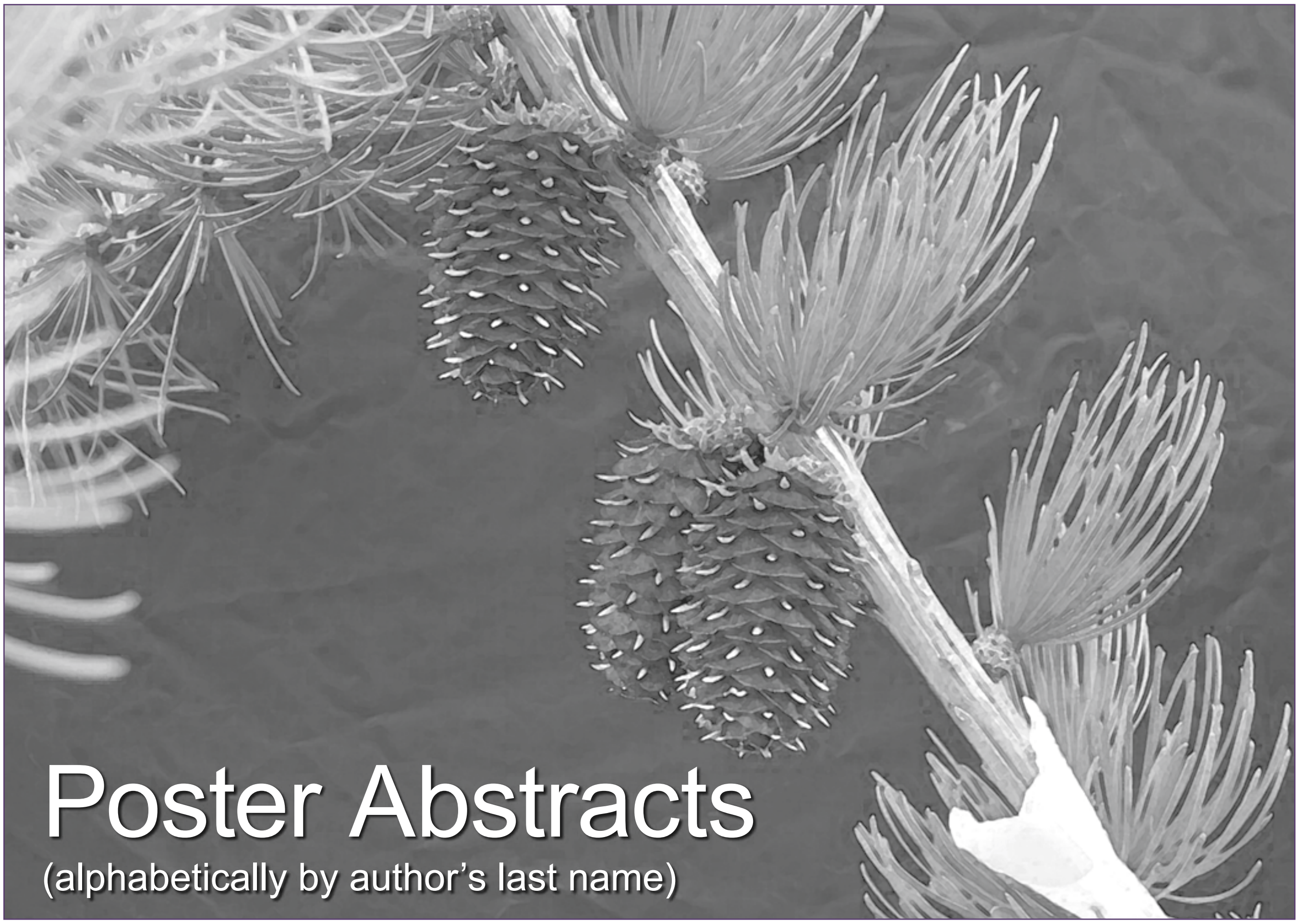
What has changed recently is the concern that pest and disease occurrences (with notable outbreaks) are increasing and are likely to continue to increase. Moreover, it might be difficult to know what particular disease or pest may become the new threat, particularly with climate change and the increased movement of goods around the world, and the subsequent introductions of exotic pests. We can go about tree breeding, in a re-active approach, as we have for the past 50 years, hoping that a genetic option can be developed within one cycle of selection and testing; however, it is not uncommon for this to take a decade or more. At some point soon, funding agencies and managers will start asking how useful is pest resistance breeding as a strategic and viable option for future forest health and biosecurity programs. The other approach may be that we more aggressively look for situations of cross resistance against future ‘classes of threats’ rather than the traditional “one-tree/one-pest” program(s) of the past. The risk we face here is that some pests (diseases in particular) will be quite host or genotype specific and we may provide no additional protection to future crops. While this is a risk, it seems that, particularly with a growing body of research showing substantial cross resistance is present, breeding trees which may have more “durable” forms of resistance is probably a timely endeavor in forest tree breeding. At the very least, it should be seriously considered in particular programs.

Breeding *Eucalyptus* for Disease Resistance

Edival A. V. Zauza, Suzano Papel e Celulose; edivalzauza@suzano.com.br; **Acelino Couto Alfenas**, Departamento de Fitopatologia/BIOAGRO, Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, MG, Brazil; and **Lúcio Mauro da Silva Guimarães** and **João Flávio da Silva**, Suzano Papel e Celulose

The *Eucalyptus* plantations cover about 1.5% of the agricultural area in Brazil and contribute to 4% of GDP and 3% in exports of forest products. Technological and research advances in silviculture and genetic improvement have increased productivity up to 80 m^3 of wood/ha/year, with an average of 35 to 45 m^3/ha/year. However, the greatest challenge to the introduction and commercial use of exotic species is the adaptation of species to climatic conditions. Even when maladapted species are able to survive, they are subjected to continuous stress that limits the expression of their maximum genetic potential. The incidence of insects and diseases frequently represent another limiting factor, because the exotic tree species have not co-evolved with most of the local insect and pathogen pests. In recent decades, the expansion of *Eucalyptus* plantations in Brazil has been frequently associated with frequent disease outbreaks. Prominent diseases, such as ceratocystis wilt, ralstonia wilt, rust, and bacterial fungal leaf blights have limited the establishment and growth of plantations that contain susceptible species or clones in regions favorable to disease establishment. Plantations of hybrid clones or elite-resistant varieties represent the most efficient strategy for disease control. In general, fungicide application is restricted for the control of nursery diseases, and rarely for rust control in the field. The *Eucalyptus* clonal forests offer potential to establish homogeneous, disease-free stands of high yield. Currently, the hybrid clones of *E. urophylla* × *E. grandis* ("urograndis") are among the most planted material in Brazil because of their high adaptability, wood quality for pulping, and resistance to chrysoporthe canker. However, other interspecific hybrids have been developed in an attempt to incorporate specific traits from other species, such as drought and frost tolerance, disease resistance, and high pulp yield. Primary breeding strategies are based on selecting species/provenances, coupled with individual genetic selection within populations to capture the natural variability among and within populations. The recurrent selection method and its variants are applied to genetic improvement of *Eucalyptus* to obtain new clones of interspecific and intraspecific hybrids. Backcrosses, mutation induction, and polyploidy have also been applied to develop new genotypes. These strategies help ensure the sustainability of commercial plantations and the continual contributions from the improvement program through the selection in advanced generations and superior clones. With interspecific breeding to incorporate contrasting traits, it is usually necessary to make continued backcrosses to the species of interest as the recurrent donor to recover the desired forest features and industrial properties. For each breeding generation it is essential to perform disease screening under controlled conditions to select disease-resistant genetic materials for commercial cloning or identifying sources of resistance for other crosses. For disease-resistance evaluations, reliable inoculation protocols are essential. In recent years, approximately 90% of elite-clones were susceptible to at least one of the main diseases tested using artificially inoculations under controlled conditions. Determining the genetic basis and the mode of inheritance of disease resistance is an essential step to obtain disease-resistant plant material for the breeding program. The breeding strategy varies according to the inheritance model. To reduce losses caused by rust, efforts have been conducted to select and plant rust-resistant clones and determine the mode of inheritance. In *E. grandis* the segregation pattern of resistance to rust (*Puccinia psidii*) is controlled by one locus with major effect, *Ppr*-1 (*Puccinia psidii* resistance, gene 1), which is tightly linked to RAPD marker AT9-917. Controlled inoculations of other families allowed the identification of a homozygous, rust-resistant mother parent of *E. grandis*, currently used to obtain rust-resistant progenies, regardless of pollen source.

Screen tests for resistance to Ceratocystis wilt showed a continuous variation in resistance, ranging from highly susceptible to highly resistant, which indicates a pattern typical of horizontal resistance. Additional studies are needed to determine the genetic basis, the new sources of resistance, and the inheritance patterns of resistance of *Eucalyptus* spp. to other diseases. Furthermore, a better understanding of the population genetic structure of pathogens is needed to determine the role of pathogen variability in disease resistance that is targeted by the breeding strategies.



Poster Abstracts

(alphabetically by author’s last name)

Development of Weevil Resistant Sitka Spruce in British Columbia, Canada

René I. Alfaro, Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 W. Burnside Rd., Victoria, British Columbia, Canada, V8Z 1M5; RAlfaro@nrcan.gc.ca; and **John N. King**, British Columbia Ministry of Forests and Range, Research Branch, P.O. Box 9519, Stn. Prov. Govt., Victoria, British Columbia, Canada, V8W 9C2

Sitka spruce (*Picea sitchensis* (Bong.) Carr), white spruce (*P. glauca* (Moench) Voss), and Engelmann spruce (*P. engelmanni* (Parry)) plantations in British Columbia, (B.C.) Canada, have come under serious attack from the white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae). This pest destroys the terminal leader of the tree, causing serious growth losses and stem deformities. Since 1993, we have conducted a series of replicated screening trials to search for spruce with resistance to the white pine weevil. These included tree selections from across the range of the species from Alaska to California. In order to accelerate the screening process, local insect populations were augmented by adding reared weevils to the site. Results indicate that screening for weevil resistance can be effectively accomplished by using the weevil population augmentation method. Consistent selections could be obtained in trials with as few as 10 replicates per family. If weevil attack rates of 50% cumulative attack are obtained, then consistent selections may be obtained in as little as four years, which is a fairly quick turnaround time for studying resistance in trees. These trials allowed us to delineate geographic areas with high frequency of resistant trees, select and breed resistant trees, and produce the first Sitka spruce F1 progeny generation resulting from controlled-crosses of parents with demonstrable weevil resistance. We have used these populations to calculate the heritability of resistance and study nature of the underlying resistance mechanisms. Based on our results, resistant seed can now be obtained from local seed orchards and BC seed dealers and resistant trees are now operationally planted in BC.

