

Pathology Specialist Report

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Tom Kubisiak and Dana Nelson – Southern Institute of Forest Genetics

Fusiform rust pathosystem:

The SIFG has been collaborating with Henry Amerson (NCSU), John Davis (UFL), and more recently Jason Smith (UFL) to further investigate the importance of specific host-pathogen gene interactions. That progress has largely depended on the use of genetically-defined host families and single-genotype isolates of the pathogen.

SUP lines

Single aeciospore- or urediniospore-derived lines are necessary to limit genetic variation in the pathogen, and thus facilitate or permit investigation of specific host resistance genes. The ability to quickly and reliably produce single-genotype isolates is paramount for our current and future rust research endeavors. To facilitate production of single-genotype isolates a simple and efficient single uredinial pustule (SUP) oak inoculation protocol coupled with pathogen-specific simple sequence repeat markers was developed to provide QA/QC. Single-genotype SUP isolates are being made available to the USDA-FS Resistance Screening Center for use in experimental and operational fusiform rust disease resistance screening trials, i.e., further gene discovery work and technology transfer.

Mapping Avr1

A loblolly family segregating for a single resistance gene and a mixture of two single-genotype isolates, one known to be avirulent and another virulent to the corresponding resistance gene, were used to show that only virulent spores can cause infection on marker assessed resistant trees whereas both avirulent and virulent spores can cause infection on marker assessed susceptible trees, exactly what would be expected given a gene-for-gene interaction. Given this information, we are currently mapping a single avirulence gene in the pathogen and have identified several markers linked to Avr1. We will be using these markers to probe a fosmid library for Cqf for positive inserts and begin building a physical map of the Avr1 region. Eventually SNPs within candidate genes will be used in an association study to link SNP genotype with phenotype. Ultimately, we are interested in the evolution of Avr genes/alleles, allele distribution across the geographic range of the pathogen, and the development of allele-specific markers for assessing allele frequencies at local spatial scales.

Long-term research goals are to genetically map all of the currently known avirulence genes, clone them, and develop markers that will be predictive of avirulence and virulence alleles. Such information would provide a reliable means by which more informed management decisions could be made regarding the deployment of host resistance genes. We are currently developing the genomic tools necessary to realize this goal such as additional molecular markers, a fosmid library, and a physical map of the Cqf genome. A future goal is to leverage these resources in a proposal to be submitted to DOE/JGI to sequence the genome of Cqf.

Chestnut blight pathosystem:

SIFG is a collaborator on an NSF funded project titled "Genomic Tool Development for the Fagaceae" spearheaded by Ron Sederoff at NCSU. Our part in this project is to help develop SSR and SNP markers from EST sequence data. We will assess the utility of these markers across various members of the Fagaceae, map 500 of these markers in a Chinese chestnut x Chinese chestnut cross, and perform comparative genetic analyses across species. Eventually, markers developed during the course of this project that appear to have putatively Chinese chestnut-specific alleles will be used to identify genomic regions influencing a resistance response in BC2-F2 progenies and used for association mapping in BC3 families derived from single sources of resistance. SIFG is collaborating with Donald Nuss (U Maryland), Michael Milgroom (Cornell), and Alice Churchill (Cornell) with annotating the chestnut blight fungus, *Cryphonectria parasitica*, genome sequence data. Our part in this project will be to help link the current recombinational map with the genome sequence data and developing additional markers (primarily SSRs) from the newly generated genome sequence data. We are also interested in identifying vegetative incompatibility (vic) genes and further investigating a particularly interesting region of high heterogeneity and reduced recombination that maps near the mating type (*MAT*) locus.

Sarah Covert – University of Georgia

Biocontrol of Annosum Root Disease - To support EPA registration of *Phlebiopsis gigantea* as a biocontrol agent for Annosum root disease of pines, we have collected 55 *P. gigantea* isolates from 5 southeastern states and we are testing them for their biocontrol efficacy in the lab. We have also developed microsatellite markers for *P. gigantea* and are using these to assess genetic diversity within the southeastern *P. gigantea* population, and between the U.S., Canadian and European populations. In the coming months, we plan to install field trials to assess the impact of *P. gigantea* release on the natural prevalence of airborne *P. gigantea* spores, and to measure the biocontrol effectiveness of selected southeastern *P. gigantea* isolates. Our research cooperators are Vic Ford (MeadWestvaco), Michelle Cram (U.S. Forest Service), and Verdera Oy (a Finnish company that sells an annosum biocontrol product in Europe). The project is supported by Georgia's Traditional Industries in Pulp and Paper Program.

