Resistance Mechanisms in *Pinus strobus* to *Cronartium ribicola*, Causal Agent of Blister Rust

Jason A. Smith, Robert A. Blanchette, Todd A. Burnes, James J. Jacobs,
Department of Pathology, University of Minnesota
LeeAnn Higgins, Bruce A. Witthuhn, Department of Biochemistry,
Molecular Biology/Biophysics, University of Minnesota
Andrew J. David, Department of Forest Resources, University of Minnesota
and Jeffrey H. Gillman, Department of Horticultural Science, University of Minnesota

Successful development and deployment of blister-rust resistant eastern white pine (*Pinus strobus*) depends on an understanding of the underlying mechanisms of resistance. In this study, mechanisms of resistance were explored utilizing germplasm selected by previously by the late Dr. Robert Patton of the University of Wisconsin. Epicuticular wax on needles was evaluated for its influence on *Cronartium ribicola* infection of resistant and susceptible selections of *Pinus strobus*. Environmental scanning electron microscopy comparisons revealed that needles from a resistant selection of eastern white pine, P327, had a significantly higher percentage of stomata that were occluded with wax, fewer basidiospores germinating at 48 h after inoculation, and fewer germ tubes penetrating stomata than needles from a susceptible selection H111. In addition, needles from seedlings that failed to develop symptoms 6 weeks after inoculation, from a cross between P327 and susceptible parent H109, had a significantly higher percentage of stomata occluded with wax compared with needles from seedlings that developed symptoms. In experiments where epicuticular waxes were removed from needles before seedlings were infected, resistant seedlings without wax developed approximately the same number of infection spots (as measured by spot index) as susceptible seedlings with wax intact. Gas chromatography/ mass spectrometry comparisons of extracted epicuticular waxes revealed several peaks that were specific to P327 and not found in susceptible H111 suggesting biochemical differences in wax composition. These results implicate the role of epicuticular waxes as a resistance mechanism in *P. strobus* selection P327 and suggest a role for waxes in reducing spore germination and subsequent infection through stomatal openings. Although infection incidence of resistant selection P327 is low, successful infections are often arrested due to a hypersensitive-like reaction. In order to characterize this reaction, a proteomic comparison of needles from resistant and susceptible seedlings was undertaken using two-dimensional gel electrophoresis (2-DE). The results revealed 19 polypeptides specific to resistant seedlings and seven of these specific to infected resistant seedlings. There were 13 polypeptides up-regulated (≥3-fold increase) in resistant family P327 in comparison to needle tissue from susceptible and mock-inoculated seedlings. Electrospray ionization liquid chromatography and tandem mass spectrometry was used to sequence 11 proteins from the 2-DE gels. Sequences obtained from electrospray ionization liquid chromatography and tandem mass spectrometry were used for MSBLAST and Pro-ID database searches allowing identification with a 95 to 99 percent confidence level. Six proteins were determined to be homologs of proteins with known roles in disease resistance, five were determined to be homologs of members of the leucine-rich repeat (LRR) superfamily, and one was a homolog of heat shock protein 90, a protein that serves as a cofactor for certain LRR proteins. This is the first report of members of the LRR family with functional homologs in *Pinus strobus* and of a molecular basis for white pine blister rust resistance in *Pinus strobus*.