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Cold hardiness of interspecific hybrids between *Pinus strobus* and *P. wallichiana* measured by post-freezing needle electrolyte leakage

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Summary Interspecific hybrids between eastern white pine (*Pinus strobus L.*) and Himalayan blue pine (*P. wallichiana* A. B. Jacks.) were developed in Ontario, Canada, to introduce blister rust (*Cronartium ribicola* Fisch.) resistance genes to *P. strobus*. There is concern that introducing blister rust resistance has resulted in reduced cold hardiness of the progeny compared with non-hybridized eastern white pine. To test the efficacy of backcrossing with *P. strobus* to improve cold hardiness, 1-year-old seedlings from hybrid crosses differing in *P. strobus* genome composition were artificially freeze-tested. In Experiment 1, unhardened seedlings were allowed to acclimate to progressively lower temperatures in a growth room, whereas in Experiment 2, seedlings were hardened outdoors under natural weather conditions in Sault Ste Marie, Ontario. Needle cold injury was determined by calculating relative electrical conductivity based on post-freezing electrolyte leakage. Results indicated that needle fascicles from unhardened seedlings of all genotypes in the greenhouse tolerated −5 °C for 3 hours with little or no injury. Cold hardiness increased in parallel with declining growth room minimum temperature over the 7-week period of hardening. Cold hardiness was improved for hybrid crosses with increased *Pinus strobus* genome composition in Experiment 2, but the results were less conclusive in Experiment 1.

Keywords: blister rust resistance, Cronartium ribicola, Himalayan pine, white pine.

Introduction

White pine blister rust (caused by *Cronartium ribicola* Fisch.) often constitutes a major challenge to the regeneration of eastern white pine (*Pinus strobus* L.) in parts of eastern Canada and the USA where weather conditions are conducive to blister rust infection on pine needles in the fall (Gross 1985, Dahir and Carlson 2001, Samman et al. 2003). Because of a lack of natural resistance to this introduced pathogen, infection of young *P. strobus* trees often results in high mortality (Pitt et al. 2003, Sinclair 2003, Daoust and Beaulieu 2004). Breeding for genetic resistance to blister rust in *P. strobus* is, therefore, of ecological and economic importance.

Interspecific hybridization was explored in Ontario, Canada, to introduce blister rust resistance genes to *P. strobus*. Progenies between *P. strobus* and Eurasian white pine species in the subgenus *Strobus* were screened for blister rust resistance through artificial rust inoculation studies and field tests (Heimburger 1962, 1972, Zsuffa 1979, 1981, 1985). Among the selections, interspecific hybrids between *P. strobus* and the Himalayan blue pine (*P. wallichiana* A. B. Jacks.) showed promise (Zsuffa 1981, 1985, Lu et al. 2005).

Despite substantial gains in blister rust resistance, long-term field trials indicate that some first-generation hybrids between *P. strobus* and *P. wallichiana* had less than satisfactory adaptation on northern sites, although they grew and flowered normally in southern Ontario. When backcrossed with *P. strobus*, the progenies showed considerable improvement in field performance (unpublished data), probably a result of an increased proportion of *P. strobus* genome in the hybrids. It is hypothesized that cold hardiness of interspecific hybrids between *P. strobus* and *P. wallichiana* may be further improved through additional backcrossing with *P. strobus*.

Cold hardiness of conifers is frequently evaluated after artificial freezing of needle or shoot tissues (Rehfeldt 1980, Rehfeldt et al. 1984, Colombo et al. 1984, 1995, Odlum and Blake 1996, Aitken and Adams 1996, 1997, Anekonda et al. 2000, Burr et al. 2000, O'Neill et al. 2001). Although a visual assessment of tissue damage based on color changes after freezing is rapid (days to weeks) and applicable to large sample sizes, the electrolyte leakage (EL) test provides a quantitative measure of tissue injury below the threshold resulting in visible injury (Odlum and Blake 1996). Numerous studies with conifer seedlings have demonstrated the applicability of the EL method for predicting cold hardiness (e.g., van den Driessche 1976, Colombo and Cameron 1986, Sutinen et al. 1992, Colombo et al. 1995). The objective of our study was to evaluate cold tolerance of *P. strobus* backcross hybrids with *P. wallichiana* based on EL from needles of seedlings subjected to artificial freezing treatments.

Materials and methods

White pine genotypes and seedling culture

Open-pollinated (OP) seeds were collected from pure *P. strobus* trees and crosses between *P. strobus* and *P. wallichiana*,