Screening for Resistance to Fusiform Rust in Southern U.S. Forest Trees

Josh Bronson, USDA Forest Service, Resistance Screening Center, 1579 Brevard Road, Asheville, NC 28805; jjbronson@fs.fed.us

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, U.S. 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 things 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.

Variation in the Development of Current Season Needle Necrosis on Noble, Nordmann, and Turkish fir Christmas Trees in the U.S. Pacific Northwest

Gary A Chastagner and **Kathy Riley**, Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA 98731; chastag@puyallup.wsu.edu; and **Chal Landgren**, Oregon State University, North Willamette Research and Extension Center, 15210 NE Miley Rd, Aurora, OR 97002

Current season needle necrosis (CSNN) is a poorly understood disease that affects a number of *Abies* spp. that are grown as Christmas trees. CSNN has been reported on noble (*A. procera*), Nordmann (*A. nordmanniana*), and grand fir (*A. grandis*) in Europe and these species, plus Turkish fir (*A. bornmuelleriana*), in the U.S. Pacific Northwest. In 2002 and 2004, a series of replicated genetic field trials were established at the Washington State University Research Center in Puyallup, WA. This is a low elevation site that is very conducive to the development of CSNN. These trials contain 91 sources of noble fir, 15 sources of Nordmann fir, and 4 sources of Turkish fir. Over a 4- to 6-year period, symptom severity was rated on a scale of 0 to 10 during late summer/early fall.

There was significant yearly variation in the overall average CSNN ratings for the trees in these trials. Over 6 years, ratings for the 37 sources of noble fir in the 2002 planting ranged from an average low of 1.5 in 2009 to an average high of 3.4 in 2006. Over 5 years, the yearly overall average rating for the 54 sources of noble fir in the 2004 trial ranged from a low of about 1.1 in 2005 to a high of 3.3 in 2008. Compared to the noble fir, limited CSNN developed on the Nordmann and Turkish fir trees. Over 4 years, the average ratings for the sources of Nordmann and Turkish fir ranged from 0.3 to 0.9.

In the 2002 noble fir plot, the percentage of trees that were resistant to CSNN ranged from 5 to 80% depending on the source. In the 2004 noble fir plot, the range was 4 to 65%. No CSNN developed on over 80% of the trees from seven of the sources of Nordmann/Turkish fir. Although there was significant yearly variation in the severity of CSNN, Spearman rank order correlation analysis indicated that there was a highly significant correlation between the yearly susceptibility rankings of the noble, Nordmann, and Turkish fir sources in each of these plots.

The effect of site on CSNN development was examined by comparing the severity of CSNN development on trees in the 2004 noble fir genetic test plot at Puyallup to the same sources at Silver Mountain Christmas Trees near Sublimity, OR in 2009. The overall plot CSNN rating for the trees at Silver Mountain was much lower than Puyallup. Over 94% of the trees at Silver Mountain had no CSNN, compared to only 23% at Puyallup. Even though much less CSNN developed at Silver Mountain, Spearman rank order analysis indicated that there was a highly significant correlation in susceptibility rankings of the sources at both sites. Regression analysis of CSNN ratings from Nordmann and Turkish fir planted in a “valley” and “hill” at Puyallup also indicated that there was a highly significant correlation between the 4-year average ratings of the individual sources at the valley and hill sites.

These results indicate that there is considerable variation in the susceptibility of different sources of noble, Nordmann, and Turkish fir to CSNN and that the relative susceptibility of different sources of trees to CSNN can be determined after one or two years at conducive sites.

Molecular Characterization of Red Gum Lerp Psyllid (*Glycaspis brimblecombei*) Pest Insect Population through ITS1 Marker

Suzana Regina Silveira Costa, Instituto de Biociências, Depto de Genética/CAGEN, UNESP, Botucatu, SP - 18618-970; costasrs@ibb.unesp.br; **Carlos Frederico Wilcken** Faculdade de Ciências Agronômicas, Depto de Defesa Fitossanitária, UNESP, Botucatu, SP - 18610-307; and **Celso Luis Marino**, Instituto de Biociências, Depto de Genética/ CAGEN, UNESP, Botucatu, SP - 18618-970

The *Eucalyptus* genus is derived from Australia and was introduced in Brazil in 1856, where it found favorable edaphoclimatic conditions for development. Currently, Brazil has the largest *Eucalyptus* crop area in the world, mainly aimed at production of pulp, paper, fiber boards, charcoal, and energy. *Eucalyptus* plantations occupy large areas, forming monocultures that facilitate infestation with pest insects. In 2003, the occurrence of red gum lerp psyllid, *Glycaspis brimblecombei*, Moore (Hemiptera, Psyllidae) was detected in Brazil, which causes defoliation in *Eucalyptus spp.* plantations. Because of its high capacity for adaptation and reproduction, and short lifespan, a quick spread of the plague was observed in the country. As a rule, psyllids feed on host plant sap and have a strong preference for new leaves and sprouts. The *Glycaspis* genus is native of Australia and is characterized by feeding exclusively on *Eucalyptus* species. An attack by *G. brimblecombei* causes discoloration of leaves, induces the appearance of sooty mold, reduces the photosynthetic zone, dries the tops, slows down the growth, and may cause expressive mortality in trees after consecutive attacks. The origin of such incursion remains uncertain, but probably the organisms now settled here are native of countries in North and Central Americas.

Entomologists are struggling to typify the different populations of red gum lerp psyllids due to the low morphological variation that such organisms present. Nowadays, molecular biology tools are key for a better understanding on pest population variability. Upon the advent of polymerase chain reaction (PCR) and DNA sequencing, techniques for ribosomal DNA studies are becoming somewhat easy to apply, and low-priced, and are now used to a great extent in phylogenetic studies at all taxonomic levels, enabling a better evaluation of molecular characters homology. In this study, four populations of *G. brimblecombei* were used, amounting to 75 organisms. Such populations are native of Quadra, Pedra Bela, and Botucatu (SP), and one of them is lab-grown. In order to extract DNA, protocol with resin Chelex® 100 (Sigma) was followed. The extracted DNA quantity and quality certification was conducted by using spectrophotometer NanoDrop®. A specific oligonucleotide from ribosomal gene ITS1 (*Internal Transcribed Spacer*) was developed to amplify fragments with approximately 600bp that reach portion 3' in gene 18S, the whole ITS1 zone, and portion 5' in gene 5.8S.

Obtained fragments were submitted to sequencing, and generated consensus sequences were compared with those available at GenBank, showing a 96% rate of similarity to the 18S ribosomal gene (U06482) of *Trioza eugeniae* (Hemiptera: Psyllidae), which is a phytophagous insect of sucking habit as well. Tests conducted with software ChromasPro version 1.5 evinced that the existing intra- and inter-population variation is too low. This narrow genetic base demonstrates that these are homogeneous populations, confirming the recent introduction coming from a genetic drift. Comparison tests on different populations from North and Central Americas must be conducted in the sense of inferring on the exact source of the red gum lerp psyllid found in Brazil.

Frequency of Hypersensitive Reaction and Stem Infections in a Large Full-sib Family of *Pinus monticola*

Robert Danchok, **Richard A. Sniezko**, **Sally Long**, and **Angelia Kegley**, USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR 97424; rsniezko@fs.fed.us; and **Jun-Jun Liu**, Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, British Columbia, V8Z 1M5, Canada

Western white pine (*Pinus monticola*) and the other native white pine species of North America are highly susceptible to white pine blister rust, caused by the non-native fungus *Cronartium ribicola*. Research and applied programs to develop resistant populations have been underway for more than 60 years. Several types of resistance have been noted in white pines, but the most documented is the hypersensitive reaction (HR) in needles. HR has generally been described as a form of complete resistance, conditioned by single separate dominant genes in the three species for which it has been described: sugar pine, western white pine, and southwestern white pine. In most cases, seedlings with HR show needle infections, but no stem infections. However, it has been previously noted, at least for sugar pine, that stem infection can occur, but development of the pathogen is often stopped with little or no damage to the seedling.

Most studies describing HR utilized small numbers of seedlings per family. The study reported here summarizes the recent assessments on thousands of seedlings from a single full-sib family of *P. monticola*, a cross between two parents heterozygous for Cr2, the gene conditioning HR in western white pine. Artificial inoculation was very successful with 100 percent of seedlings showing needle spots. We report on the frequency of HR in this family, the frequency of stem infections from seedlings showing HR and non-HR (susceptible), and the number of stem infections on susceptible and HR seedlings. Progress of the stem infections on HR seedlings is still being monitored.

The Evaluation of Biological Control Potential of *Ophiostoma novo-ulmi,* Causal Agent of "Dutch Elm Disease" by *Bacillus subtilis* In Vitro

Mehrdad Ghodskhah Daryaei and **Shghaygh Zolghadry**, University of Guilan, Forestry Department, Somehe Sara, P.O. Box 1144; mehrdad_daryaei62@yahoo.com

Bacteria play an important role in biological control of plant diseases, bacillus spp. with extensive existence in environment, tolerance of high temperature and formation of resistant spores, are important and safe for biological control. Recent findings about the existence of this bacterium as endophyte trough plant vessel such as maple have opened up new possibilities for using of this bacteria for biological control of vascular diseases. The antagonistic effect of three isolates of *bacillus subtilis* on *ophiostoma novo-ulmi*, the causal agent of Dutch elm disease, was studied *in vitro.* Antibiosis of antagonistic mechanism as volatile metabolites and culture filtrate was evaluated. Microscopic studies indicated that all of bacillus isolates caused growth inhibition and deformation of mycelium of causal agent by production of antifungal metabolites. All isolates could produce antifungal volatile metabolites and growth inhibitors in PDA which decreased mycelia growth and spore germination of the fungus significantly. In most experiments, isolate B7 was better than other isolates in antagonistic activity.

Resistance to *Ophiostoma novo-ulmi* in *Ulmus carpinifolia* var. *umbraculifera*, *Ulmus glabra*, and *Ulmus parvifolia*

Mehrdad Ghodskhah Daryaei and **Shghaygh Zolghadry**, University of Guilan, Forestry Department, Somehe Sara, P.O. Box 1144; mehrdad_daryaei62@yahoo.com

Dutch elm disease is one of the most important vascular diseases, causing sever epidemics. With regard to occurrence of aggressive species and races of the disease, nowadays applying resistant species and varieties of elm is the best method to manage practices against this disease. Estimating the wilt and defoliation percent of saplings of *Ulmus carpinifolia* var. *umbraculifera* and *U. glabra* showed very high susceptibility rates and almost die-off after eight weeks, but saplings of *U. parvifolia* showed a few wilt percent following the same time laps. This survey has indicated isolates of *O. novo-ulmi* have very high pathogenesis ability, in spite of the fact that Chinese elm indicated high relative resistance.

