

# Confirmation of Dominant Gene Resistance (Cr2) in U.S. White Pine Selections to White Pine Blister Rust Growing in British Columbia

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**Abstract**—To demonstrate the existence of the dominant Cr2 blister rust resistance gene in Dorena derived stock already producing seed in British Columbia, about 50 seedlings/parent tree were inoculated and examined for hypersensitive needle spots. Seedlings from 33 of 42 canker-free parents produced hypersensitive spots, confirming the presence of Cr2 in the parent trees. To determine if an existing pathotype might have already overcome the Cr2 gene in British Columbia, seedlings from three suspect trees (because they were canker-free for 10 years, but recently became cankered) were inoculated. The Cr2 gene was absent in these three trees. Additionally, seedlings from two seedlots known to possess the Cr2 gene were subjected to two consecutive annual inoculations. This double inoculation was repeated on five different occasions with a composite inoculum. Canker-free seedlings following the first inoculation remained canker-free after a second inoculation, consistent with the uncompromised expression of Cr2.

**Key words:** Cr2, blister rust, resistance genes

## Introduction

As early as the 1960s a single dominant gene for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch.) was suspected in western white pine (*Pinus monticola* D. Don) from the Champion mine region of Oregon. Canker-free trees from this source were incorporated into the USDA Forest Service seed orchard at the Dorena Genetic Resource Center, near Cottage Grover, Oregon. By 1984 this resistance had failed (McDonald and others), which was later attributed to the failure of a single dominant gene, called Cr2 (Kinloch and others). We obtained two seed collections from the Dorena seed orchard - one bulked collection of 32 Champion Mine-derived parents (Champion), and one bulked from six Washington State parents (WA). We out planted the resulting seedlings in plantations across south-

ern British Columbia (BC) in 1986 and 1987 (Hunt 1987). Unaware that these lots could possess the dominant Cr2 gene, we reported that these two lots had greater resistance than other lots in two coastal and two interior plantations (Hunt 1994). Since then, we have tried to (a) confirm that these healthy trees possess the Cr2 gene; (b) determine if a virulent pathotype might exist in BC which overcomes the Cr2 gene; and (c) determine whether the resistant phenotype would be maintained under repeated inoculation.

## Materials and Methods

The seven plantations with the Champion and WA seedlots have been monitored for rust incidence annually, or biennially (Hunt 1994). Some of the surviving trees from these two Dorena seedlots have produced seed, particularly in the two most rusted coastal plantations. We collected seed from 42 of these parents and three trees that were canker-free for 10 years but then developed rust. In 2000 50 seeds/parent were stratified and 1-0 seedlings bearing only primary needles were inoculated in a ribes (disease) garden with a composite of six BC rust sources initially collected from throughout the range of western white pine on Vancouver Island. The seedlings were examined for hypersensitive spots, the manifestation of the Cr2 gene (Kinloch and others 1999), in February 2001.