Jason Smith - University of Florida

Hired in late Oct. 2006 on a 60% research and 40% extension appointment. Planning to teach a forest pathology course for the 07-08 year. Has one current graduate student, Tyler Dreaden, who is working on annosum root rot in Florida pines. Marc Hughes will begin his M.S. in August on Laurel Wilt Disease.

Works on a wide range of research from disease biology/diagnosis/epidemiology to pathogen taxonomy to disease resistance mechanisms. Given the limited number of forest pathologists in the country his research is driven by current needs.

Summary of current research projects:

(1) Co-evolution of pathogens and hosts - Plant diseases have been recognized as being responsible for their significant role in the development of the earth's terrestrial biodiversity. Pathogens can exert

tremendous pressure on natural and cultivated landscapes. However, how these plant-microbe interactions relate to plant diversity and forest ecology are still largely unknown. In addition, how the spectrum from symbiosis to pathogenesis fits into the process of co-evolution is in need of further study. This research has far reaching implications and can be used to guide forest management strategies to improve the health and productivity of Florida's forests.

Since the initiation of the lab in October, 2006 we have begun studies on the host-pathogen relationships of major forest diseases in the state of Florida. The first of these involves identifying how various factors influence the incidence and severity of annosus root disease in Florida pine forests. Tyler Dreaden, a M.S. student in the lab since January, 2007 will conduct research on this topic for his thesis. We will be collaborating with the Florida Division of Forestry and private consultants on this project. We will investigate how hosts differ in response to *H. annosum* and other root diseases and how forest management practices influence disease incidence and severity.

Another project we have just begun involves comparing the population genetics and biogeographic patterns of the pitch canker pathogen, *Fusarium circinatum* in the southeastern U.S.. Utilizing and building on a comprehensive culture collection, we will look at how biogeography, host species and other factors influence the population genetics of this important pathogen.

One ongoing study is to better understand the relationship between *Melampsora* rusts and their *Salix* hosts. Despite differences in geography, host ranges (both uredinial and aecial) and tissue specificity, all willow rusts in North America have been classified as one collective species, *M. epitea* sensu lato. However, regardless of taxonomic confusion, there is a lack of understanding of what forces have shaped the evolution of this pathosystem. Although it is likely that none of the nearly 600 species of willow are free of infection, there are significant differences in aecial host availability with highly diverse plant families being represented in the aecial host range. In previous work comparing arctic and temperate isolates of *M. epitea* from four host species, distinct molecular and morphological characteristics provided evidence for at least host-species level divergence. As part of a collaborative effort, we will continue this work to determine levels of specificity and divergence in this pathosystem. Some of this work was published in a 2004 article in *Mycologia*. Knowledge of how pathogens and hosts co-evolve and adapt to changes could be a key factor for developing trees for sustainable human use.

(2) Disease resistance mechanisms - As a collaborative project between UF (J. Smith and John Davis), NC State (Henry Amerson) and the Southern Institute of Forest Genetics (Tom Kubisiak and Dana Nelson) we will utilize genetic information of the fusiform rust-pine pathosystem

to better understand gene-for-gene interactions in this system. Although this work is still in its infancy, there are plans for a joint grant proposal in the coming year.

A second project that is currently being developed involves characterizing resistance responses/mechanisms in slash pine families selected for resistance to pitch canker disease, an economically important disease of many pine species world-wide. This work will provide some of the first information on host responses to this pathogen in southern pines.

