Nine Year Survival of 16 *Phytophthora lateralis* Resistant and Susceptible Port-Orford-Cedar Families in a Southern Oregon Field Trial

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Abstract

Port-Orford-cedar (*Chamaecyparis lawsoniana*) has suffered high mortality from the pathogen *Phytophthora lateralis* in portions of its natural range in southwest Oregon and northwest California, as well as in horticultural plantings in North America, and more recently in Europe. A program to develop genetically resistant populations of Port-Orford-cedar is underway. This operational program began in 1996 and utilizes artificial inoculation of the roots of young seedlings (or rooted cuttings) to rate parent trees for resistance. A key step to any such program is to establish field trials: to validate the results of artificial inoculation trials; to examine resistance at a range of sites and environments conducive to the pathogen; and to monitor the durability of resistance. The field trial ('Foggy Eden' site) examined here consists of 16 Port-Orford-cedar families established in southern Oregon in 2002 at a site on the Rogue River-Siskiyou National Forest. The Foggy Eden (FE-02) site was chosen because of the notable recent heavy *P. lateralis* related mortality of the natural Port-Orford-cedar trees in the immediate vicinity. Sixteen families had been selected to cover a range from highly susceptible (100 percent mortality) to highly resistant (0 percent mortality) in a previous short-term greenhouse artificial inoculation ('root dip,' RD) trial. Some of the families were also included in two short-term raised bed (RB) trials, where the raised bed had been previously infested with *P. lateralis*. By summer 2010, overall mortality at FE was 42.7 percent (263 of 616 trees). Most of the mortality occurred by 2006 (244 trees). Family variation in survival ranges from 20.8 to 93.8 percent. The top three families for survival involve a common parent and all have less than 11 percent mortality. The comparison of the results from FE-02 with the short-term 'root dip' and raised bed tests showed a strong relationship between these trials. The results are encouraging at this point, but the trial needs to be followed longer, and the results from the additional trials in other environments conducive to *P. lateralis* need to be examined to determine the efficacy of resistance in different environments. Resistant seed from wind-pollinated orchards is now available to aid restoration and reforestation.

Key words: Port-Orford-cedar, *Chamaecyparis lawsoniana*, *Phytophthora lateralis*, genetic resistance, field trials

Introduction

Port-Orford-cedar (POC) (*Chamaecyparis lawsoniana* (A. Murr.) Parl.), a long-lived conifer, has experienced high mortality from the non-native root pathogen *Phytophthora lateralis* (Tucker and Milbrath) in portions of its natural range in southwest Oregon and northwest California (USDA FS and USDI BLM 2004), as well as in horticultural plantings in North America and Europe. The use of growing stock with genetic resistance is key to restoration of POC in these areas as well as in general reforestation and horticultural plantings where *P. lateralis* may be problematic. An applied program to develop genetically resistant populations of POC is underway (Sniezko 2006, Sniezko et al. 2009; Sniezko et al., Operational program to develop *Phytophthora lateralis* resistant populations of Port-Orford-cedar (*Chamaecyparis lawsoniana*), these proceedings). This resistance program utilizes several methods of short-term testing to rapidly evaluate hundreds of field selections for resistance. However, there are few reports detailing differential survival of resistant and susceptible POC

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families in field tests or the correspondence between genetic resistance in short-term seedling tests and field tests (Sniezko et al. 2006).

In this paper, we examine field survival 9 years after the planting of 16 POC families at a site where *P. lateralis* mortality of the existing POC in the forest stand had been moderately high. We also examine the relationship between field mortality in this trial with seedling mortality in four short-term greenhouse and raised bed trials.

Materials and Methods

Seedlings for all trials were grown for 1 year in 164 ml tubes using a soilless nursery medium (mixture of peat, vermiculite, composted fir bark, perlite, and pumice) in a greenhouse at Dorena Genetic Resource Center (DGRC) in Cottage Grove, Oregon. The 'Foggy Eden' field site (42° 48.195' N, 123° 53.340' W, 731 m elevation; fig. 1) on the Powers Ranger District of the Rogue River-Siskiyou National Forest was planted at 1.83 m x 1.83 m spacing on April 5th and 6th 2002 using 616 1-year- old seedlings from 16 families (representing 20 parents). Seedlings were planted in family row plots with 4 to 12 trees per family in each of the four blocks of the randomized complete block design. The parent trees were forest selections from within varying parts of the natural range in Oregon and California. The nine open-pollinated (wind-pollinated seed lots from a clone bank at DGRC) and six full-sib families had been chosen to represent a wide range of resistance (0 to 100 percent survival) based on a previous short-term (7 month) seedling test (RD-00, see below). The Foggy Eden site (FE-02) was chosen due to the presence of recent mortality from *P. lateralis* of approximately 75 percent of the POC in the overstory and surrounding area. For each of the seven assessments since the 2002 planting, seedlings were rated as alive, 'fading,' or dead. We report on the cumulative mortality of POC through the July 2010 assessment.

