

# ***In vitro* and *in vivo* Investigation into the Characterization of Resistance Against White Pine Blister Rust**

**David Noshad<sup>1\*</sup>, John N. King<sup>2</sup> and Abul Ekramoddoullah<sup>1</sup>**

<sup>1</sup>Natural Resources Canada, Canadian Forest Services, 106 West Burnside Rd, Victoria, BC V8Z 1M5 Canada

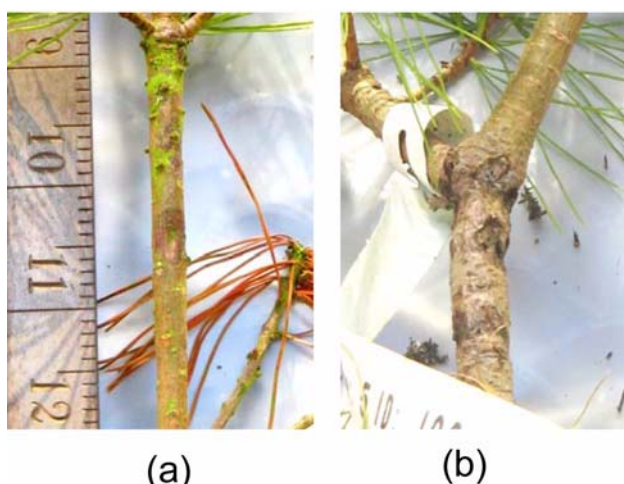
<sup>2</sup>BC Forest Service PO Box 9519, Stn Prov Govt, Victoria, BC V8W 9C2 Canada

\*Corresponding author: dnoshad@nrcan.gc.ca

White pine blister rust (WPBR) caused by the rust fungus *Coronartium ribicola* J.C. Fischer is one of the most devastating disease of white pine (*Pinus monticola* Dougl.) trees of the Canadian forests (Kinloch, 1999; Hoff, 1980). Different types of resistance with a very low frequency have been observed in a 4 yr field screen program (Figure 1-a & b). The resistant plants have been categorized in 4 major groups: difficult to infect (DI), bark reaction (BR), slow canker growth (SCG) and needle shed (NS). We developed a disease assessment index (DI), based on both *in vitro* and *in vivo* techniques, to evaluate specific reactions to the pathogen (Kinloch, 2003; Hoff, 1980).

The *in vitro* method provides a new approach to study inoculation in an axenic environment under controlled condition.

The first step in developing our *in vitro* screening program was to establish an *in vitro* culture protocol for the pathogen, ribes (alternative host) and white pine explants (Figures 2 and 3). Then we developed a disease assessment index, based on both *in vitro* and *ex vitro* techniques, to evaluate specific reactions to the pathogen of the resistant plants.



**Figure 1.** (a) Bark reaction resistance; (b) Slow canker growth resistance/tolerance.



**Figure 2.** *In vitro* rooted ribes leaf after 4 wk in culture.



**Figure 3.** *In vitro* western white pine-new microshoots have been observed after 5 wk in culture.

In: Noshad David; Noh Eun Woon; King, John; Snieszko, Richard A. (Eds. 2009) Breeding and Genetic Resources of Five-Needle Pines. Proceedings of the Conference 2008, Yangyang, Korea. Korea Forest Research Institute, Seoul 104p. ISBN 978-89-8176-605-4 (93520)

The preliminary results indicate: 1) white pine needle and seedling explants can grow successfully on both WPM (McCown and Lloyd, 1983) and GD based media and produce callus and roots; 2) Ribes leaves can grow independently in culture and produce roots on MS (Murashige and Skoog, 1962) based media; 3) the pathogen can infect and grow successfully on the *in vitro* cultured leaves.

By using molecular techniques e.g. SNPs we are trying to further characterize the resistance and understand the molecular mechanisms behind them. The results of this research could potentially help us to answer to some of the fundamental questions about the WPBR resistance systems.

## Acknowledgement

The authors would like to thank Dr. Rich Hunt and Dr. Simon Shamoun of the Pacific Forestry Centre, Canadian Forest Service, for their valuable review comments.

## References

- Gresshoff, P.M., Doy, C.H. 1972. Development and differentiation of haploid *Lycopersicon esculentum*. *Planta* 107: 161-170.
- Hoff, R., Bingham, R.T., McDonald, G.I. 1980. Relative blister rust resistance of white pines. *Eur. J. For. Pathol.* 10: 307-316.
- Hunt, R.S., Meagher, M.D. 1989. Incidence of blister rust on “resistant” white pine (*Pinus monticola* and *P. strobus*) in coastal British Columbia plantations. *Can. J. Plant Pathol.* 11: 419-423.
- Kinloch, B.B., Sniezko, R.A., Barnes, G.D., Greathouse, T.E. 1999. A major gene for resistance to white pine blister rust in western white pine from the Western Cascade Range. *Phytopathology* 89: 861-867.
- Kinloch, B.B. 1982. Mechanisms and inheritance of rust resistance in conifers. Pages 119-125 in: *Proc. Third Int. Workshop on the Genetics of Host-Parasite Interactions in Forestry*. Pudoc, Wageningen, the Netherlands.
- Lloyd, G., McCown, B. 1981. Commercially-feasible micropropagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip. *Int. Plant Prop. Soc. Proc.* 30: 421-427.
- Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.