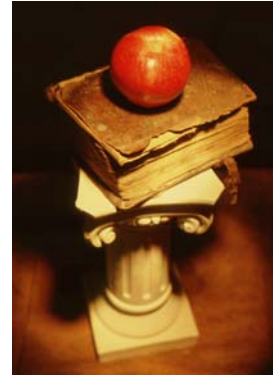


*Welcome to the First Annual*

# Ph.D. Symposium



*sponsored by the*

## Department of Plant Pathology



**Friday 26 September 2008**

**8:30am – 1:30pm**

**J.C. Raulston Arboretum  
York Auditorium in the  
Ruby C. McSwain Education Center**

## First Annual Ph.D. Symposium, Department of Plant Pathology

Friday 26 September 2008

## PROGRAM

8:30–9:00		Continental breakfast
9:00–9:10	Welcome	Dr. James W. Moyer
9:10–9:20	Introduction	Dr. Larry F. Grand
9:20–9:40	Courtney Gallup	Investigating sources of genetic variability of <i>Phytophthora nicotianae</i> , the causal agent of black shank of tobacco in NC.
9:45–10:05	Laura Hudson	Cell wall synthesis gene activity in root-knot nematode infection sites.
10:10–10:30	Amanda Kaye	Population genetic analysis of <i>Tomato spotted wilt virus</i> on peanut in North Carolina and Virginia.
10:30–10:50	Break, View Posters	
10:55–11:15	Faith Bartz	Fungal plant growth regulation: the influence of phenylacetic acid and its derivatives on two solanaceous hosts of <i>Rhizoctonia solani</i> AG-3.
11:20–11:40	Monica Blanco-Meneses	Genetic structure of populations of the tobacco blue mold pathogen, <i>Peronospora tabacina</i> , in North America, Central America and the Caribbean and Europe.
11:45–12:05	Ryan Georgianna	So close, yet so far away: Comparative genome analysis reveals surprising similarity between a friend and a foe.
12:10–12:30	Brantlee Spakes Richter	Cellulase activity and microbiology of cultural systems for <i>Phytophthora</i> root rot control in Fraser fir.
12:30–12:35	Wrap-Up	Drs. James Moyer and Lane Tredway
12:35–1:30	Lunch, View Posters	

### **Investigating sources of genetic variability of *Phytophthora nicotianae*, the causal agent of black shank of tobacco in NC.**

Courtney A. Gallup

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The black shank pathogen occurs in all tobacco-producing areas of North Carolina. The state has experienced rapid race shifts since the wide-spread deployment of single-gene resistance. Previous investigations suggested that this pathogen is highly variable, potentially contributing to the rapid rate of race shifts in the population. It is unknown whether the high level of population diversity is due to asexual recombination alone, or if sexual sporulation contributes to the epidemiology of the disease. Current investigations conducted in the laboratory, greenhouse, and field indicate high levels of variability contributed by asexual sporulation. Also, a state-wide survey of NC revealed that both mating types of *Phytophthora nicotianae* occur throughout the state. Both mating types were present in at least 28% of the fields surveyed, and sexual compatibility was identified between isolates within 8 of the fields surveyed. In a multiyear microplot study, plots were infested with either compatible A1 and A2 isolates or with each mating type alone. Over the three year study, mating type shifts were observed in both directions, from A2 to A1 and vice versa. However, there is a clear trend toward the A1 mating type in all plots. A subset of these isolates will be genetically characterized to compare variability generated by asexual and potentially sexual sporulation over time. Characterizing the level of genetic variability arising from asexual and sexual recombination will help elucidate how variability is generated and how it influences pathogen phenotypes, including races. This information can be used by breeders and pathologists interested in the development and deployment of different types of resistance genes and their short and long term viability for disease management.

