

A First Look at Genetic Variation in Resistance to the Root Pathogen *Phytophthora cinnamomi* using a Range-wide Collection of Pacific Madrone (*Arbutus menziesii*)

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Introduction

Phytophthora cinnamomi (Oomycetes) causes root disease and basal canker on a number of hardwood and conifer hosts, including Pacific madrone (*Arbutus menziesii* Pursh) (figs. 1, 2), a broadleaf evergreen species whose range extends from coastal British Columbia to southern California (Reeves 2007). Increasing mortality of Pacific madrone and the related shrub species manzanita (*Arctostaphylos* spp.) has been seen in California forests (Fichtner et al. 2009) in recent years.

Considered to be one of the world's most invasive species, *P. cinnamomi* is thought to have originated in southeast Asia and spread worldwide, causing decline in Australian eucalypt forests, crop losses on avocado in California and Europe, and on Christmas tree plantations in the southeast United States. It has a wide host range and infects many ornamental trees and shrubs in landscape plantings. In this situation, it is thought that the pathogen moves in contaminated soil on nursery stock (Smith 1988).

Materials and Methods

Between 2006 and 2008, Pacific madrone (*Arbutus menziesii* Pursh) fruit was collected from 237 trees at various sites throughout its natural distribution range in British Columbia, California, Oregon, and Washington and extracted seed was added to the Washington State University (WSU) Puyallup madrone seed collection. From this collection, seedlings were grown from 34 families representing seven ecoregions for the study (table 1). One to five families per geographic location within one or two locations in each ecoregion were sampled. Twelve one-year-old seedlings per family were arranged randomly in rows on the greenhouse benches. Six seedlings per family were inoculated with an isolate of *P. cinnamomi* collected from madrone roots in Sonoma County, California, and the remaining six were not inoculated. After inoculation, all plant pots were placed in 946 ml plastic cups which were then flooded with water. Plants remained submerged in water for 2 days.

Seedlings were then observed for wilting and browning symptoms (fig. 3) over a 6- week period after which roots and stems of inoculated and check plants were sampled for dry weight (table 2). Principal components analysis was performed on mean values of all variables arranged by seedlot (family) after standardization (z-score). Non-parametric ANOVA (Kruskal-Wallis test) was performed on the original variables grouped by site and ecoregion, and Dunn's post-hoc test was performed if the differences were significant at $P = 0.05$.

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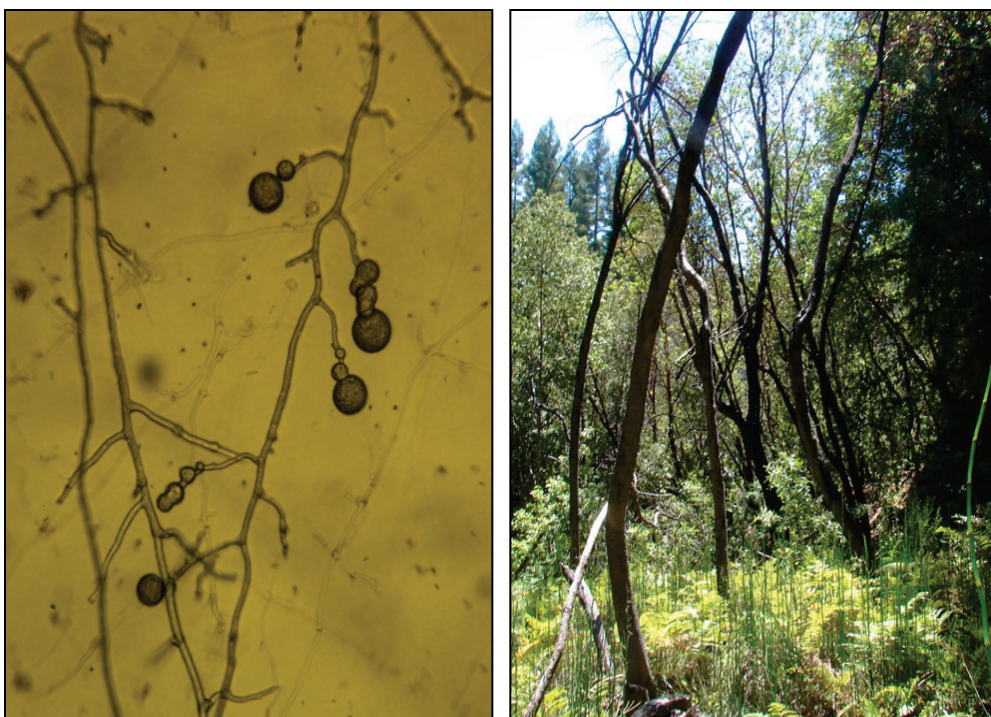


Figure 1 (left)—*Phytophthora cinnamomi* in V8 culture The organism can be identified by clusters of chlamydospores and coraloid hyphae. (Photo: Jeff Kepley)

Figure 2 (right)—Madrones in Soquel State Demonstration Forest, California. These trees have symptoms of crown dieback and general decline. *Phytophthora cinnamomi* was baited out of soil from this area. Horsetail (*Equisetum* spp.) is growing on the site, which is near the bottom of a slope, and is indicative of a wet site.