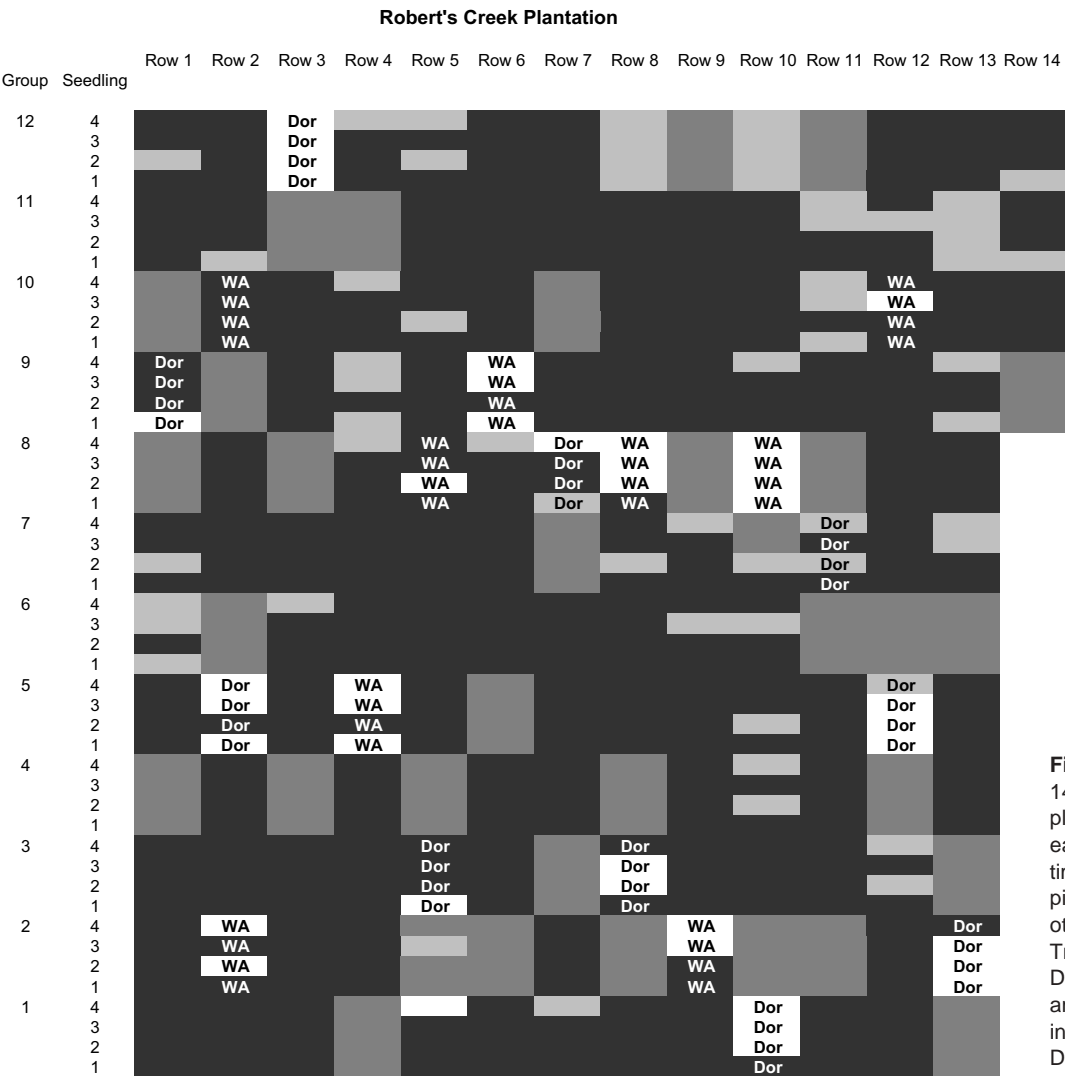
To determine if repeated inoculation would nullify the dominant resistance, or whether it would be maintained, two seedlots known to possess the Cr2 gene respectively were obtained from Dorena (Kinloch and others 1999; registration # 119-15045-845 X OP (our seedlot #1) and #119-15045-845X15045-841 X OP (our seedlot #2)), sown and grown in 1994, 1996, and 1997. Some were first inoculated at age 4-months (only primary needles present) others at 16-months (secondary needles present) in the ribes (disease) garden. Seedling survival was attributed to the Cr2 gene. All survivors were re-inoculated the following year. From the 1994 inoculation, 40 grafted ramets were produced from seven seedlings, and these were inoculated.

## Results

The most severely damaged plantation had 49 percent of the Dorena derived trees canker-free compared to only 0.2 percent for the other provenances after 15 years (fig. 1); comparable numbers for the other plantation were 66 and 13

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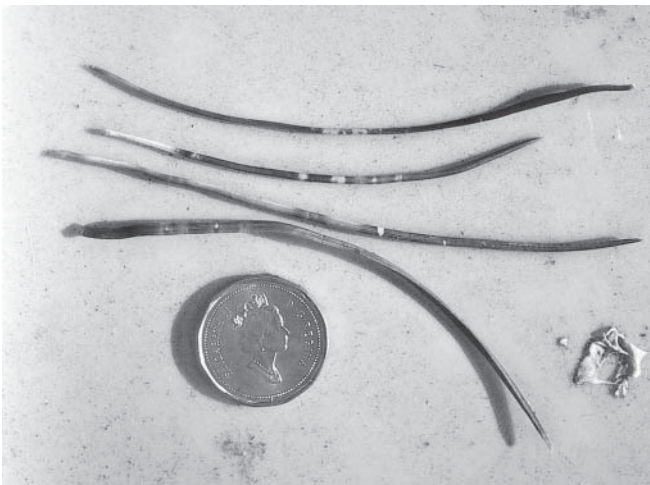
The authors are with the Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.