Our lab is also collaborating with the University of Minnesota Forest Pathology Laboratory on work with the white pine blister rust pathosystem. In this work we are focusing on resistance traits in *Pinus strobus* to the introduced pathogen *Cronartium ribicola*. We have discovered the importance of epicuticular wax in reducing infection frequency on a resistant genotype of *P. strobus*. In addition, proteomic comparisons of total protein extracted from infected needles revealed up-regulation of important classes of resistance proteins including homologs of heat-shock protein 90 and TIR-NBS-LRR proteins. These studies are summarized in articles published in *Phytopathology* and *Molecular Plant-Microbe Interactions*. This work has provided some new perspectives on white pine blister-rust resistance; however, it also provides many more questions for further study. In the near future, we intend to begin studies to identify and characterize key compounds in wax from resistant needles and determine how these compounds affect basidiospore germination. In addition, studies will begin to obtain gene sequences for the putative resistance proteins identified. This work has provided a basis for selection of resistant *P. strobus* for reforestation and landscape planting.

(3) New and Emerging Threats - Our overall goal is to improve and protect the health of Florida's forests. Due to the paucity of forest pathologists in the U.S. at present and the number of rapidly emerging forest pests, one of our primary focus areas is responding to new disease threats to our forests. This work involves developing better diagnostic techniques, elucidating disease etiology and epidemiology.

Laurel Wilt Disease of Red Bay (*Persea borbonia*)
Laurel Wilt Disease (LWD) is a new and very destructive fungal disease of species in the Lauraceae. This disease is devastating native red bay (*Persea borbonia*) in the southeast and is spreading at an alarming rate.

A multi-state, multi-agency task force has been developed to orchestrate a coordinated plan of action against this disease. Our research involves selecting for and characterizing resistance to this disease in native red bay populations in an effort to preserve and re-introduce red bay germplasm in areas where the species is nearly being extirpated. This work will be funded (at least in part) by the Forest Health Protection unit of the U.S. Forest Service. We are also developing a rapid qPCR detection system for this pathogen and GFP-tagged isolates for

host-pathogen studies. In the state of Florida, we are also working with other agencies to develop a series of extension publications regarding this new disease.

(4) Viral diseases in native trees - The forest pathology laboratory at UF is currently involved in better understanding the potential threat from a new viral diseases of native ash (*Fraxinus* spp.) and *Celtis* spp. that may further threaten this trees in North American forests. We are currently collaborating with Dr. Ben Lockhart, virologist at the Department of Plant Pathology on this new and potentially destructive disease. This work is being summarized in a paper that will be submitted for publication soon.

(5) Diseases of *Populus*

With the rapid expansion of *Populus* cultivation in North America and its potential use for biomass and bioenergy, a better understanding of diseases and disease resistance of *Populus* species and hybrids is needed. We are currently initiating projects to better understand the biology and host responses to important diseases of *Populus* in North America

(6) Decay in urban trees in Florida

The ISA has funded a project to investigate the diversity of decay fungi in urban forests and determine their significance and role in hazard tree designations.

Henry Amerson - North Carolina State University (supposed to be retired - but we all know Henry and his unparalleled dedication) Henry is currently trying to wrap up three separate projects. One is the ReMatrix work for fusiform rust where NCSU and SIFG are cooperating to solidify R gene and Avr gene designations in families and isolates previously tested at NCSU. Another is a fusiform rust experiment in conjunction with Dudley Huber UFL, where they are testing to see if Fr1 resistance is expressed in a slash/loblolly hybrid, and the third is the rust Avr1 mapping project (SIFG and NCSU). Henry's quote "Remember, I am retired so I am suppose to be doing as little as possible." Based on this statement he does not appear to be fishing much.....

Jeff Dean - University of Georgia:

FY07 grant for \$66,000 from the Georgia Traditional Industries Program - Pulp & Paper (TIP3) for a study entitled "*Sirex noctilio*: Genetic Approaches to Managing a Newly Introduced Insect Pest of North American Pines and Conifers." This first year of the project we're looking at various bioassay and transcriptional profiling approaches to screening southern pine responses to the fungal pathogen, *Amylostereum areolatum*, transmitted by this woodwasp, and in subsequent years we intend to use some of the same approaches to looking at the effect of the wasp on these trees. Not much to report yet, but given the recent discovery that the pest has established itself in the eastern Great Lakes region, we're expecting this research area to heat up rapidly in the near future.