Investigation on “Dutch Elm Disease” in Some Forest Areas of Guilan Province

Mehrdad Ghodskhah Daryaei, **Shghaygh Zolghadry**, and **Javad Torkaman**, University of Guilan, Forestry Department, Somehe Sara, P.O. Box 1144; mehrdad_daryaei62@yahoo.com

Forest elm trees of the Guilan province, such as Mazandaran, Golestan, and Arasbaran, have declined due to the causal agent of “Dutch Elm Disease” during the last two decades. Since decline of *Ulmus glabra* Huds. and *U. carpinifolia* Borkh. (two important elm species) had been reported in recent years, some Guilan forest areas were continuously inspected for “Dutch Elm Disease”, from 2008-2010. During these inspections, different symptoms such as; yellowing, wilt, defoliation, dieback, complete or partial decline, and, most importantly, feeding symptoms of bark beetles (the only source of pathogen transmission), were observed frequently. Samples were transferred to laboratory. The results indicated that the causal fungus exists in a predominance of areas. In this study, *Ophiostoma novo-ulmi* isolates showed the highest frequency. Investigating some of the morphological and physiological characteristics between *O. ulmi* and *O. novo-ulmi* also proved that most isolates of *O. novo-ulmi* have striate, fibrous to petaloid colonies with more daily growth rates (3.6 mm/day), fewer optimal growth temperatures (20-22° C), and dry weight of biomass, distinguished from *O. ulmi* with mean daily growth rates (2.5 mm/day).

A Survey on Histopathology of *Ulmus glabra* and *Ulmus carpinifolia* Inoculated by *Ophiostoma nova-ulmi* Fungs

Mehrdad Ghodskhah Daryaei, **Shghaygh Zolghadry**, and **Javad Torkaman**, University of Guilan, Forestry Department, Somehe Sara, P.O. Box 1144; mehrdad_daryaei62@yahoo.com

Dutch elm disease is one of the most important vascular diseases of elm. However, it has always being considered to survey its different perspectives such as distribution, biology, pathogencity, histopathology, disease management, etc. in order to investigate host-pathogen interaction. This research was carried out with title survey on histopathology of two elm species, *Ulmus glabra* and *U. carpinifolia* inoculated by *Ophiostoma novo-ulmi* against seedlings. Transversal sections were prepared, and after different stages of staining, microscopic slides were prepared. The results of microscopic investigations showed that the causal agent of Dutch elm disease has caused disorder in physiology and flow of vascular sap by spreading into vessels, obstructing xylem vessels, and destroying them. The results also showed that seedlings with tylose production caused obstruction of vessels and prevented spreading of the pathogen.

Operational Disease Screening Program for Resistance to Vascular Wilt in Hawaiian Koa

Nick Dudley, Hawaii Agriculture Research Center, Forestry, Honolulu, HI; ndudley@harc-hspa.com; **Robert James**, Plant Disease Consulting Northwest, Vancouver, WA; **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR; **Phil Cannon**, USDA Forest Service, Forest Health Protection, Vallejo, CA; and **Aileen Yeh**, **Tyler Jones** and **Michael Kaufmann**, Hawaii Agriculture Research Center, Forestry, Honolulu, HI

Koa (*Acacia koa* A. Gray) is a valuable tree species economically, ecologically, and culturally in Hawaii. With significant land use change and declines in sugarcane, pineapple, and cattle production, there is an opportunity and keen interest in utilizing native koa in reforestation and restoration efforts. The presence of vascular wilt disease caused by *Fusarium oxysporum* f. sp. *koae* (FOXY) causes widespread mortality throughout much of koa's range. Koa wilt also causes moderate to high mortality rates in many plantings and has restricted the use of koa in many reforestation and restoration efforts. *F. oxysporum* is a relatively common agricultural and nursery fungus, but the origin of strains of FOXY virulent to koa in Hawaii is unknown.

A statewide survey was conducted to determine distribution of koa wilt disease across the four main Hawaiian Islands: Kauai, Maui, Oahu, and Hawaii. A total of 386 samples were taken at 46 different sites covering approximately 13,830 acres of natural and planted koa forest. Koa trees and seedlings infected by *F. oxysporum* were found on all of the major islands in forest tree seedling nurseries, natural, and plantation forests. From these samples more than 500 isolates of *F. oxysporum* were obtained. Of these, 160 isolates have been tested for virulence on koa seedlings in controlled greenhouse inoculation tests. From isolate screening tests, 10 highly virulent isolates have been identified for use in screening selected koa families for disease resistance.

Identifying and developing koa populations that are genetically-resistant to virulent strains of FOXY may be the key to successful koa restoration and reforestation. Great differences in mortality among seed sources in young koa field trials planted in the 1990s was the impetus for developing a seedling screening test and investigating genetic resistance to FOXY. An operational screening program is in place and has made significant progress towards indentifying resistant koa families and producing seed for use in areas where the disease is present and causes wide spread mortality.

Between 2006 and 2011, more than 250 koa families were evaluated for their potential FOXY resistance in high-throughput greenhouse tests. Most of the seed lots came from wild populations. However, several seed lots were from survivors of family level progeny trials at the Maunawili Experiment Station. All seed lots were open-pollinated. Seedling wilting and mortality in the greenhouse was monitored over a 90-day period for each test. Seedling mortality among families varied widely (4 to 100%) and averaged 61.5%. These results indicate that while natural resistance to FOXY is low in wild populations, several highly resistant families have been identified.

A First Look at Genetic Variation in Resistance to the Root Pathogen *Phytophthora cinnamomi* Using a Range-Wide Collection of Pacific Madrone (*Arbutus menziesii*)

Marianne Elliott, **Gary Chastagner**, and **Gil Dermott**, Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA 98731; melliott@puyallup.wsu.edu; and **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR 97424

Phytophthora cinnamomi causes root disease and basal canker on a number of hardwood and conifer hosts, including Pacific madrone, a broadleaf evergreen species whose range extends from coastal British Columbia to southern California. In recent years, increasing mortality of Pacific madrone and the related shrub species manzanita (*Arctostaphylos* spp.) has been seen in California forests attributed to this disease. One-year-old seedlings from a range-wide collection of Pacific madrone seedlots from individual parent trees sampled throughout the species range were screened for resistance to *P. cinnamomi* root disease in a greenhouse study. Roots of potted seedlings were inoculated with an isolate of *P. cinnamomi* taken from a madrone tree in California and observed for wilting and browning symptoms over a 6-week period and then roots and stems of inoculated and check plants were sampled for dry weight. Additional data was collected on seedlings from the same seedlots that were grown outdoors, including seedling height, diameter, cold damage, and foliar disease severity. There was a negative correlation between root dry weight of uninoculated plants and disease severity of inoculated plants. Madrones from seed sources that had larger root mass had less severe disease symptoms. The experiment is being repeated in summer 2011 using seedlings from the same seedlots.

Range-Wide Genetic Variability in Pacific Madrone (*Arbutus menziesii*): Examining Disease Resistance, Growth, and Survival in a Common Garden Study

Marianne Elliott, **Gary Chastagner**, and **Gil Dermott**, Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA 98731; melliott@puyallup.wsu.edu; **Alan Kanaskie**, Oregon Department of Forestry, 2600 State Street, Salem, OR 97310; **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR 97424; and **Jim Hamlin**, USDA Forest Service, Umpqua National Forest, 2900 NW Stewart Parkway, Roseburg, OR 97471.

Pacific madrone is an important hardwood species in PNW forests that provides food and habitat for wildlife as well as erosion control in disturbed areas. The species has been in decline for several decades due to a combination of factors such as climate change, forest management, and several endemic fungal pathogens. It is also a host for the invasive pathogen *Phytophthora ramorum*. Pacific madrone is a wide ranging species, covering a large latitudinal gradient from California to British Columbia. Very little is known about the genetics of this species, such as its variation in resistance to pathogens and adaptation potential in the face of changing climate.

Using material from the Washington State University Pacific madrone seed collection, common garden plantings consisting of 119 families collected from 7 ecoregions will be planted in four locations in California, Oregon, and Washington. Smaller demonstration plantings with a few trees taken from a range of widely different geographic seed sources, and including other *Arbutus* species from the western states, are planned at other locations to be determined.

This project will use the range-wide collection of Pacific madrone to examine genetic variability. Specific objectives are the following: (1) Screen for resistance to multiple pathogens such as *P. ramorum*, *P. cinnamomi*, and endemic canker and foliar pathogens. (2) Examine variation in growth and adaptive traits. 3) Identify seed sources or populations that may contain individuals that are best adapted to climate change and for urban and restoration plantings.

Information about this project can be found on the web at http://www.puyallup.wsu.edu/ppo/madrone/index.htm.

Development of Screening Trials to Rank *Pinus radiata* Genotypes for Resistance to Defoliation by Monterey Pine Aphid (*Essigella californica*)

Stephen Elms, HVP Plantations, P.O. Box 385 Churchill, Victoria, Australia 3842; selms@hvp.com.au; **Peter Ades**, University of Melbourne, Victoria, Australia 3010; and **Nick Collett**, DPI Victoria, Melbourne, Victoria, Australia

Monterey Pine Aphid (*Essigella californica*) is a recent arrival in Australia, being first detected in 1998. It quickly spread throughout the national radiata pine (*Pinus radiata*) plantation estate causing seasonal defoliation and compromising tree growth in many areas. Selection of resistant radiata pine for deployment commenced in 2002 based on heritable defoliation in mature progeny trials. Further development of the breeding program requires efficient methods for screening later generation germplasm.

Glasshouse and nursery-based screening trials involving infestation of potted radiata pine plants with aphids were used to develop guidelines for operational scale screening of defoliation resistance.

Three types of plant material were tested: grafted and physiologically aged clones (10-30 years), one-year-old clones derived from somatic embryogenesis, and one-year-old seedling families.

All of the plant types examined were shown to support dramatic increases in aphid populations under suitable environmental conditions. Variability between plants within clone and between clone was high. For example, over a period of 28 days, 1 m tall grafted plants infested with 30 aphids yielded between 26 and 3,804 aphids (mean 584).

Significant and repeatable differences in aphid population counts between different radiata pine genotypes and families were obtained using trial durations of 18 to 35 days.

Treatment options including environmental conditions, seasonality, initial infestation levels, including reliance on background infestation levels only and replication requirements were examined.

Specific attention needs to be paid to managing peak aphid production levels by infestation level and experiment duration in order to minimize alate initiation.

Glasshouse screening results were tested by counting aphids on one-year-old clonal cuttings in the field, and on one-year-old stoolbeds, and both showed good correlation with glasshouse trial results (r=0.45 and 0.93).

