Altered Distribution of Susceptibility Phenotypes Implies Environmental Modulation of Genetic Resistance

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Resistance to disease is determined by the genetic capacity of a plant to recognize and respond to a pathogen, as modified to varying degrees by the environment in which the interaction occurs. Physical factors such as temperature and moisture can limit the ability of a pathogen to infect and cause disease, and may also influence the response of the host through effects on gene expression and/or by imposing constraints on physiological activities required to deliver an effective defense. Recent research has also drawn attention to the potential for the biotic environment to modulate susceptibility to disease. Thus, resistance may be enhanced by endophytic microbes and also by sub-lethal exposure to a plant pathogen. For example, studies under controlled conditions document that systemic induced resistance (SIR) to pitch canker, caused by *Fusarium circinatum*, is operative in *Pinus radiata* (Monterey pine). Evidence for SIR in natural populations of *P. radiata* derives from studies showing that trees are more resistant to pitch canker in areas where the disease is of long residence than trees in areas where the disease is only recently established. Likewise, in a given stand, trees tend to become more resistant with time after establishment of pitch canker. These observations suggest that susceptibility to a disease may be influenced as much by the history of exposure to a pathogen as by inherent genetic resistance. As a test of this hypothesis, we compared the distribution of susceptibility phenotypes (based on lesion lengths induced by inoculations with a common strain) in a population of standing trees to seedlings reared from seed collected from the same trees. Seedlings were grown in a greenhouse in Davis, CA and were not exposed to *F. circinatum*. Lesion lengths on inoculated seedlings were normally distributed around a median of 31.0 mm, which is consistent with resistance to pitch canker being a quantitatively inherited trait. In contrast, among parent trees the distribution of susceptibility phenotypes was strongly skewed toward shorter lesions lengths, with a median of 8.0 mm. Although the pitch canker pathogen is presumably the most important of the biotic drivers of altered susceptibility, other plant-associated microbes may contribute as well.

Towards the Development of a Laurel Wilt Screening Program in Redbay (*Persea borbonia*)

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Laurel wilt is a highly destructive disease of redbay (*Persea borbonia*) and other Lauraceous natives in the southeastern United States. The disease and associated vector, the redbay ambrosia beetle (*Xyleborus glabratus*), has spread through the U.S. coastal plain. The presence of surviving and asymptomatic individuals in severely affected stands illustrates the possibility of natural resistance by surviving redbays.

In 2008, a field survey was initiated to locate and identify healthy, asymptomatic redbays in areas of severe mortality. Six heavily affected sites were chosen along the redbay-dense barrier islands of Florida, Georgia, and South Carolina. Over 80 trees with a 3"+ diameter at breast height were selected as putatively resistant candidates. A 1/5 acre plot was then established around each candidate tree along with measurements of redbay plot disease severity and redbay ambrosia beetle activity, based on trapping studies. Branch cuttings were taken and used in novel experiments to investigate methods of redbay vegetative propagation and disease resistance screening. A mean of 20% rooting was achieved in the propagation experiment, although genotype had a large effect on rooting success. The vegetatively propagated clones of live parent trees are currently being tested for resistance/tolerance to the laurel wilt fungus by inoculation experiments. A preliminary study tested 10 clones, and a single clone (FG-C1) has survived at 10 months post-inoculation, suggesting a possible tolerance response. Further testing of FG-C1 and over 50 new clones is under way. Additional studies on the genetic/pathogenic variability of the pathogen and effects of inoculum concentration on symptom expression are underway.

A Major-Gene Element to Weevil Resistance in Sitka Spruce? What We Can Learn from Crop Breeders.

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The weevil resistance breeding program against the white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae), particularly for Sitka spruce (*Picea sitchensis* (Bong.) Carr), is arguably one of the most successful pest resistance breeding programs for plantation forest species and it has done a lot to rehabilitate this important western conifer. Nearly all planting stock currently comes from this breeding program. So far the resistance seems effective, durable, and stable. We have used this program to also study causes behind this resistance including: various hindrance mechanisms such as induced and constitutive resin cells, sclereid or stone cells, and terpene defenses. All of these appear to be factors in resistance, but none singly is strongly predictive to resistance - the strongest are sclereid cells. All of these factors are in their nature complex, multifaceted, and appear to offer some partial solution that is likely controlled by complex multigenic systems. We do note, however, that we have very strongly expressed and complete resistance in some individuals. This and some preliminary data investigation may indicate that there is also a major gene element in our observed resistance. Such elements are well described against rusts and other pathogens in forestry, but are also well described for insects and nematodes in crop breeding. Particularly interesting is the Hessian fly in wheat which has a similar life strategy to the weevil. We describe here how we might uncover this element using molecular techniques and pedigree analysis as well as how we might go about discovering the underlying phenotypic expression of this resistance. Finally, we describe how we might effectively incorporate a major gene resistance element with these more complex multigenic systems to preserve the strength and durability to resistance against the white pine weevil.