Table 1—Sites and ecoregions in western North America where Pacific madrone seed sources used in this experiment originated

Site	Ecoregion	Number of seed sources
BC16	Coastal western hemlock/Sitka spruce	1
BC14	Pacific and Nass ranges	1
FB, FW	Strait of Georgia, Puget lowland	10
WV	Willamette Valley	2
CP, KL	Klamath mountains	10
PL	Sierra Nevada	5
CC, SC	California coastal sage, chaparral, and oak woodlands	5



Figure 3—Madrone seedlings in greenhouse 21 days after inoculation. Some variability in symptom expression can be seen. The seedling on the left is dead where the seedling on the right is relatively healthy.

Table 2—Data collected on Pacific madrone seedlings inoculated with *Phytophthora cinnamomi* in the greenhouse trial

Variable	Description
BR	Disease severity - 0-10: 0 = 0% brown foliage, 1= 1-10% brown, 2=11-20% brown, 10= 91-100% brown, dead plant.
WR	Wilt rating - 0-4: 0 = no disease, 1= slight disease, 2 = Moderate disease, 3 = severe, 4= dead plant.
RR	Root ball size rating - 1-5: 1 = smallest, 5 = largest
RDW	Root dry wt, grams
SDW	Stem dry wt, grams

Results and Discussion

Variability within seed sources was high for root dry weight in both inoculated and check treatments and lower for other variables. *Phytophthora cinnamomi* was isolated from the roots of all inoculated plants and not from the roots of check plants.

No relationship between ecoregion and any of the variables was observed. There were significant differences in disease severity on inoculated plants among sites, with the least disease seen on seedlings obtained from parent trees at Fidalgo Bay, Washington (fig. 4). There was no significant difference in disease severity among families when considered separately; however, agglomerative hierarchical clustering produced three groups with low, intermediate, and high disease severity (table 3).

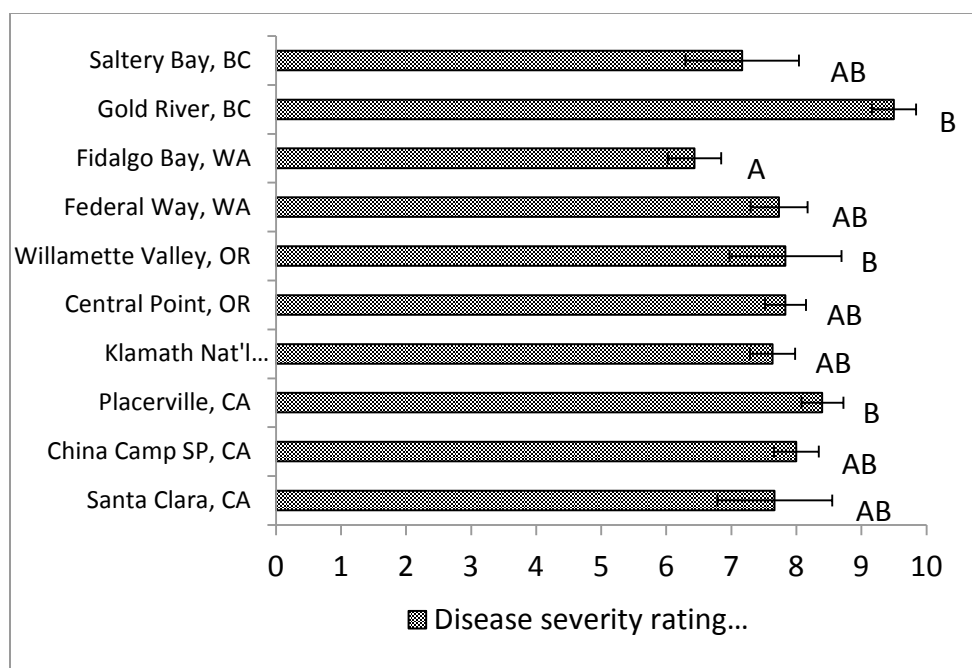


Figure 4—Severity of browning and wilting symptoms on madrone seedlings from seed collected at 10 sites. (Kruskal-Wallis test with Dunn's multiple comparisons. Bars with different letters are significantly different at $p = 0.05$).