Damage to foliage and eventual loss of foliage was observed where aphid counts were high, suggesting that selection and deployment of genetic material which does not support rapid aphid population buildup is likely to result in reduced defoliation and reduced growth losses in plantations due to *Essigella* activity.

Tree-Mediated Interactions between the Jack Pine Budworm and a Mountain Pine Beetle Fungal Associate

Nadir Erbilgin, University of Alberta, Department of Renewable Resources, 4-42 ESB, Edmonton, Alberta, Canada T6G2E3; erbilgin@ualberta.ca; and **Jessie Colgan**, University of Alberta, Edmonton, Alberta, Canada

Coniferous trees deploy a combination of constitutive (pre-existing) and induced (post-invasion) structural and biochemical defenses against invaders. Induced responses can also alter host suitability for other organisms sharing the same host, which may result in indirect, plant-mediated interactions between different species of attacking organisms. Current range and host expansion of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins; MPB) from lodgepole pine-dominated forests to the jack pine-dominated boreal forests provides a unique opportunity to investigate whether the colonization of jack pine by MPB will be affected by induced responses of jack pine (*Pinus banksiana* Lamb.) to a native herbaceous insect species: the jack pine budworm (*Choristoneura pinus pinus* Freeman; JPBW). We simulated MPB attacks with one of its fungal associates, *Grosmannia clavigera*, and tested induction of either herbivory by JPBW or inoculation with the fungus followed by a challenge treatment with the other organism on jack pine seedlings and measured and compared monoterpene responses in needle. There was clear evidence of an increase in jack pine resistance to *G. clavigera* with prior herbivory, indicated by smaller lesions in response to fungal inoculations. In contrast, although needle monoterpenes greatly increased after *G. clavigera* inoculation and continued to increase during the herbivory challenge, JPBW growth was not affected, but JPBW increased feeding rate to possibly compensate for altered host quality. Jack pine responses varied greatly and depended on whether seedlings were treated with single or multiple organisms, and their order of damage.

The Chapter Breeding Program of the American Chestnut Foundation

Sara Fitzsimmons, 206 Forest Resources Lab, Pennsylvania State University, University Park, PA 16802; **Kendra Gurney**, U.S. Forest Service Northern Research Station, 705 Spear St., South Burlington, VT 05403; **William White**, Department of Biology and Environmental Science, University of Tennessee - Chattanooga, 615 McCallie Ave., Chattanooga, TN 37403; and **Katy McCune**, Virginia Department of Forestry Central Office, 900 Natural Resources Dr., Charlottesville, VA 22903; Fred@acf.org

A unique feature of the American Chestnut Foundation breeding program is the use of volunteers to conduct most of the regional breeding that will help increase genetic diversity and preserve local adaptation in the products of our program. This effort is coordinated by the four authors of this abstract, who are employees of the Foundation. The Foundation has 16 state chapters spread over almost the entire range of the American chestnut, and 14 have active breeding programs. The chapter with the most advanced program, Pennsylvania, has been planting a B3-F2 seedling seed orchard for a number of years, and is starting to harvest B3-F3 seed from the selections. The stages of breeding at other chapters range from making B3 crosses, to making selections, to beginning to plant B3-F2 orchards.

The chapters make one backcross onto local American chestnut trees using pollen from B2 and B3 trees at the Foundation's professionally operated Meadowview facilities. Most chapters have been using the 'Graves' and 'Clapper' sources of blight resistance since these exist in about 30 unique American backgrounds each. The chapters endeavor to cross pollen from 20 backgrounds onto 20 local chestnut trees; 20 backgrounds yields a reasonable prospect of avoiding population collapse from inbreeding depression, with an inbreeding effective population size of about 72. Collectively across all units, the inbreeding Ne for 'Graves' and 'Clapper' combined exceeds 500.

Full recovery of local adaptation may not occur with only one cross onto local trees, but may enable that adaptation to be recovered by natural selection. As the number of Regional Science Coordinators has increased, and the collective knowledge of the chapters, their ability to embark on more complex breeding endeavors has increased. Increasing additional sources of resistance into 20 American backgrounds with two cycles of backcrossing should be possible.

The Regional Science Coordinators also are developing a curriculum to train volunteers to plant and monitor forest test plantings. This will need to be a focused, long-term effort should the performance of the trees be sufficient. It should be an interesting process, hopefully increasing awareness of the forest as well as producing valuable data.

Genetic Variation of Piperidine Alkaloids in *Pinus ponderosa* From a Common Garden

Elizabeth A. Gerson, **Rick G. Kelsey**, and **J. Bradley St Clair**, USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331; rkelsey@fs.fed.us

Most species of pine and spruce synthesize and accumulate variable quantities of alkaloids in their tissues. These compounds express numerous types of biological activities in bioassay and could potentially offer resistance against enemies, although this function has never been confirmed for any known enemies of pine or spruce under natural conditions.

The present study takes a complementary and intensive, common garden approach to examine genetic variation in *Pinus ponderosa* var. *ponderosa* alkaloid production. It also investigates the potential trade-off between seedling growth and alkaloid production, and associations between topographic/climatic variables and alkaloid production.

Piperidine alkaloids were quantified in foliage of 501 nursery seedlings grown from seed sources in west-central Washington, Oregon, and California, roughly covering the western half of the native range of ponderosa pine. A nested mixed model was used to test differences among broad-scale regions and among families within regions. Alkaloid concentrations were regressed on seedling growth measurements to test metabolite allocation theory. Likewise, climate characteristics at the seed sources were also considered as explanatory variables.

Quantitative variation from seedling to seedling was high, and regional variation exceeded variation among families. Regions along the western margin of the species range exhibited the highest alkaloid concentrations, while those further east had relatively low alkaloid levels. Qualitative variation in alkaloid profiles was low. All measures of seedling growth related negatively to alkaloid concentrations on a natural log scale; however, coefficients of determination were low. At best, annual height increment explained 19.4% of the variation in ln (total alkaloids). Among the climate variables, temperature range showed a negative, linear association that explained 41.8% of the variation.

Given the wide geographic scope of the seed sources and the uniformity of resources in the seedlings' environment, observed differences in alkaloid concentrations are evidence for genetic regulation of alkaloid secondary metabolism in ponderosa pine. The theoretical trade-off with seedling growth appeared to be real, however slight. The climate variables provided little evidence for adaptive alkaloid variation, especially within regions. This study was published in full in the Annals of Botany 103: 447-457; 2009.

Gene Expression in the Tanoak-*Phytophthora ramorum* Interaction

Katherine J. Hayden and **Matteo Garbelotto**, University of California, Berkeley, Environmental Science, Policy, and Management, 137 Mulford Hall, Berkeley, CA 94702; khayden@berkeley.edu; **Hardeep Rai**, Utah State University, Provo, UT; **Brian Knaus** and **Richard Cronn**, USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR; and **Jessica W. Wright,** USDA Forest Service, Pacific Southwest Research Station, Davis, CA

Disease processes are dynamic and involve a suite of changes in gene expression in both the host and the pathogen. As such, they lend themselves well to transcriptomic analysis. Moreover, a single tissue contains both host and pathogen RNA, allowing for the study of the transcriptional responses of both species to each other. Here we focus on a generalist invasive pathogen (*Phytophthora ramorum*) and its most susceptible California Floristic Province native host (tanoak, *Notholithocarpus densiflorus*). The advent of new sequencing technologies has made the study of non-model systems possible at a scale never before possible. We argue that this non-model system is ideal for studying the interactions between host and pathogen using massively parallel mRNA sequencing, in part because the *P. ramorum* genome has been fully sequenced.

Here we present early data in a project developed to elucidate the molecular genetic interactions between these two species. We used the Illumina Genetic Analyzer system to sequence all mRNA present in a single tanoak genotype after inoculation with *P. ramorum* and from a non-inoculated control. We separated sequences from the dataset that originated from the pathogen or from highly conserved regions by aligning the reads to the *P. ramorum* genome; this first set includes the genes expressed *in vivo* by the pathogen. The remaining sequences were used to generate a *de novo* reference transcriptome for tanoak, and then to subsequently pinpoint gene regions of interest with increased or decreased expression after infection. Each of the three outcomes—the set of expressed *P. ramorum* gene regions, the tanoak gene regions showing changes in expression, and the tanoak transcriptome reference itself—are an important step in understanding the interactions of forest pathogens and their hosts at the molecular level.

Blister Rust Resistance among 20 Families of Whitebark Pine, *Pinus albicaulis*, from Oregon and Washington—Early Results from an Artificial Inoculation Trial

Angelia Kegley, **Richard A. Sniezko**, and **Robert Danchok**, USDA Forest Service,
Dorena Genetic Resource Center, Cottage Grove, OR; akegley@fs.fed.us

Whitebark pine is considered one of the most susceptible white pine species to white pine blister rust, the disease caused by the non-native pathogen *Cronartium ribicola*. High mortality from blister rust and other factors in much of the range in the U.S. and Canada have raised serious concerns about the future viability of this tree species. A major effort is now underway to collect seed for gene conservation, to evaluate trees for genetic resistance to blister rust, and to implement restoration plantings.

Seed collected from 20 whitebark pine parent trees at four locations in Oregon and Washington were sown in 2005. The number of families represented in this study varied by geographic source: eight from Mt. Rainier National Park, three from the Colville National Forest, two from the Deschutes National Forest, and seven from the Fremont National Forest. Seedlings were inoculated with *C. ribicola* twice, initially in 2006, and again in 2009, using the well-established protocols at Dorena Genetic Resource Center (DGRC). The first inoculation was very effective in producing needle spots (>99 percent of the seedlings), but, unexpectedly, less than 10 percent of the seedlings later developed cankers. The second inoculation also was very effective in producing needle spots (>99 percent of the seedlings), but also subsequently led to very high levels of stem infection. This second inoculation will be particularly interesting to follow; the trees were 5-years-old and much larger than any whitebark pine previously subjected to artificial inoculation at DGRC. As of November 2010, 76.6% of trees had stem infections, with family means ranging from 46.9 to 100%. Large differences in percent stem infection were also apparent by geographic source: the Mt. Rainier National Park families averaged 57.6% stem infection, while the Deschutes National Forest families averaged 97.1%. The number of stem infections at the November 2010 assessment averaged 16.4 per infected tree, with family means ranging from 7.3 to 28.3 stem infections per infected tree. One individual had 90 stem infections. Some bark reactions (complete and partial) were apparent; families from the Colville and Mt. Rainier populations had the highest levels.

Canker severity is currently low (3 on a 9 point scale), but is expected to increase dramatically over the next year. Mortality from blister rust is currently low (4.2 percent), but is expected to continue with high mortality expected over the next 2 years. Seedlings will be assessed annually over the next years for additional stem infections and mortality. The high levels of canker-free seedlings in some families at this stage are encouraging. Seedlings from top families will be grafted for gene conservation, and land managers will be contacted concerning the potential to collect seed from the top parents to use for restoration.