Screening Western White Pine for Partial Resistance against Blister Rust Using *In vitro* Techniques

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Western white pine has been highly impacted by the introduction of *Cronartium ribicola*, the causative agent of white pine blister rust. Various categories of partial resistance have been identified based on the phenotypic observation or response of seedlings undergoing artificial inoculation. Among these observed seedling responses are: needle shed, slow canker growth, and a category called "difficult-to-infect." Difficult-to-infect (DI) are families that prove to be hard to infect even when using high artificial inoculation loads. However, a comprehensive evaluation of this and other seedling response traits is difficult because of the 'unwanted variations' e.g., the environmental factors in the screening of seedlings. To control unwanted variations, genetic variation in field plants and pathogens, and low incidence of DI resistance, we developed a disease assessment index based on both *in vitro* and *in vivo* techniques, in order to evaluate specific reactions in resistant plants to the pathogen. The results from our *in vitro* screening experiments indicated a significant difference in the number of successful infections between DI and control populations. Further morphological investigation with electron microscopy into the mechanism(s) responsible for these variations revealed a considerable difference in the morphology of stomata. These adaptations could provide a greater structural defense system against white pine blister rust. The results of this experiment indicated that *in vitro* screening is an efficient method for screening western white pine. This method can help us to evaluate and effectively assess and characterize this and other partial resistance traits in a controlled environment.

The Effect of Hemlock Woolly Adelgid Infestation on Water Relations of Carolina and Eastern Hemlock

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In North America, hemlock woolly adelgid (HWA; *Adelges tsugae* Annand) is an exotic insect pest from Asia that is causing rapid decline of native eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) populations. The exact physiological mechanisms that cause tree decline and mortality are not known, despite substantial research efforts on ecological impacts and potential control measures of HWA. Eastern and Carolina hemlock may be reacting to infestation in a manner similar to the response of Fraser fir (*Abies fraseri* (Pursh.) Poir.) to infestation by balsam woolly adelgid (BWA; *Adelges picea* Ratz.). It is known that Fraser fir produces abnormal xylem in response to BWA feeding. This abnormal xylem obstructs water movement within the trees, causing Fraser fir to die of water stress.

In this study, water relations within 15 eastern and Carolina hemlock were evaluated to determine if infestation by HWA was causing water stress. Water potential, carbon-13 isotope ratio, stem conductivity, and stomatal conductance measurements were conducted on samples derived from those trees. In addition, branch samples were analyzed for possible wood anatomy alterations as a result of infestation. Pre-dawn branch water potential measurements were more negative in infested hemlock than in non-infested trees. Carbon isotope ratios of the branches were more positive for infested trees, while stomatal conductance was lower in infested trees. These results indicate that infested eastern and Carolina hemlock are experiencing drought-like symptoms. Wood anatomy of the branches provided evidence that infested hemlocks are experiencing abnormal wood production in the xylem, including lower earlywood to latewood ratios and increased frequency of false rings. The significant reduction in conducting sapwood area, terminal branch growth, and leaf area in infested trees were sufficient to influence sap flux and whole-tree water use.

Simultaneous Laurel Wilt Disease Biology and Resistance Research

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Laurel wilt (LW) is a devastating, emerging disease of native and non-native members of the Lauraceae family in the southeastern United States. Currently, the fungal pathogen (*Raffaelea lauricola*) and its vector (*Xyleborus glabratus*) are found in Florida, Georgia, Mississippi, and North and South Carolina. LW is decimating native stands of redbay (*Persea borbonia*) and causing significant damage to planted and native sassafras (*Sassafras albidum*) and avocado (*Persea americana*). Research has addressed significant knowledge gaps that exist for the biology and management of this disease. To date, effective fungicidal management of LW has been limited to the expensive, preventative treatment of high-value landscape trees with systemic fungicides. Long-term management in landscape and avocado plantings may rely on a combination of sanitation practices and the use of disease-resistant germplasm. The susceptibility/tolerance of different taxa and avocado germplasm has been assessed in artificial inoculation work in the field. Host range experiments have assessed the response of 35 taxa in the Annonaceae, Fagaceae, Lauraceae, Magnoliaceae, Moraceae, and Sapindaceae, which include known hosts of the vector in southeast Asia and their relatives. In general, members of the Lauraceae that are native to the southeastern U.S. have been most susceptible, whereas those in the family from Asia and in other families have been resistant. Forty-one cultivars of avocado, representing the three races of the species (Guatemalan, West Indian, and Mexican) and hybrids thereof, have been screened for disease response. Unfortunately, West Indian cultivars that predominate in Florida have been most susceptible. Host-pathogen interactions have been examined in greenhouse and field studies. Results from this work indicate that: (i) systemic colonization by the pathogen, without apparent internal or external symptom development, occurs in some hosts; (ii) wilting is associated with reduced hydraulic conductivity in the xylem; (iii) vascular dysfunction results from host responses, not occlusion of vessels by the pathogen; and (iv) the pathogen does not produce wilt-inducing toxins. In ongoing research, greater understandings are sought for how susceptible and tolerant/resistant host plants respond to this disease.