Table 3—Groupings of families by severity of browning symptoms on plants inoculated with *Phytophthora cinnamomi* using agglomerative hierarchical clustering (AHC)

Class	Disease severity rating ^a	Families
1	6.0	FB3, FB5, FB8, FW10, KL8
2	7.5	BC14, CC2, CC3, CC7, CP18, CP21, CP23, CP24, FB1, FB6, FW5, FW7, FW9, KL1, KL2, PL11, PL4, SC9, WV2
3	8.9	BC16, CC1, CP11, FW3, KL10, KL7, PL3, PL5, PL9, WV5

^a Disease severity rating is given as the class centroid for each group.

Root ball size rating (RR) and root dry weight (RDW) of inoculated plants were negatively correlated with the disease severity rating ($r = -0.459$ RR, -0.424 RDW). The root dry weight, stem dry weight, and root ball size rating on check plants showed no relationship with disease ratings on inoculated plants from the same seed sources. The first two principal components accounted for 63% of the variation in the data set (Figure 5). The first principal component was related to root and shoot weights and root ball rating (inoculated and check plants), and the second to disease severity (inoculated plants). Thus, plant size was not a predictor of disease development in this experiment.

This initial study indicated that while madrones from some seed sources had less severe symptoms than others, there was no significant relationship of disease severity with plant characteristics measured, such as root ball size and stem dry weight. To confirm these results, this experiment was repeated in fall 2011 using seedlings from the same seedlots. Results from 2011 were similar to those from 2010.

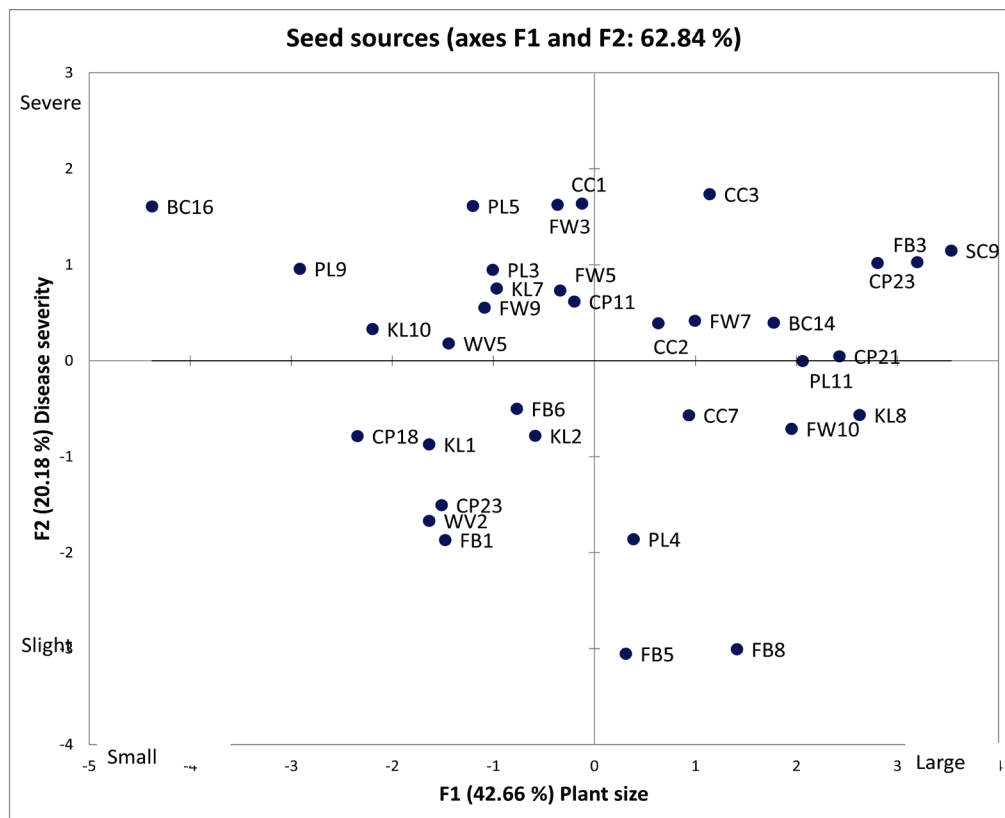


Figure 5—Scatter plot showing results of principal components analysis on the variables measured in this study. Individual seed sources are labeled by site and tree number.

Literature Cited

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