Interaction of an Invasive Bark Beetle with a Native Forest Pathogen: Range Expansion of Mountain Pine Beetle in Jack Pine Forests and Impact of Dwarf Mistletoe

Jennifer Klutsch and **Nadir Erbilgin**, University of Alberta, Department of Renewable Resources, ESB 442, Edmonton, Alberta T6G 2E3; klutsch@ualberta.ca

In recent decades, climate change has facilitated shifts in species ranges that have the potential to significantly affect ecosystem dynamics and resilience. Understanding the interactions between native and invasive species will be important in determining how the range expansions of invasive species will affect a novel ecosystem (and their properties) and how to mediate these effects. In this research, the expansion of mountain pine beetle (*Dendroctonus ponderosae*) into a new host and forest ecosystem will be used as a model to evaluate tripartite interactions.

Mountain pine beetle is expanding east from in British Columbia, where it has killed millions of lodgepole pine (*Pinus contorta*) during the last 10 years of outbreak populations, into portions of the lodgepole pine x jack pine hybrid zone in Alberta. There is a threat that mountain pine beetle will expand into the novel host, jack pine (*P. banksiana*), which extends from Alberta into eastern Canada and the Great Lakes Region, which is an integral component of boreal communities and provides important ecosystem services. With an extensive distribution in jack pine, dwarf mistletoe (*Aceuthobium americanum*) is a native parasitic plant of jack pine that significantly reduces tree vigor and contributes to tree mortality. The expansion of mountain pine beetle into jack pine forests will interact with this native plant-pathogen system.

Infection by dwarf mistletoe may alter jack pine suitability by changing its physical and chemical defenses to mountain pine beetle and may thus affect initial establishment and survival of low mountain pine beetle populations in jack pine forest. The work that will start in 2011 will evaluate how dwarf mistletoe-induced changes in jack pine affect growth and development of mountain pine beetle and its associated fungi. Interactions between jack pine, dwarf mistletoe, and mountain pine beetle will be identified by addressing a number of research objectives. First, changes in dwarf mistletoe-induced chemistry will be evaluated in jack pine and how these changes will influence mountain pine beetle fitness will be established. Second, the “cross-talk” of defense chemical pathways between pathogen and mountain pine beetle-associated fungi will be explored. Third, we will examine the “invasiveness” of mountain pine beetle by identifying transgenerational plasticity of beetle responses to dwarf mistletoe-infected jack pine. Finally, we will assess whether changes in jack pine chemistry will affect pheromone-induced aggregation of mountain pine beetle

This presentation will explain the methods that will be used to investigate research objectives and discuss a number of potential implications of interactions between a native host-parasite system with an invasive phytophagous insect. With this information, management options could be prioritized to reduce the susceptibility of invasion by mountain pine beetle in areas. Furthermore, the role of tree-mediated interactions in jack pine will be quantified and will contribute to our understanding the regulation and coordination of tree defense chemistry against two disturbance agents. Resulting information from such tripartite interaction among trees, pathogens and insects will enhance our understanding of complex interactions that no doubt occur in all forest ecosystems.

Using Dutch Elm Disease-Tolerant Elm to Restore Floodplains Impacted by Emerald Ash Borer

Kathleen S. Knight and **James M. Slavicek**, USDA Forest Service Northern Research Station, Delaware, OH; ksknight@fs.fed.us; **Karen Menard**, Metroparks of the Toledo Area, Toledo, OH; and **Terry Robison**, Cleveland Metroparks, Cleveland, OH

American elm (*Ulmus americana*) was a dominant species in floodplains and swamps of the Midwest before Dutch elm disease (*Ophiostoma novo-ulmi*) reduced its populations. In many areas, ash (*Fraxinus* spp.) became dominant in these ecosystems. Emerald ash borer (EAB) (*Agrilus planipennis*), an introduced insect pest, is now spreading rapidly through the Midwest and killing up to 99% of ash trees in infested areas. We have begun a restoration experiment to study methods of reforestation for ash-dominated floodplains impacted by EAB through plantings of other native tree species, including Dutch elm disease-tolerant American elm. We will test the effect of planting trees before, during, or after ash mortality by planting in sites in northern Ohio across a gradient of EAB infestation duration. We will examine the effects of microhabitat of the planted tree seedlings, including light, moisture index, and herbaceous competition, on their growth and survival.

Compatibility of Some Widespread Diseases and Insect Herbivores in Oak (*Quercus robur*) Trees and Their Connection with Leaves Biochemistry

L. V. Polyakova, **S. G. Gamayunova**, and **J. A. Boltenkov**, Ukrainian Research Institute of Forestry and Forest Melioration, Kharkov, Ukraine; polyakova_lv@mail.ru; and **P. T. Jurova**, National Nature Park “Svyaty Gory”

Biochemical analysis of centuries-old trees (600- and 300-years old) during a5-year period not only showed the difference in accumulation of biochemical substances in leaves, but also difference in the character of diseases and herbivores that annually damaged these trees. According to the character of leaf damage, a 600-year-old tree was regularly infected with *Apiosporum sp.* and only a little with *Microsphaera alphitoides* (powdery mildew). The following herbivores were noticed: *Diaspidiotus zonatus Frauenf., Archips crataegana Hbr.,* and *Curculio pyrrchoceras Marsh.,* and some others were rarer. 300-year-old trees were annually heavily infected by powdery mildew (10-50% leaves) and damaged noticeably by *Erannis sp., Andricus foecundatrix Hart., Neuroterus numismalis Fourc., Eriophyes ilicis quercinus Can.*, and others.

Biochemical analysis included determination of some groups of first metabolites—chlorophyll (CHL) and protein (PR)—and second metabolites—flavonoles (FL), condensed tannins (CT),connected form of proanthocyanidins (PA), and hydrolysable tannins (HT). It appeared that 600-year-old trees accumulate more content of some first metabolites (138% CHL, 110% PR, summer 2010 y.) and noticeably lower content of second metabolites (63% FL, 30% CT, 73% HT) in their leaves compared to 300-year-old trees. The quantitative biochemical difference between these trees is reliable and the genotypic distinction was confirmed by Correspondence Analysis.

In early July 2010 (induced level of all compounds content as a result of diseases, herbivore action, and hot summer) common oak trees in a natural population were studied (16 trees, near 100- years old, eight leaves from each tree were used for individual analysis). According to the character of damage, all trees were divided into three groups: 1 – genotypes similar to previously studied 300-year-old trees (eight trees); 2 – genotypes similar to 600-year-old trees (five trees), and 3 – some trees of intermediate type (three trees, which suffered more from different kinds of damage). Biochemical characteristics of these groups coincided with previously studied centuries-old trees. The trees of 2-group were less damaged by powdery mildew and *Erannis sp.* They were in good correlation with biochemical peculiarity of the 600-year-old trees. Content of the first metabolites was higher than average for this population (127% CHL, 100% PR), but the second metabolites content was lower (88% Fl, 64% PA, 90% HT).

Genotypes that are similar to 300-year-old trees (1- group) were dominating among analyzed trees. They were heavily infected by powdery mildew (5-30%) and by *Erranis* sp. (15-30%). These trees were characterized by lower content of the first metabolites (85% CHL, 90% PR) and higher levels of the second ones (112% FL, 150% PA, 120% HT). It can be concluded that high levels of tannin do not prevent these trees from disease development and leaf damage from herbivores. Such kinds of accumulation of both group compounds in leaves was noticed earlier for near 50-year-old-tree populations of *Quercus pubescentis* damaged with *Tortrix viridana* L. (15 trees , 2008-2009 y.y., middle of May, constitutive level of compounds accumulation). It appeared that trees more susceptible for insect damage accumulated less of the first metabolites in leaves (94% of CHL, 85% of

PR) and more HT: 110% of pentagalloylglucose and 128% of digalloylglucose in comparison to resistant trees. This fact confirmed that a high quantitative level of phenolics (CT, HT) is not always a guarantee of reliable resistance to herbivores or diseases. Additionally, it is obvious that disease and herbivore spread is not accidental in oak populations, and that they have a tight connection with accumulations of second and first metabolites in individual trees.

Improvement Strategies for Disease Resistance in Forest Tree Species

Paolo Raddi (Emeritus) and **Roberto Danti**, Institute of Plant Protection – C.N.R., Via Madonna del Piano 50019, Sesto fiorentino, FI, Italy; p.raddi@ipp.cnr.it

At the Institute of Plant Protection (IPP) of the Italian National Research Council (CNR) we have been working for more than 35 years on breeding programs for disease resistance in forest trees which are of interest for Italy. "Forest tree improvement" is defined as the control of the parentage and of other combined forest management activities with the aim to select superior candidates. It clearly involves knowledge of forest genetics and forest tree breeding and both of these must be combined with the best use of the appropriate forest management. The strategy of the improvement for resistance must always consider at least three important components: the host , the pathogen, and the environment. The main objective of the IPP's improvement programs for resistance is to obtain trees that can resist or tolerate biotic and abiotic stress factors and that can grow well under different environmental conditions. But it is known that the selection of "totally" resistant trees against diseases or pests is very expensive and time consuming and that the improvement program is economically feasible only when the disease is very destructive and significant for rural income, and only when eradication and chemical preventive and curative treatments are not effective.

IPP programs were dealing with four pathosystems dealing with forest trees:

1. *Cupressus sempervirens-Seiridium cardinale*;
2. *Ulmus* spp.-*Ophiostoma ulmi* s.l.;
3. *Pinus pinaster- Cronartium flaccidum*; and
4. *Castanea sativa- Cryphonectria parasitica*.

This paper describes the feasibility of the genetic improvement program for resistance, which depends on the pathosystem considered (knowledge of physiology and resistance variability of the host; pathogen variability in pathogenicity; and correlation host-pathogen-environment.) The four pathosystems described required four different improvement strategies to obtain the best and less expensive results, which are always linked to the named several variables: host, pathogen, environment, and also to the experience in genetics, pathology, statistics, and, more recently, in biotechnologies of the research staff.

Several varieties of cypress and elm were selected for their resistance to cypress canker and to DED, patented, and put on the market with success.

Quantitative Trait Loci for Resistance to Two Fungal Pathogens in *Quercus robur*

Cécile Robin, **Amira Mougou-Hamdane**, **Jean-Marc Gion**, **Antoine Kremer**, and **Marie-Laure Desprez-Loustau**, INRA, UMR 1202 BIOGECO, INRA-Université Bordeaux 1, 69 route d'Arcachon, 33612 Cestas, France; robin@bordeaux.inra.fr

Powdery mildew, caused by *Erysiphe alphitoides* (*Ascomycete*), is the most frequent disease of oaks, which are also known to be host plants for *Phytophthora cinnamomi* (Oomycete), the causal agent of ink disease. Components of genetic resistance to these two pathogens, infecting either leaves or root and collar, were investigated in a full-sib family of *Quercus robur* L, that was vegetatively propagated by cuttings.