**Cell Wall Synthesis Gene Activity in Root-Knot  
Nematode Infection Sites.**

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Root-knot nematodes (*Meloidogyne* sp.) are sedentary endoparasites that infect roots of a wide range of plant species and cause considerable economic loss to many crops. Root-knot nematodes (RKN) transform selected root vascular cells into enlarged, multinucleate feeding sites called giant-cells that arise from repeated karyokinesis without cytokinesis. Giant-cells undergo extensive modifications of the cell wall architecture including cell wall thickening and the formation of ingrowths that act to increase the surface area of the plasma membrane to facilitate solute uptake by the nematode. Extensive cell division is stimulated around the giant-cells to give rise to the root gall that is characteristic of RKN infection sites. The ten-member cellulose synthase (*CesA*) gene family of *Arabidopsis thaliana* was analyzed to monitor cell wall deposition in RKN infection sites. *CesA* gene promoter::GUS constructs and developmental quantitative RT-PCR indicated that *CesA* genes responsible for both primary and secondary cell wall synthesis were temporally and quantitatively expressed in the same pattern, with peak activity in RKN infection sites at five days post-inoculation. Sections of RKN infection sites in *CesA* promoter::GUS roots indicated that upregulated secondary cell wall *CesA* genes were localized within giant-cells and primary cell wall *CesA* genes were primarily localized to the surrounding dividing cells within the infection site. The number of galls and RKN female development were decreased in *Arabidopsis* mutants of each of the ten *CesA* genes, and complementation studies are in progress to further analyze this result.

**Population genetic analysis of *Tomato spotted wilt virus*  
on peanut in North Carolina and Virginia.**

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*Tomato spotted wilt virus* (TSWV) is an important pathogen of peanut worldwide and can cause severe yield loss and plant death. In 2007, 180 leaf samples were collected from peanuts exhibiting symptoms of TSWV from fields in North Carolina and Virginia. One hundred thirty-five samples that tested positive for TSWV with ELISA were used for extraction of RNA and cDNA synthesis. Primers specific to three optimal regions (RdRp, 1000nt; M, 699nt; N, 720nt) of the TSWV genome were used to amplify and sequence cDNA from each sample. Sequence data from 2007 were combined with sequence data from TSWV-infected peanuts sampled in 2005 and 2006 and subjected to phylogenetic analysis using the Suite of Nucleotide Analysis Programs. Application of these programs identified over 100 haplotypes for each genomic region. Measurements of haplotype diversity,  $H_d$ , indicated diversity levels of over 92% in each region. Noncontiguous, non-recombining regions of sequence were distilled from the larger sequence data set for use in calculation of population parameter statistics. Applying Hudson's  $PermtestK$  and Nearest Neighbor statistic,  $S_{nn}$ , to these regions showed that these samples are not subdivided by geographical area and comprise a panmictic population. Tests of neutrality (Tajima's  $D$ , Fu and Li's  $D^*$ , Fu and Li's  $F^*$ , Fu's  $F_s$ ) indicated significant negative selection pressure acting upon on the virus population. Topology of inferred maximum parsimony and maximum likelihood phylogenetic trees suggests exponential population growth. Future analysis will apply the coalescent process to infer evolutionary relationships of the sampled isolates.

**Fungal plant growth regulation: the influence of phenylacetic acid and its derivatives on two solanaceous hosts of *Rhizoctonia solani* AG-3.**

Faith E. Bartz

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The plant growth regulator phenylacetic acid (PAA) has been associated with the parasitism and infection process of *R. solani* on plant hosts. The metabolic pathway that leads to the production of PAA and its hydroxy and methoxy derivatives shares two intermediates with a carbon catabolism pathway that is induced by the presence of the substrate quinic acid (QA). Thus, the induction of the QA pathway could sequester the shared intermediates from the pathway involved in PAA biosynthesis. This leads to the hypothesis that the induction of the QA pathway by the presence of QA in the growth medium of *R. solani* would reduce PAA production and reduce plant disease symptom development. Experiments were conducted to determine the effects of pure PAA and each of its derivatives on the growth of Yukon Gold potato and Micro-Tom tomato. The form and concentration of PAA affected the number of shoots formed on potato micro tubers, as well as shoot and root system length. A significant ( $p < 0.0001$ ,  $\alpha = 0.05$ ) reduction in the germination of tomato seeds was seen at PAA concentrations of 70  $\mu\text{g/ml}$  and above. However, symptoms typical of *Rhizoctonia* infection were not observed when PAA and derivatives were applied to five-week old tomato plants at concentrations ranging from zero to 1000  $\mu\text{g/ml}$ . The goal of this research is to better understand the role of PAA in *Rhizoctonia* disease and to generate information that can be useful in the development of *Rhizoctonia* disease management strategies.