News of personnel changes, new additions, retirements, important contacts, job announcements

Mr. Josh Bronson was hired in late 2006 by the USDA FS to manage the Resistance Screening Center upon Carol Young's retirement.

Carol Young manager of the USDA Forest Service, Forest Health Protection, Resistance Screening Center retired in January of 2007.

Dr. Henry Amerson, Associate Professor of Forestry (NCSU Department of Forestry), retired in January 2007 but Henry continues to work as an Emeritus faculty member.

Mr Saul Garcia has joined the NCSU Industry Cooperative Tree Improvement Program and will continue to work on fusiform rust resistance gene discovery in advance generation selections of loblolly pine.

Dr. Jason Smith was hired by the School of Forest Resources at the University of Florida as an Assistant Professor of Forest Pathology.

POSITION ANNOUNCEMENT

Forest Pathology Research Position

Available July or August 2007

University of Georgia, Warnell School of Forestry and Natural Resources

The candidate will use a combination of molecular genetic techniques, lab assays and field experiments to develop a biocontrol product for annosum root disease. Annosum root disease was a major cause of pine mortality in Georgia in 2006 and is expected to continue as a serious problem throughout the southeast in the coming years. Microsatellite markers will be used to assess the genetic diversity of *Phlebiopsis gigantea*, a wood-rotting fungus shown previously to protect pines from annosum root disease. Wood competition assays pitting *P. gigantea* against the annosum pathogen will be used to assess the biocontrol efficacy of different *P. gigantea* isolates in the lab and the field. In addition, there will be an opportunity to work with an international biotechnology company on the registration of this biocontrol product with the U.S. EPA. The successful candidate will have molecular genetic experience and a willingness to work in the field. Experience with mycology and/or population genetics is also desirable. The position is funded for at least a year and will be classified as a Post-doctoral Associate or Research Technician III, depending on the candidate's qualifications. Interested candidates should send a c.v. to Sarah Covert via email (covert@uga.edu)

Publications of potential interest:

Aegerter, B.J., and T.R. Gordon. 2006. Rates of pitch canker induced seedling mortality among *Pinus radiata* families varying in levels of genetic resistance to *Gibberella circinata* (anamorph *Fusarium circinatum*). *Forest Ecology and Management* 235:14-17.

Baker LG, Spaine P, and Covert SF. In Press. Effect of surface wettability on germination and gene expression in *Cronartium quercuum* f.sp. *fusiforme* basidiospores. *Physiol. Mol. Plant Path. Mol. Plant Path.* 68:168-175.

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- Ekramoddoullah, A.K.M., Liu, J.-J., and A. Zamani. 2006. Cloning and characterization of a putative antifungal peptide gene (*Pm-AMP1*) in *Pinus monticola*. *Phytopath.* 96:164-170.
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- Grondin, J., Bourassa, M., and R. C. Hamelin. 2005. First report of the aecial state of *Melampsora larici-populina* on *Larix* spp. in North America. *Plant Dis.* 89:1242
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- Kaitera, J., and H. Nuorteva. 2006. Finnish *Cronartium ribicola* does not infect alternate hosts of *Cronartium flaccidum*. *For. Path.* 36:247-252.
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- Kayihan, G.C., Huber, D.A., Morse, A.M., White, T.L., and J.M. Davis. 2006. Genetic dissection of fusiform rust and pitch canker disease traits in loblolly pine. *Theor. Appl. Genet.* 110:948-958.
- Kubisiak TL, Amerson HV, and Nelson CD. 2005. Genetic interaction of the fusiform rust fungus with resistance gene *Fr1* in loblolly pine. *Phytopath.* 95:376-380.
- Li, H., Amerson, H., and B. Li. Genetic models of host-pathogen gene interaction based on inoculation of loblolly pine seedlings with the fusiform rust fungus. *New forests.* 31: 245-252.
- Liu, J.-J., Ekramoddoullah, A.K.M., Hunt, R.S., and A. Zamani. 2006. Identification of characterization of random amplified polymorphic DNA markers linked to a major gene (*Cr2*) for