Resistance to powdery mildew was assessed by two methods. First, inoculations of *E. alphitoides* were performed under controlled conditions on excised leaves removed from cuttings grown in the greenhouse. The level of host-pathogen compatibility was assessed by recording infection success and mycelial growth. Second, the progeny, planted in a comparative test, was assessed for susceptibility to powdery mildew using field evaluation under natural infection conditions over 3 years. Resistance to ink disease was estimated by inoculating *P. cinnamomi* on stems on 2-year-old cuttings grown in glasshouse, and by measuring the length of the induced lesion in two experiments.

Preliminary results showed that quantitative trait loci (QTL) associated with the response to both pathogens were located on the genetic linkage maps available for the two parents of the F1 family. However we could not identify QTL involved in both diseases.

Although the genetic architecture of resistance to *E. alphitoides* varied between years and infection conditions, stable QTL were detected. The infection by this fungus being strongly dependent on the phenological status of its host, co-locations between QTL for resistance, and QTL for phenology were investigated.

The Italian Elm Breeding Program for Dutch Elm Disease Resistance

Alberto Santini and **Francesco Pecori**, Institute of Plant Protection – C.N.R Via Madonna del Piano, 10, 50019 Sesto fiorentino, Italy; a.santini@ipp.cnr.it; **Andrew Brookes**, University of Portsmouth, Portland Street, Portsmouth, PO1 3AH England, UK; and **Lorenzo Mittempergher** (Emeritus), Via Andrea di Bonaiuto, 14, 50143 Firenze, Italy

In the 20th century, elms across Europe and North America were devastated by two pandemics of Dutch Elm Disease (DED), caused by the introduction of two very different fungal pathogens: *Ophiostoma ulmi* (Buisman) circa 1910, followed 60 years later by the three times more lethal *O. novo-ulmi* (Brasier).

Research into a resistance to DED in the native elm species and populations began in The Netherlands in 1928, and in the U.S. in 1937. No worthwhile resistance was ever found within the native European or American elms. The Dutch team resorted to hybridizing European elms, from which the first cultivars exhibiting a resistance were obtained during the 1930s, but these clones did not have sufficient resistance to the second pandemic. It was the use of Asian species, with their higher resistance to DED, which accelerated progress on both sides of the Atlantic. Eventually a number of second-generation resistant clones derived from hybrids of native and Asian (or often exclusively Asian in the U.S.) species, were released to commerce.

In Italy, an elm breeding program was started in 1975 by the Institute of Plant Protection, C.N.R., in Florence, born of a conviction that the Mediterranean environment would demand its own selections. The favorable adaptation of species such as the Siberian elm (*U. pumila* L.) in Italy since the 18th century, and the unsuitability of the Dutch selections to the more arid regions, encouraged the wider assessment of the Asian elms. The purpose was twofold: to examine more fully adaptability to the climate, and to broaden the genetic base of the native species.

Selection of superior genotypes ostensibly reduces genetic variation in cultivated species. However, when breeding is designed for obtaining plants adapted to different environmental conditions and for different uses, the outcome can actually result in increased variation. The case of elm breeding for resistance to DED is paradigmatic. The uses of elm are in fact manifold. For this reason, breeding for resistance is not enough: many other features are required, including fast growth and aesthetic factors such as attractive shape and foliage. To satisfy all these needs, whilst maintaining enough genetic variability to buffer the effects of climate change and possible arrival of new strains of DED or other diseases, it was decided to undertake a fundamental broadening of genetic resources, or, as it will be called later, “incorporation.” A base of native elms with enough good characters to act as parents was hybridized with those disease-resistant Asian species able to acclimatize.

This elm breeding program has produced DED-resistant elm varieties able to adapt to arid conditions, yet endowed with some remarkable ornamental characteristics. Four of these clones have already been patented and released to commerce: ‘San Zanobi,’ ‘Plinio,’ ‘Arno,’ and ‘Fiorente,’ a new variety, ‘Morfeo,’ will be released shortly. ‘Morfeo’ is a robust, attractive tree extremely resistant to DED; it is also fast-growing, tolerant of both summer drought *and* winter floods, thus proving as well adapted to the climate of northwestern Europe as that of the Mediterranean. Indeed, following trials in England, ‘Morfeo’ is now considered potentially the most important cultivar in the conservation of several invertebrates there endangered by the consequences of DED.

Projecting Population Demography and Resistance Gene Frequencies in Pines in the Presence of White Pine Blister Rust: A Population Genetic Model for High-Elevation Five-Needle Pines

A.W. Schoettle, USDA Forest Service, Rocky Mountain Research Station, 240 W Prospect Rd, Fort Collins, CO; aschoettle@fs.fed.us; **J.G. Klutsch**, Department of Agricultural and Resource Economics, Colorado State University, Fort Collins, CO and USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO; **M.F. Antolin**, Department of Biology, Colorado State University, Fort Collins, CO; and **S. Field**, Department of Biology, Colorado State University, Fort Collins, CO

The slow growth and long generation time of the five-needle pines have historically enabled these trees to persist on the landscape for centuries, but without sufficient regeneration opportunities these same traits hinder the species' ability to adapt to novel stresses such as the exotic disease white pine blister rust (WPBR). Increasing the frequency of resistance to WPBR is the foundation for options to sustain five-needle pine species in the presence of the pathogen. Depending on the condition of the ecosystems, increasing resistance can be achieved via outplanting resistant seedling stock and/or stimulating natural regeneration. The objective of management intervention in the high elevation ecosystems is to promote pine population resiliency for many generations. However, greater understanding of the regeneration cycle and the potential for increasing the frequency of resistance are needed. This is especially critical for the WPBR pathosystem as WPBR kills trees of all ages and therefore affects multiple stages of the regeneration cycle.

We developed a population genetic model, parameterized for high elevation five-needle pines, to improve our understanding of pine population dynamics in the presence of WPBR and identify key processes that affect resistance gene frequencies over time. This model allows us to address questions such as: (1) What frequency of resistance to WPBR is enough to sustain a population under different initial condition? (2) What regeneration densities are sufficient to promote increased frequency of resistance over time? (3) What is the effect of competition on population sustainability and frequency of resistance over time? (4) During what state of infestation and impact by WPBR is intervention most effective? The matrix model includes six age classes (cohorts): seeds, primary seedlings (1-4 yrs old), secondary seedlings (5-20 yrs), saplings (21-40 yrs), young adults (41-90 yrs), and mature adults (>90 yrs). The model includes non-linear functions for the effects of competition (leaf area index) on germination and cone production. Population size, incidence of infection by WPBR, and frequency of a simply inherited dominant WPBR resistance gene by age class over time are all included in the model output. Model predictions under different WPBR infection probabilities on population dynamics and resistant allele frequencies over time will be presented.

Patterns of Resistance to *Cronartium ribicola* in *Pinus aristata*, Rocky Mountain Bristlecone Pine

A. W. Schoettle, USDA Forest Service, Rocky Mountain Research Station, Ft Collins, CO; aschoettle@fs.fed.us; **R. A. Sniezko**, **A. Kegley**, and **R. Danchok**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR; and **K.S. Burns**, USDA Forest Service, Forest Health Management, Lakewood, CO

The core distribution of Rocky Mountain bristlecone pine, *Pinus aristata*, extends from central Colorado into northern New Mexico with a disjunct population on the San Francisco Peaks in northern Arizona. Populations are primarily at high elevations and often define the alpine treeline; however, the species can also be found in open mixed conifer stands with ponderosa and/or pinyon pines in some locations. On dry, exposed sites the stands are open and sparse and Rocky Mountain bristlecone (hereafter referred to as bristlecone) is commonly the only species present. The combination of the pine's adaptive traits with infrequent disturbance has enabled trees on these sites to attain ages of over 2,500 years. These same traits and conditions will inevitably hinder the ability of bristlecone pine to adapt to novel anthropogenic stresses such as climate change and infection by the non-native pathogen (*Cronartium ribicola*) that causes the lethal disease white pine blister rust (WPBR). Infection of bristlecone pine by *C. ribicola* was first documented in the field in 2004 in south-central Colorado. Rapid climate warming and the associated increase in mountain pine beetle activity are also affecting these high elevation ecosystems. These threats and the species' unique aesthetic and ecological roles make bristlecone pine a species of conservation interest. WPBR invasion is relatively recent compared to the generation time of bristlecone pine, thus we still have a window of time to gain and utilize new knowledge of this species and their ecosystems under natural conditions and develop proactive conservation strategies.

Genetic resistance to WPBR is a key factor that will affect the trajectory of bristlecone pine populations in the future. This presentation will report results from our studies of rust resistance for bristlecone pine families. Seeds collected in 2001 from 184 individual trees across 11 sites along the full latitudinal gradient in Colorado were sown in 2002. The 3-year-old seedlings were inoculated with *C. ribicola* at Dorena Genetic Resource Center in 2005. Seeds for an additional smaller test, also conducted at Dorena Genetic Resources Center, were sown in April 2009 and seedlings inoculated with *C. ribicola* in September 2009. Needle infection lesions (spots) were easily identified on needles of the seedlings inoculated at a young age (2009 trial), but were less obvious on needles of the seedlings inoculated at an older age (2005 trial). However, WPBR stem symptoms did develop on many of the older seedlings in the 2005 trial for which infection spots were not observed, suggesting that needle lesions are not a good early measure of infection in older bristlecone pine seedlings. Multiple WPBR resistant phenotypes are expressed by bristlecone pine including canker-free seedlings, seedlings with partial bark reactions, and seedlings developing stem infections later or showing longer survival with stem infections. Survivorship of seedlings in the large study, 5 years post-inoculation, range from 0% to 92% among families and 17% to 60% among geographic areas. There was no apparent relationship between resistance and latitude of the source trees.

Development of Methods for the Restoration of the American Elm in Forested Landscapes

James M. Slavicek, USDA Forest Service, Forestry Sciences Laboratory, 359 Main Road, Delaware, OH 43015; jslavicek@fs.fed.us

The American elm was once widely distributed throughout the eastern United States and was a preferred tree for use in the urban landscape. The Dutch elm disease (DED) fungal pathogen *Ophiostoma ulmi* (Buisman) Nannf., introduced into the U.S. in 1930, and *Ophiostoma novo-ulmi,* Brasier has destroyed tens of millions of American elm trees in the U.S. and Canada.