**Genetic structure of populations of the tobacco blue mold pathogen, *Peronospora tabacina*, in North America, Central America and the Caribbean and Europe.**

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Tobacco blue mold, caused by the oomycete pathogen *Peronospora tabacina*, causes a yearly epidemic in tobacco (*Nicotiana tabacum*). The genetic structure was examined through gene sequencing of specific *cox2* mitochondrial and *Igs2* rDNA and *Ypt1* nuclear regions. Populations from Europe (EU), U.S.A. and Central America-Caribbean-Mexico (CCAM) were analyzed. The blue mold populations in North Carolina were analyzed and the Burley and Flue-cured populations were compared. All of the regions showed high genetic variability across all worldwide populations. The neutrality tests were significant for all populations that were analyzed and the equilibrium model of neutral evolution was rejected, indicating an excess of recent mutations or rare alleles. Hudson's tests were performed to quantify population genetic structure within and among populations. On the CCAM the Hudson's test was significant for each region and show possible migration between Mexico, Dominican Republic, Guatemala and Nicaragua. Within the U.S.A. population the Hudson's test was significant as well as when it was pooled with CCAM there was possible migration between Dom. Rep. and Georgia, N. Carolina and Virginia. Within the European population the Hudson's test was not significant and showed no migration between European countries and the Western Asia tobacco fields.

**So close, yet so far away: Comparative genome analysis reveals surprising similarity between a friend and a foe.**

D. Ryan Georgianna

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*Aspergillus flavus* is one of the most notorious members of the genus *Aspergillus* and is well studied for its production of the highly carcinogenic secondary metabolite aflatoxin, and for its pathogenicity to plants and animals. A closely related species, *Aspergillus oryzae*, has been used for centuries in food fermentation and is generally regarded as safe (GRAS). The genomes of these two species were analyzed using bioinformatics and comparative genome hybridization (CGH) to identify genomic divergence that may have contributed to the different ecologies of these two fungi. Examination of the recently sequenced genomes of *A. flavus* NRRL3357 and *A. oryzae* RIB40 revealed only 332 and 306 unique genes in each respective species. An examination of two additional strains within each species using comparative genome hybridization (CGH) showed the genomes of these two species are even more similar. CGH among the six strains identified only 129 unique *A. oryzae* genes and 43 unique *A. flavus* genes. Further analyses of these six strains showed potential species-specific polymorphic sites in 607 of 13,548 predicted genes. Polymorphism at these sites was verified by comparison of probe sequence to the sequenced strains. Our data show that these two fungal species are very similar; however, they can be distinguished from each other by species-specific features. Current work examining gene expression on substrates commonly encountered by *A. flavus* or *A. oryzae* will further reveal the functional changes each species has undergone to thrive in their respective ecological niche.

**Cellulase activity and microbiology of cultural systems for *Phytophthora* root rot control in *Fraser fir*.**

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*Phytophthora* root rot of Fraser fir, caused by several *Phytophthora* spp., is a severe problem in Christmas tree production. As fungicides and host resistance are ineffective in long term disease control, cultural control systems are being tested at five naturally infested field sites. Treatments include wood chips (WC), wood chips plus compost (WCC), or pine bark (PB) as raised beds, and compost or sulfur tilled into soil. Microbial populations and activity were characterized by dilution plating, fluorescein diacetate hydrolysis (FDA), and cellulase enzyme activity. Bacterial and fungal counts were generally higher in mulches than in soil, while counts of cellulose degraders were less consistent and rarely showed significant differences. Fungal counts were higher in upper mulch than lower mulch at two of three sites. Total microbial activity and cellulase activity were also higher in mulch than in soil. Both total and cellulase activity remained relatively stable in soil over time, but while total activity increased in mulch over the first two growing seasons, cellulase activity decreased. Tree survival was significantly higher in mulched plots at two ( $\alpha=0.05$ ) or three ( $\alpha=0.10$ ) of five sites; WC and WCC had higher tree survival than control or compost plots at all sites.