- resistance to *Cronartium ribicola* in *Pinus monticola*. *Phytopath.* 96:395-399.
- Liu, J.-J., and A.K.M. Ekramoddoullah. The CC-NBS-LRR Subfamily in *Pinus monticola*: Targeted Identification, Gene Expression, and Genetic Linkage with Resistance to *Cronartium ribicola*. *Phytopath.* 97:728-736.
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Informative websites:

USDA FS Forest Health Protection - Southern Region
<http://www.fs.fed.us/r8/foresthealth/>

USDA FS Forest Health Protection - Forest Health Monitoring
<http://fhm.fs.fed.us/>

FHP Insect and Disease Risk Maps
http://www.fs.fed.us/foresthealth/technology/invasive_species.shtml

Forest Insect and Disease Leaflets
http://na.fs.fed.us/pubs/fidl_hp.shtml

Forest & Shade Tree Pathology
<http://www.forestpathology.org/index.html>

List of Forest Pathology Web Sites Composed by Bill Livingston at UMaine:
http://www.umaine.edu/fes/Classes/int256/home/web_sites.htm

North American Forest Commission Exotic Forest Pest Information System
<http://spfnic.fs.fed.us/exfor/index.cfm>

The American Chestnut Foundation:
<http://www.acf.org>

California Pitch Canker Site:
http://frap.cdf.ca.gov/pitch_canker/

California Oak Mortality Taskforce Website:
<http://nature.berkeley.edu/comtf/>

California Forest Pest Council Website:
<http://www.caforestpestcouncil.org/>

Technology transfer-type publications:

The Early Warning System for Forest Health Threats in the United States:

A general overview of the Early Warning System (EWS) and a detailed application of the EWS framework to forest insect and disease threats can be found at:

http://www.fs.fed.us/foresthealth/publications/EWS_final_draft.pdf

2006 Forest Insect and Disease Conditions for the Southern Region:

A report summarizing forest insect and disease conditions for 2006 can be found at

http://www.fs.fed.us/r8/foresthealth/2006conditions/2006_conditions.pdf

Preparing for Invasive Species Outbreaks:

A useful tool for forest managers and landowners which contains general guidelines for how to create networks, locate resources, and develop a strategy for rapid response to detection of an invasive species. The workbook can be found at:

<http://www.stateforesters.org/pubs/invasivesWorkbook.pdf>.

Genome Sequencing Projects and Proposals:

Phytopathogenic fungi being sequenced at DOE/JGI in 2006-2007:

- Heterobasidion annosum* (annosum root rot fungus)
- Cryphonectria parasitica* (chestnut blight fungus)
- Melampsora larici-populina* (poplar leaf rust fungus)
- Phytophthora capsici* (root rot fungus of vegetable crops such as cucurbits, tomatoes, and peppers)
- Mycosphaerella fijiensis*, (fungus causing leaf streak disease in bananas)

Phytopathogenic fungi being sequenced at the Broad Institute 2006-2007:

- Botrytis cinerea* the asexual stage of the teleomorph *Botryotinia fuckeliana* (fungus causing gray-mold rot on vegetable and fruit crops)
- Fusarium graminearum*, the anamorph of *Gibberella zeae* [fungus causing head blight (scab) of wheat and barley]
- Fusarium verticillioides* (causal agent of kernel and ear rot of maize)
- Puccinia graminis* (causal agent of stem rust of cereals)

Phytopathogenic fungi being sequenced at Genoscope the French National Sequencing Center 2006-2007:

- Botrytis cinerea*, the asexual stage of the teleomorph *Botryotinia fuckeliana* (fungus causing gray-mold rot on vegetable and fruit crops)
- Xanthomonas albilineans* (causal agent of little leaf scald of sugar cane)

Sequencing Proposals:

In March 2007 Scott Gold (UGA) and Mee-Sook Kim (USDA FS) submitted a proposal to the DOE/JGI Community Sequencing Program under the auspices of the American Phytopathological Society's Forest Pathology Committee to sequence the genome of six forest tree pathogens that were listed as priority organisms based on broad input from society membership. These species include *Armillaria ostoyae*, *Armillaria mellea*, *Cronartium ribicola*, *Cronartium quercuum* f.sp. *fusiforme*, *Rhizoctonia solani* anastomosis group 4, and *Sclerotium rolfsii*. Unfortunately, the proposal was not selected for funding (reviews are yet to be received). We will likely submit a new proposal this fall. We expect that the next CSP call for proposals will be issued in November, 2007 with letters of intent due in Jan 2008 and full proposals in Feb 2008, but this may change, so watch JGI's website.