One line of research on the American elm from the 1970s to the present focused on the identification of American elm isolates that could withstand the DED pathogen. Over 100,000 American elm trees were tested for resistance to Dutch elm disease. No trees were found that were resistant to DED; however, at least eight selections were identified that exhibited good levels of tolerance to the disease.

To develop methods to restore the American elm in forested landscapes five DED tolerant selections, the Valley Forge, Princeton, New Harmony, R18-2, and Delaware 2, were used in experimental plantings in areas where the trees could exist undisturbed and be allowed to regenerate. Plantings sites were on state forests, natural parks, and conservation lands managed by the Army Corps of Engineers and private foundations. Additional selections will be added to the sites as they become available with the goal of having 25 different genotypes at each test site.

The following aspects will be monitored at the restoration sites: tree growth, determination of basis for loss of planted trees, dates of first seed formation, number of regenerating trees and their distance from the planting site, incidence of DED at the restoration site in planted and seedling trees, and genetics of survivor trees. In addition, information on the suitability of selected cultivars for different regions will be discerned as well as identification of currently unknown pathogens and pests that could impact a future American elm restoration effort.

Test sites were established in 2003 in collaboration with the Ohio Department of Natural Resources (ODNR) at the Mohican Memorial State Forest, with Columbus Metro Parks at Highbanks Metro Park, and with The Wilds on conservation land. In 2004 sites were established at Maumee State Forest in partnership with the ODNR and with Columbus Metro Parks at Glacier Ridge Park. In 2005 three sites were planted in collaboration with the U.S. Army Corps of Engineers at Eagle Island in the Mississippi River in Wisconsin, with Carpenter St. Croix Nature Center in Hasting, Minnesota, and with Luther College in Decorah, Iowa. In 2007 an additional site was established in Wisconsin with the U.S. Fish and Wildlife Service near Cassville, and in 2009 with Worthington City Parks in Ohio. In 2010 three sites were established in floodplains in collaboration with The Nature Conservancy in Vermont, and fifteen more sites are planned for Connecticut River floodplains in New Hampshire, Massachusetts, and Connecticut. To date, 13 experimental American elm restoration sites have been established in five states.

Generation of American Elm Trees with Tolerance to Dutch Elm Disease through Controlled Crosses and Selection

James M Slavicek, USDA Forest Service, Forestry Sciences Laboratory, 359 Main Road, Delaware, OH 43015; jslavicek@fs.fed.us

The American elm was once widely distributed throughout the eastern United States and was a preferred tree for use in the urban landscape. The Dutch elm disease (DED) fungal pathogen *Ophiostoma ulmi* (Buisman) Nannf., introduced into the U.S. in 1930, and *Ophiostoma novo-ulmi,* Brasier has destroyed tens of millions of American elm trees in the U.S. and Canada. With the emerald ash borer now threatening the ash component of riparian forests there is a critical need to identify American elms that can be used to help restore affected forests. From six to 10 selections of American elm have been identified, tested, and shown to have tolerance to Dutch elm disease; however, this number is inadequate for restoration purposes.

To attempt to increase the number and the genetic base of DED-tolerant selections, three approaches are being used: 1) controlled crosses between known DED tolerant selections, 2) controlled crosses between known DED tolerant and putative survivor selections, and 3) screening of putative survivor trees. American elm clonal selections with significant tolerance to DED include Valley Forge, New Harmony, Delaware 2, R18-2, and Princeton. These clones were crossbred to generate progeny with potentially enhanced levels of DED tolerance or resistance. The first set of crosses made were R18-2 x Valley Forge, New Harmony x Delaware 2, New Harmony x R18-2, and New Harmony x Valley Forge. Replicate field plots consisting of the progeny, parents, unselected seedlings, and DED susceptible controls were established, and the trees were inoculated with a mixture of the *O. ulmi* and *O. novo-ulmi* pathogens. The total percentage of the crown showing DED foliar symptoms was visually assessed for each tree at 4 weeks and at 8-9 weeks. Significant numbers of progeny were identified as potential candidates for propagation as DED-tolerant elms. Several progeny showed few symptoms even at 8-9 weeks: 38 of 321 progeny at 5% or less, 73 of 321 progeny at 10% or less. Progeny of the R18-2 x Valley Forge cross had a significantly lower mean percentage of foliar symptoms compared to progeny of the other three crosses at both reading dates. Mortalities of the susceptible elm selection and the average of the crosses were 38% and 7%, respectively at 2 years post-inoculation. Additional crosses of Valley Forge x R18-2, Valley Forge x Princeton, and Princeton x Valley Forge were performed, progeny trees were generated and established in replicate blocks, and the trees will be tested for DED tolerance.

To attempt to generate cold-hardy, DED tolerant, and site-adapted American elm strains for the Chippewa National Forests, controlled breeding was performed using DED tolerant selections (Valley Forge and R18-2) and local survivor trees from the Chippewa National Forest. Progeny trees were generated and planted in replicate blocks at three sites on the Chippewa National Forest, and these trees will be tested for DED tolerance in the future.

While the vast majority of large American elm trees have been lost from forested areas in the Eastern U.S., some large trees remain. Some of these trees are those that have escaped the disease; however, after 8 decades of disease pressure some of these trees may have sufficient tolerance to the disease to survive it. Seventeen large putative survivor American elm tree selections from MI, IL, IN, and OH were identified, clonally propagated, established in replicate blocks, and will be tested for DED tolerance.

Nine Year Survival of 16 *Phytophthora lateralis* Resistant and Susceptible Port-Orford-Cedar Families in a Southern Oregon Field Trial

Richard A. Sniezko, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR; rsniezko@fs.fed.us; **Jim Hamlin**, USDA Forest Service, Umpqua National Forest, Roseburg, OR; **Everett M. Hansen**, Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR; and **Sunny Lucas**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR

Port-Orford-cedar (*Chamaecyparis lawsoniana*) has suffered high mortality from the pathogen *Phytophthora lateralis* in portions of its natural range in southwest Oregon and northwest California, as well as in horticultural plantings in North America, and more recently in Europe. A program to develop genetically resistant populations of Port-Orford-cedar is underway. This operational program began in 1996 and utilizes artificial inoculation of the roots of young seedlings (or rooted cuttings) to rate parent trees for resistance. A key step to any such program is to establish field trials: to validate the results of artificial inoculation trials, to examine resistance at a range of sites and environments conducive to the pathogen, and to monitor the durability of resistance.

The field trial ('Foggy Eden' site) examined here consists of 16 Port-Orford-cedar families established in southern Oregon in 2002 at a site on the Siskiyou National Forest. The Foggy Eden (FE) site was chosen because of the notable recent mortality of the natural Port-Orford-cedar trees in the immediate vicinity. The 16 families had been selected to cover a range from highly susceptible (~100 percent mortality) to highly resistant (~0 percent mortality) in previous short-term greenhouse artificial inoculation ('root dip,' RD) trials. Most of the families were also included in a raised bed (RB) trial, where the raised bed had been previously infested with *P. lateralis*. The raised bed environment was established to examine its potential use as an alternative to establishing more expensive field trials.

By summer 2010, overall mortality at FE was 42.7% (263 of 616 trees). Most of the mortality to date occurred by 2006 (244 trees). Family variation in mortality varies from 6.2 to 79.2%. The top three families for survival involve a common parent and all have less than 11% mortality. Where possible, the cause of mortality was determined by examining dying trees by scraping the bark and looking for tell-tale cinnamon color staining and/or sending samples to OSU for PCR testing and lab culturing for presence of *P. lateralis*. In many cases, confirmation of *P. lateralis* was made, but in other cases it was not, possibly due to the long time period between mortality and sample collection. The results are encouraging at this point, but the trial needs to be followed longer, and the results from the additional trials in other environments conducive to *P. lateralis* need to be examined to determine the efficacy of resistance in different environments. A comparison of the results from FE, and the RD and RB trials is underway.

White Pine Blister Rust Resistance of 12 Western White Pine Families at Four Field Sites in the Pacific Northwest

Richard A. Sniezko, **Angelia Kegley**, **Robert Danchok**, **Sally Long**, and **James Mayo**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR; rsniezko@fs.fed.us; and **Jim Hamlin**, Umpqua National Forest, Roseburg, OR

Western white pine, *Pinus monticola*, a wide ranging forest tree species in western North America, is highly susceptible to *Cronartium ribicola*, the fungus causing white pine blister rust. In the Pacific Northwest (Oregon and Washington) the USDA Forest Service has a regional program underway at Dorena Genetic Resource Center (DGRC) to develop genetic resistance to this non-native pathogen. Resistance is identified through artificial inoculation of two-year-old seedlings from seed collected from parent trees selected in the forest or from control crosses among selected parents. Several types of complete and partial resistance have been documented in these seedling tests. However, until relatively recently, there were few multi-site field trials that closely monitored the field performance of families with these different types of resistance. A large series of field trials have been established in the Pacific Northwest since 1996. We report here on 12 western white pine families from the DGRC rust resistance screening program that have been included in four field trials established between 1996 and 2002 on sites in Oregon and northern California. Based on results from previous artificial inoculation at DGRC, the 12 families were selected to represent varying levels and types of complete and partial resistance, and include a highly susceptible checklot. Two of the 12 families have R gene resistance from Cr2, which imparts complete resistance (from a hypersensitive type reaction in the needles, HR) in the absence of a race of rust with corresponding virulence gene, vcr2. The remaining nine families have varying types and levels of partial resistance.

The timing and frequency of assessments varied to coincidence with the appearance and development of natural rust infection at the four sites. Trees were assessed in multiple years for growth as well as number of stem infections, types of stem infection (normal canker, bark reaction, partial bark reactions), severity of infection, and mortality. By 2010, all four sites had moderate to high levels of blister rust infection, and moderate levels of mortality were present at some sites. The high level of infection of the two families with the Cr2 gene at three of the sites suggests that it is likely that a virulent vcr2 race of rust is present. Analyses of the trials are now underway and we will report at this workshop on the levels of field resistance found in the families and whether the resistances are effective at these diverse sites. Infected trees in families with putative partial resistance traits will continue to be followed to examine their subsequent survival under field conditions. These current results and future assessments will provide valuable information on the level of rust resistance and survival that land managers can expect over a range of sites. Long-term monitoring of these sites will be essential to evaluate durability of the various resistance types.

