SIMPLY INHERITED RESISTANCE TO *Phytophthora lateralis* IN PORT-ORFORD-CEDAR: GREENHOUSE TESTING.

R.A. Sniezko[1], E.M. Hansen[2], and S.E. Kolpak[1]
[1]USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424
[2]Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR 97331

Poster Design by John S. Parker

Results and Discussion

Dramatic differences exist in survival among seedling families tested (Tables 1, 2, and 3). All seedling families of parent 117490 show very high survival (Table 2) regardless of what other parent is involved. Only one of the crosses exhibited mortality that is significantly greater than expected ($\pi = 0$). The data indicates that 117490 is a homozygous dominant genotype for a major resistance gene.

Seedling families of other parents (such as CF1 & 510015) show intermediate ranges of survival depending what other parent is involved in the cross (Table 1). All crosses are within the expected chi-square ratios except for progeny when CF1 or 117502 are selfed. Data on these parents suggest that they are heterozygous for a major resistance gene.

Seedling families of susceptible parents show little or no survival (Table 3) unless involved in matings with parents such as 117490, CF1, 510015, or 117502 (Table 1,2) None of the crosses exhibited mortality lower than expected ($\pi = 1$). The data indicates that these parents are homozygous recessive for a major resistance gene.

The greenhouse data indicate a major gene for resistance to *P. lateralis* exists. A few exceptions in the suggested inheritance of this resistance occur here, and further work is underway to examine these.

Table 1. Observed and expected segregation ratios (live/dead) for putative heterozygous parents. Proportion of seedlings dying, 2000 to 2002 test years. Crosses between heterozygotes or heterozygotes selfed were evaluated against an expected 3:1 segregation ratio. Crosses between heterozygotes and homozygotes were evaluated against an expected 1:1 segregation ratio. The Chi-square value tested for deviations from the expected segregation ratios.

Table 1.

Family	Parent: Female	Parent: Male	Test Year	No. of seedlings	Proportion dying	Test ratio	x^2	*P*	
510015	510015	PO-DOR-70020	2001	24	**0.29**	*3:1*	2	**0.157**	
	510015	510015	2001	18	**0.28**	*3:1*	1	**0.317**	
	510015	CF1	2001	24	**0.29**	*3:1*	2	**0.157**	
CF1	CF1	CF1	2001	24	**0.04**	*3:1*	10	**0.002**	*
	CF1	CF1	2002	24	**0.13**	*3:1*	6	**0.014**	*
	CF1	118569	2003		**NA**	*1:1*			
117502	510044	CF1	2000	12	**0.42**	*1:1*	2	**0.157**	
	CF1	117502	2003		**NA**	*3:1*			
	117502	117499	2000	24	**0.46**	*1:1*	2	**0.157**	
	117502	117502	2000	24	**0.46**	*3:1*	10	**0.002**	*

* significantly different than the expected ratio
heterozgotes denoted in blue, homozygous recessive denoted in red

Infected Port-Orford-Cedar (P.O.Cedar) **in the wild.**

Example of Disease @ the base of P.O.Cedar in the wild.

Figure 1: Root Root Dip procedure @ OSU.

Figure 3: Checking root system of survivor for disease.

Figure 2: Stem lesion on diseased seedling.

Table 2. Parent 117490 (homozygous dominant) crossed with heterozygotes, homozygous recessive, and uncharacterized parents. Proportion of seedlings dying, 2000 to 2002 test years. The 95 % confidence intervals are in parentheses.

Table 2.

Parent: Female	Parent: Male	Test Year	No. of seedlings	Mortality ratio		
117490	117344	2000	24	**0**	-	
117490	117490	2000	12	**0**	-	
117490	117499	2000	24	**0**	-	
117490	117502	2000	18	**0.06**	**(0.11)**	
117490	117503	2000	18	**0**	-	
117490	CF2	2000	18	**0**	-	
117509	117490	2000	24	**0.04**	**(0.08)**	
510049	117490	2000	18	**0.06**	**(0.11)**	
117490	117505	2001	24	**0**	-	
117490	117650	2001	24	**0**	-	
117335	117490	2001	24	**0.04**	**(0.08)**	
118054	117490	2001	18	**0.06**	**(0.11)**	
117490	117344	2002	24	**0**	-	
117490	117490	2002	48	**0.02**	**(0.04)**	
117490	117499	2002	24	**0**	-	
117490	118569	2002	24	**0.04**	**(0.08)**	
117490	510015	2002	24	**0**	-	
117490	PO-DOR-70231	2002	24	**0.08**	**(0.11)**	
117490	CF1	2002	48	**0**	-	
117490	CON1	2002	24	**0.21**	**(0.16)**	*
			Average	*0.03*		

* significantly different than $p = 0$
-homozygous dominant denoted in green, heterozygotes in blue
homozygous susceptible in red

Goal: Healthy forests.

Table 3. Crosses between recessive parents. Proportion of seedlings dying, 2000 to 2002 test years. The 95 % confidence intervals are in parentheses.

Table 3.