**Figure 1**—A plantation depicting 14 provenances of western white planted in four-tree row plots, with each provenance replicated 10 times. White denotes canker-free pine, black cankered pine, grey other conifers and missing trees. Trees labelled Dor = a bulked Dorena seed orchard collection, and WA = a collection from Washington State trees growing in the Dorena seed orchard.

percent. Seventeen of 18 canker-free plantation trees from the Champion seedlot produced seedlings with hypersensitive spots (fig. 2) typical for Cr2 (family mean percent Cr2 49; range 21 to 100). None of the seedlings produced by the parent lacking Cr2 possessed Cr2. From the WA seedlot 16 of 24 plantation trees also produced hypersensitive spots (family mean percent Cr2 41; range 21 to 60). The seven WA plantation trees lacking Cr2 processed few seedlings with hypersensitive spots (family mean percent 1; range 0 to 5). The percentage of Cr2 in the offspring of the three recently cankered parents was 0, 0, and 5.

Many of the seedlings from the two registered Dorena seedlots possessing the Cr2 gene died from fusarium root rot making survival rates variable. Nevertheless, the over-all mean survival was greater than 50 percent for each seedlot. None of the canker-free survivors from the first inoculation developed rust cankers from a second inoculation (table 1). In addition, none of the 40 ramets became infected, but in six the rootstock became infected. In these, the rust failed to cross the graft union over 4 years (fig. 3).



**Figure 2**—Blister rust infection spots in pine needles. The top two needles show normal spotting; the bottom two have necrotic halos typical of hypersensitive spots in western white pine as a result of the Cr2 gene.

**Table 1**—Percentage Cr2 gene in two western white pine families based on survival after an initial inoculation with *Cronartium ribicola* and canker-free survivors after a second inoculation. No survivors from the first inoculation became cankered from a second inoculation, but numbers were reduced by fusarium root rot.

Seedlot	Seedlings	Inoculation	Canker-free	Cr2
	(no.)	(year)	(no.)	(%)
1	44 <sup>a</sup>	1995&6	10	29
1	24 <sup>a</sup>	1996&7	12	54
1	20 <sup>b</sup>	1997&8	10	50
1	446 <sup>a</sup>	1997&8	214	55
1	498 <sup>b</sup>	1998&9	324	74
2	76 <sup>a</sup>	1995&6	28	74
2	40 <sup>a</sup>	1996&7	28	77
2	37 <sup>b</sup>	1997&8	22	59
2	336 <sup>a</sup>	1997&8	182	73
2	448 <sup>b</sup>	1998&9	305	80

<sup>a</sup> First inoculated at 4 months.

<sup>b</sup> First inoculated at 16 months.



**Figure 3**—Failure of *Cronartium ribicola* to infect a western white pine scion possessing the Cr2 gene from an infected root stock.

## Discussion

No apparent failure of the dominant resistance gene has been observed in seven BC plantations over 15 years. Inoculation of seedlings from 33 healthy parents derived from these plantations produced hypersensitive spots, confirming they possessed the Cr2 gene. Additionally, inoculation of seedlings possessing the Cr2 gene with a composite inoculum resulted in high survivor ratios as would be expected from a heterozygous parent pollinated with orchard pollen. Re-inoculated survivors remained canker-free, confirming the stability of the Cr2 gene. Even scions possessing the Cr2 gene failed to become infected from *C. ribicola* infected root-stock after 4 years. In contrast, seedlings from three recently cankered parents derived from the same Dorena stocks produced normal spots and thus lack the Cr2 gene. We conclude that we have failed to demonstrate the existence of a virulent pathotype of *Cronartium ribicola* to the Cr2 gene in BC and so far the Cr2 gene appears stable in BC even when nonselected stocks are devastated (fig. 1).

Most blister rust cankers in BC occur within 3 m of the ground (Hunt 1991); that is, when the plantations are young (usually less than 15 years old). It appears that the Cr2 gene could be deployed successfully in isolated areas in BC, and if the resistance failed, the trees likely will have out-grown their most susceptible age before a virulent pathotype could have an opportunity to build-up damaging levels of inoculum. However, if a series of age classes were planted in contiguous areas, a new virulent pathotype could be limiting to the younger plantations.

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