Reports of new forest tree diseases and insects:

The Mississippi Forestry Commission, MS Department of Agriculture, USDA Forest Service, and USDA Animal and Plant Health Inspection Service (APHIS) have developed a follow-up survey plan in response to the water baiting confirmation made last month from a ditch draining a *P. ramorum*-positive nursery. The plan includes immediately implementing stream baiting, soil baiting around the edge of the waterway where the positives were obtained, and vegetation surveys. As environmental conditions are not ideal this time of year for *P. ramorum* recovery, these same survey activities will be repeated in the fall when weather conditions are more conducive to pathogen detection. For more information, contact Steve Oak at soak@fs.fed.us.

A new fungal species that is being transmitted by a newly discovered non-native beetle is killing alarming numbers of redbay trees (*Persea borbonia*) along the coast of South Carolina, Georgia, and Florida. Redbay mortality rates exceeding 80 percent have been found on some of the coastal islands in the area. The beetle (*Xyleborus glabratus*) was first found in North America in 2002 through the USDA Forest Service Forest Health Protection's Early Detection and Rapid Response project and the Forest Health Monitoring program has supported follow-up detection surveys in the area. Recently, dying sassafras and a rare spicebush have also been found infected with the fungus. USDA Forest Service representatives met with personnel from APHIS and representatives from numerous local groups at Jekyll Island, Georgia in January to address the problem. For more information go to <http://www.doacs.state.fl.us/pi/enpp/ento/x.glabratus.html> or contact Don Duerr, dduerr@fs.fed.us.

A non-native *Erythrina* gall wasp, *Quadrasticus erythrinae*, was detected in south Florida in October 2006. Both native and non-native *Erythrina* species (coral trees) appear to be susceptible. Adults attack soft tissues causing swelling and physiological disruption. Severe infestations result in complete and repetitive defoliation and host mortality. For more information go to http://www.srs.fs.fed.us/4501/ED-IPRA_4-10-07mid.pdf or contact Sheri Smith at ssmith@fs.fed.us.

A non-native woodwasp, *Sirex noctilio*, was captured in Fulton, NY in February 2005. This discovery raises red flags across the nation because the invasive insect species has devastated up to 80 percent of pine trees in areas of New Zealand, Australia, South America and South Africa. The woodwasp, which is native to Europe, Asia and northern

Africa, where it rarely is a pest, kills pines, and sometimes several other types of conifers, by introducing a toxic mucus and spores of a toxic fungus when the female lays her eggs through the bark and into the sapwood of the tree. If established in the United States, it would threaten pines coast-to-coast, particularly in the pine-dense states in the Southeast. For more information go to http://www.aphis.usda.gov/plant_health/plant_pest_info/sirex/index.shtml
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Upcoming events:

American Phytopathological Society Annual Meeting
2007, July 27 - Aug. 1
San Diego, California, USA

Mycological Society of America Annual Meeting
2007, Aug. 5-8 (foray Aug. 4)
Baton Rouge, Louisiana, USA

IUFRO Root and Butt Rot Conference
2007, Aug. 12-19
Berkeley, California, USA
IUFRO Division 7.02.01

IUFRO Phytophthoras in Forests and Natural Ecosystems
2007, Aug. 26-31
Monterey, California, USA
IUFRO Division 7.02.09

International Workshop on Biological Control of Invasive Species of Forests in the United States and P. R. China
2007, Sept. 20-25
Beijing, China

Western International Forest Disease Work Conference
2007, October 15-19
Sedona, Arizona, USA

IUFRO Recognition, Identification and Management of Tropical Plantation Diseases
2007, Oct. 19-20 (tentative)
FABI, University of Pretoria, South Africa
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