Family	Parent: Female	Parent: Male	Test Year	No. of seedlings	Mortality ratio	
118569	118569	510042	2000	18	**1**	-
	510041	118569	2001	24	**0.96**	**(0.08)**
	118569	510044	2000	18	**0.94**	**(0.11)**
510044	118569	510044	2002	20	**1**	-
117499	117499	117499	2000	11	**0.91**	**(0.17)**
	117499	117499	2002	48	**0.94**	**(0.07)**
			Average	*0.96*		

* significantly different than $p = 1$
-homozygous recessives (susceptible) red

Introduction

Port-Orford-cedar (*Chamaecyparis lawsoniana*) is very susceptible to the non-native, invasive pathogen *Phytophthora lateralis*. The pathogen continues to spread through native forests in California and Oregon as well as horticultural plantings. Only in the late 1980's was there first indication of genetic resistance to *P. lateralis* (Hansen et al. 1989). Additional testing in the last eight years confirmed the presence of resistance (Sniezko et al. 2003) and its relative rarity. Through 2000, only a few parent trees had been identified that demonstrated resistance across different screening methods (Sniezko, unpublished data). The work reported here focuses on examining the inheritance of resistance of the few parent trees showing the highest levels of resistance in the prior trials.

117490

510015

CF1

Examples of resistant P.O.Cedar to be used for forest restoration.

Abstract

Phytophthora lateralis, a non-native root rot pathogen, is the primary cause of Port-Orford-cedar (*Chamaecyparis lawsoniana*) mortality throughout its native range in southwest Oregon and northwest California. Most trees in field are very susceptible, but greenhouse testing in the late 1980's demonstrated existence of genetic resistance to this pathogen. Greenhouse studies to examine the magnitude and inheritance (whether Mendelian or polygenic) of this resistance have utilized a root dip technique using young seedlings. Susceptible families show 90-100% mortality over the 10 to 11 month evaluation period, but a few full-sib families show little (0-10%) or only moderate (25-60%) mortality. Results from full-sib families from control crosses using the most resistant parents indicate that a major gene for resistance is present, with survival ratios of 3:1, and 1:1 depending on the parent and cross. There are a few deviations from the survival ratios, including two selfed crosses. A putative homozygous dominant parent, three heterozygous parents, and three homozygous recessive parents were identified from the analysis. The data cannot preclude a mixed mode of inheritance (Mendelian and polygenic) across the entire range of Port-Orford-cedar or multiple mechanisms.

Material and Methods

Test procedure:

Seedlings are grown at Dorena GRC for ~9 months in individual 10 in³ 'super cell' tubes. After randomization, up to seven seedlings are inoculated at a time in jars containing two isolates (with mycelia, sporangia, and zoospores). Two isolates (#366 & #368) are grown separately on petri plates before being combined in jars. Total inoculum per jar is ~60 ml. The bottom 2 cm of the tubes are immersed in the inoculum for two days (**Figure 1**), then are removed and placed back in the racks in the greenhouse. In general, the first signs of infection begin ~6 weeks later. In this greenhouse test ('root dip' test) infection can progress from one or more root tips present at the bottom of the tube. At the first sign of disease symptoms (yellowing or wilting) a small slice in the bark at ground-level is made and the stem is examined for a lesion (**Figure 2**). Trees with lesions are noted as infected with *P. lateralis* and are pulled and marked as dead. Seedlings are examined over the next 9 to 12 months or until there is little sign of additional symptoms and mortality. At the termination of each trial, the roots of surviving seedlings are examined for lesions (**Figure 3**.)

Data Analysis:

Full sib (from controlled pollinations) or half-sib (wind pollinated in the Dorena breeding orchard) seedling families were used for disease testing. Results from several test years are incorporated in this report in an attempt to construct a mating design such that each putative heterozygote is crossed with another heterozygote and a homozygote. A Chi-square test was used to evaluate the observed against the expected segregation ratios (live/dead). Crosses between putative heterozygotes (including selfs) were evaluated at a 3:1 ratio while heterozygotes crossed with homozygous recessives (susceptibles) were evaluated at a 1:1 ratio (Table 1). In addition, 95% confidence intervals were computed for the putative homozygous dominant parent and the homozygous recessive parents to determine if the mortality ratio deviated from zero or one respectively (Table 2,3).

References

Hansen, E.M., Hamm, P.B., and Roth, L.F. 1989. Testing Port-Orford-cedar for resistance to *Phytophthora*. Plant Disease 73(10): 791-794.
Sniezko, R.A., Elliott, L.J., Goheen, D.J., Casavan, K., Hansen E.M., Frank C., Angwin, P. 2003 (in press). Development of *Phytophthora lateralis* resistant Port-Orford-cedar for restoration in the Pacific Northwest. IN: In: Bassman, J.H., J.D. Johnson, L. Fins, and J.P. Dobrowolski, eds., Rocky Mountain Ecosystems: Diversity, Complexity and Interactions. Proc. 17th North American Forest Biology Workshop, July 15-18, 2002, Pullman, Washington. Washington State University Cooperative Extension, Pullman, Washington, U.S.A. (In Press). Proceedings of North American Forest Biology Workshop.

ACKNOWLEDGEMENTS: Many Forest Service and BLM people have been essential for various elements of the inter-agency and inter-regional resistance program, particularly for the field selections. We would also like to acknowledge the funding from th R6 Genetics and Forest Health Programs and BLM which has allowed the progress to date. Special thanks to Wendy Sutton (OSU) for providing much of the oversight on the seedling root dip evaluations, and Leslie Elliott (FS) for coordinating the other operational aspects of this project. John S. Parker provided the poster design.