Hypersensitivity Reactions in Five Needle Pines in Response to Infection by *Cronartium ribicola*

Katarina Sweeney and **Jeffrey Stone**, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; stonej@science.oregonstate.edu; **Richard Sniezko** and **Angelia Kegley**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424; and **Anna Schoettle**, USDA Forest Service Rocky Mountain Research Center, Fort Collins, CO 80526

Hypersensitive response-like (HR) needle reactions to infection by the white pine blister rust pathogen, *Cronartium ribicola,* have been reported for several species of five needle pines native to western North America. The best studied examples are in *Pinus monticola* and *P. lambertiana*. In these species a "needle spot" phenotype has been identified in which HR-like needle reactions are related to disease resistance that is conditioned by a major gene. Conventionally it is believed that the HR-like needle reactions in resistant pines prevent spread of the pathogen to vascular stem tissue by HR mechanisms commonly seen in other plant-pathogen incompatibility interactions, i.e. a rapidly induced plant cell death and subsequent localized tissue necrosis. The dead cells present a barrier to colonization by biotrophic pathogens and cause degeneration of fungal hyphae to stop further pathogenic invasion. Structural analyses of early *C. ribicola* colonization in resistant *P. monticola* have shown, however, that these symptoms and their underlying physiology are fundamentally different from the clearly defined HR described in other host-pathogen systems. Contrary to the pattern of HR responses seen in most incompatible host reactions, onset of needle lesions was first seen several weeks after initial entry of *C. ribicola.* We observed extensive proliferation of fungal hyphae in the host and penetration of the needle endodermis and vascular tissue by the pathogen prior to the onset of a discernable HR or cell necrosis. The amount of fungal tissue present and progress of needle colonization was similar for both resistant and susceptible *P. monticola*. Therefore, typical HR does not appear to function in needles as the mechanism of disease resistance in the "needle reaction" phenotype. We are also studying needle resistance reactions in other western North American pines, including *P. albicaulis* and *P. flexilis* in comparison with the reactions seen in *P. monticola*.

Occurrence of *Phytophthora cryptogea* on Rhizosphere of *Cupressus sempervirens* cv "Bolgheri" in Central Italy

A. M. Vettraino, University of Tuscia, DIPROP, via S. Camillo de Lellis, snc, 01100 Viterbo, Italy; and **R. Danti**, **A. Pecchioli**, and **G. Della Rocca**, Istituto per la Protezione delle Piante IPP-CNR, Via Madonna del Piano 10,I-50019 Sesto F.no (FI), Italy; g.dellarocca@ipp.cnr.it

Cupressus sempervirens L., also known as Italian Cypress, native of the Middle-East and the eastern part of Mediterranean basin, is currently widely used as a multipurpose tree, especially in ornamental, forest, and windbreak plantings throughout the Mediterranean region, and in other areas with similar hot, dry summers and mild, rainy winters. During a survey carried out in 2009 and 2010 in two young ornamental plantations in Tuscany and one nursery in Umbria, Italy, blight of the entire canopy and tree mortality of *C. sempervirens* cultivar "Bolgheri" have been observed, with incidence value about 10%. The cultivar Agrimed did not show symptoms. Symptoms consisted mainly in sudden yellowing and subsequent browning of the crown starting from the basal portion. With respect to the ornamental plantations, symptomatic plants were observed from May to August and were distributed in scattered groups often near a watershed. A *Phytophthora* species was isolated from the rhizosphere of dead plants and dead roots on selective medium PARPH. Analyses of morphological characteristics of 5-day-old cultures grown on carrot agar showed a petaloid and sparse aerial mycelium. No clamydospores were observed on carrot agar. Sporangia were oval to obpyriform with rounded bases, nonpapillate and persistent. Cultures were heterotallic. Ribosomal DNA (regions ITS1, 5.8S rDNA and ITS2) was amplified and sequenced and compared with sequences of known *Phytophthora* species obtained from GenBank. Morphological and molecular results confirmed this species as *Phytophthora cryptogea.*

A pathogenicity test was conducted using two cultivars of *C. sempervirens*, "Bolgheri" and "Agrimed," growing in pots containing soil inoculated with sterile millet seeds colonized by two isolates of *P. crytpogea*. For each *P. cryptogea* isolate and each host cultivar a total of 10 plants were treated and 10 plants used as controls. All plants were flooded for 24 h twice at 2-week intervals. Results of the pathogenicity test confirmed the different susceptibility of the varieties used. This is the first report of *P. cryptogea* on *C. sempervirens.*

Resistance in Nature Systems to Brown-Spot Needle Blight of Pine in China

Jianren Ye and **Xiaoqin Wu**, Forest Pathology, Nanjing Forestry University,
Nanjing, China; jrye@njfu.com.cn

Brown-spot needle blight of pines caused by *Lecanosticta acicola* (Thum.) Syd., asco state is *Mycosphaerella dearnessii* Barr, was first found in Jiangsu province of China on Japanese black pine (*Pinus thunbergii)* in 1958, but the damage was so insignificant that no one gave any attention to it.

In 1978, the disease was observed in a four-year-old slash pine (*Pinus elliottii*) plantation in the Fujian province of China, and by 1981 hundreds of hectares of young slash pine (*Pinus elliottii*) plantations had been destroyed.

Slash pine and loblolly pine (*P. taeda*) were introduced from the U.S. in the 1930s and cultivated in scattered locations in southeast China. But problems with the brown-spot disease of these pines had never been reported before 1978. The disease was gradually found in the Fujian, Zhejiang, Jiangxi, Anhui, Guangdong, and Guangxi provinces of China. The exotic pine plantations, including slash pine, loblolly pine, and Japanese black pine are severely damaged. Cultivated Caribaea pine (*P. caribaea*), long-leaf pine (*P. palustris*), shortleaf pine (*P. echinata*), and sand pine (*P. clause*) are also susceptible. The indigenous pines, including Masson's pine (*P. massoniana*) and Taiwan pine *(P. taiwanesis*), are highly resistant, and the five-needle Hainan pine (*P. fenzeliana*) has never been found infected. Seedlings and saplings of susceptible trees under 10 years old, especially under five years, may all be infected.

In severely infected slash pine plantations, slightly infected or non-infected individual trees are commonly detected. These phenotypes are probably due to genetic resistance. Resistant selections of slash pine to brown spot disease have been made since 1982. For slash pine, 122 disease-resistant phenotypes were selected in heavily infected plantations in the Fujian and Jiangxi provinces and 3 hm^2 disease resistance cutting orchards were established by grafting those phenotypes. Further screening by natural infection and artificial inoculation were conducted, and 62 clones with high resistance were obtained. A seed orchard about 10 hm^2 with disease resistance clones of slash pine was established in the Fujian province.

Resistance to *Lecanosticta acicola* and growth performance of eight families with seven-year-old progenies and five families with six-year-old progenies from the resistant seed orchard were studied. A significant difference in the ratio of affected needles among families was found in our experiments. Results also showed that the resistance breeding to brown spot needle blight achieved excellent results, hence, eminent traits of both growth performance and resistance to brown spot needle blight could be acquired by control pollination. Progeny testing is an essential process in forest breeding as the resistance to *Lecanosticta acicola* of parents varies among progenies. Families with the ratio of affected needles less than 15% are numbers 32, (32×1), 8, 24, 10, 34, and 1 with better resistance to brown spot needle blight. We found that family numbers 8, 10, and 32 have excellent and steady resistance, and thus could be used in afforestation.

The Potential of Breeding for Enhanced Inducibility in *Pinus pinaster* and *Pinus radiata*

Rafael Zas, Misión Biológica de Galicia, CSIC, Apdo. 28, 36080 Pontevedra, Spain; **Alejandro Solla**, Universidad de Extremadura, Virgen del Puerto 2, 10600 Plasencia, Spain; and **Xoaquín Moreira** and **Luis Sampedro**, Centro de Investigación Forestal de Lourizán, Apdo 127, 36080 Pontevedra, Spain; lsampe@uvigo.es

Most resistance mechanisms against pests and pathogens in pine trees involve the production of chemical defenses. Secondary metabolism is not cost free, and costs of chemical defenses are usually shown as negative genetic correlations between effective resistance and plant fitness correlates, such as growth. The existence of these negative genetic correlations imposes an important obstacle for breeding for resistance and productivity simultaneously. Increased susceptibility to pests and diseases is, indeed, a common side-effect of enhancing productivity in many tree breeding programs. For instance, in a companion communication we have reported negative correlation between constitutive resistance and growth potential of pine trees. Pines are able to respond to insect and fungus attacks eliciting a wide array of responses. Induced defences, i.e. those that are activated after biotic damage, are assumed to have evolved as a saving cost strategy, as costs of resistance materialize only when strictly necessary. Despite the fact that breeding for resistance is emerging as an important tool to diminish the impact of forest pests and diseases on managed forests, to date no attention has been paid to the possibility of improving forest resistance through breeding for improving inducibility, the ability to respond to biotic damage eliciting induced defences. Such a breeding strategy could likely improve the benefits of both resistance and productivity, as inducible defensive strategies are cost-saving. The very first step to explore whether inducibility can be increased through breeding is to determine whether these phenotypically plastic responses are under genetic control. Here we show that inducibility of chemical defenses in *Pinus pinaster* and *P. radiata*, two main forest tree species in SW Europe and other temperate regions, is genetically variable within populations.

We performed two greenhouse experiments with half-sib families of the two pine species. Plant material was a random selection of the actual breeding populations of these species in NW Spain. We simulated biotic damage by spraying a solution of methyl jasmonate, a plant phytohormone that is known to be involved in the signalling and triggering of induced defenses, and that, exogenously applied, elicit similar responses as real herbivory. Fifteen days after MJ application, we measured the concentration of diterpenes in the stem, total polyphenolics, and condensed tannins in the needles as measures of quantitative chemical defenses.

We found large genetic variation in all the defensive traits studied, but more interestingly we also found additive genetic variation in the inducibility of the stem diterpenes in both species, as revealed by the significant Family × MJ interaction. Besides, although Family MJ interaction was not significant for phenolics, the different levels of genetic variation observed in control and MJ-induced plants, with significant differences among families found only in the MJ-induced treatment, does show the existence of genetic variation in the inducibility in this trait, too. Thus, our results indicate the existence of additive genetic variation for the inducibility of the three studied defensive traits in the two pine species. This prerequisite allows both the continued evolution of defensive strategies in response to the herbivore pressure and the possibility of improving resistance of managed forests by artificially selecting for high inducibility potential, minimizing, thus, the possible undesired side effects on productivity.

This publication is available online at http://www.fs.fed.us/psw/. You may also order additional copies of it by sending your mailing information in label form through one of the following means. Please specify the publication title and series number.

Fort Collins Service Center

Web site	http://www.fs.fed.us/psw/
Telephone	(970) 498-1392
FAX	(970) 498-1122
E-mail	rschneider@fs.fed.us
Mailing address	Publications Distribution Rocky Mountain Research Station 240 West Prospect Road Fort Collins, CO 80526-2098

Pacific Southwest Research Station
800 Buchanan Street
Albany, CA 94710