Figure 1—Port-Orford-cedar *Phytophthora lateralis* resistance trial planted in 2002 in understory of forest canopy at Foggy Eden (FE-02) site on Siskiyou National Forest in Oregon. (Photo credit: Eric Martz)

Subsets of the 16 families planted in FE-02 were also tested as part of four short-term (7-month to 2-year duration) greenhouse or raised bed trials (table 1) at Oregon State University in 2000 and

2002. The two greenhouse trials (RD-00, RD-02) utilized the 'root dip' technique where roots that had emerged from the bottom of the 164 ml tubes were clipped and the bottom 1 cm of the upright tubes were immersed in a zoospore suspension for 48 hours. The suspension was a mix of two standard *P. lateralis* isolates, '366' and '368.' The seedlings remained in the greenhouse for monitoring and assessments over the subsequent 5 to 7 months (table 1; fig. 2). In the raised bed trials (RB-00, RB-02), seedlings were planted in a raised bed (fig. 3) in which the soil had been previously infested with the same two isolates of *P. lateralis*. Mortality in all four seedling trials was recorded over the ensuing 5 to 18 months as seedling foliage began to wilt or turn chlorotic (table 1). At each assessment, fading seedlings were scraped at the root collar to check for the presence of the characteristic cinnamon-colored stain (fig. 3). *Phytophthora lateralis* was also confirmed on a small subset of seedlings using a combination of culture and PCR techniques. Selective culture and PCR methods were adapted from Winton and Hansen (2001). Further details are discussed elsewhere (Oh et al. 2006). Further details on methods for root dip and raised bed testing are reported by Hansen et al., Methods for screening Port-Orford-cedar for resistance to *Phytophthora lateralis*, these proceedings.

Figure 2—Port-Orford-cedar seedlings in greenhouse following 'root dip' inoculation with *Phytophthora lateralis.* (Photo credit: Richard Sniezko)

Figure 3—Raised bed trial of Port-Orford-cedar for *Phytophthora lateralis* resistance and seedling showing brown lesion from *P. lateralis* at root collar. (Photo credit: Richard Sniezko)

The design for all trials was a randomized complete block, with the number of seedlings per family in each block varying by trial (table 1). The 2000 trials (RD-00 and RB-02) had relatively few seedlings per family compared to the other trials (table 1). Survival in all trials was analyzed with blocks considered random and families as fixed effects using SAS (SAS Institute Inc., V9.2, 2008).

Survival at the termination of the four short-term trials (RD-00, RD-02, RB-00, RB-02) and field survival through summer 2010 for FE-02 (9 years after planting) were used for all analyses. Family differences in survival were analyzed using family-plot means in Proc Glimmix (binomial distribution); plot means were adjusted by 0.01 for those values of 1.0 (adjusted to 0.99) and 0.0 (adjusted to 0.01) in order to avoid the non-convergence issue in Proc Glimmix when numerous values of 1 or 0 are encountered in the dataset. Proc Corr (product moment correlation) was used to examine family mean correlations among the respective experiments, and Proc Reg (regression) was used to plot the family mean survival among experiments. All statistical analyses were conducted using SAS (SAS Institute Inc., V9.2, 2008).

Table 1—Background information on five *Phytophthora lateralis* resistance trials: field trial (FE-02), raised bed trials (RB-00, RB-02), and root dip trials (RD-00, RD-02)

Trial	No. Families (F)	No. Blocks (B)	No. Seedlings/F/B	First Assessment	Final Assessment	No. of Assessments
FE-02	16	4	4 to 12	2002	2010	7
RD-00	15	6	1 to 6	04/2000	01/2001	22
RD-02	9	4	6 to 12	04/2002	09/2002	5
RB-00	15	6	1 to 4	04/2000	01/2001	20
RB-02	9	4	6 to 12	05/2002	11/2003	8

Results

Overall survival of POC was 57.3 percent through summer 2010 at FE-02. Most of the mortality occurred within 4 years of planting (fig. 4). Visual symptoms (cinnamon-colored staining of the phloem tissue at the base of the tree after scraping away the bark) or DNA testing using PCR indicated that *P. lateralis* was the probable cause of mortality, but because the field site was only assessed periodically, definitive confirmation of cause of death for some seedlings was not possible. A few seedlings in the 2003 examination showed the lesion only at ground level (not downward into the roots) indicating that there may have been overland water flow infecting the seedlings right at the soil line, and not through the root tips, thus, potentially bypassing some resistances.

Significant differences ($p<.05$) among families were found at FE-02 with survival varying from 20.8 to 93.8 percent (fig. 4; table 2). The three families involving parent 117490 and the two families involving CF1 (both resistant parent controls) had high survival at FE-02 (table 2). Significant differences were also found among families in all of the raised bed and root dip trials (RB-00, RB-02, RD-00, RD-02). There was a moderate to high correlation between trials for the five tests, with only the correlations of RB-00 with RB-02 and RD-02 being non-significant (table 3; fig. 5). Families involving parents 117490, CF1, 117486, 510015, and 510005 generally ranked high for survival in the trials, although a few of these families showed higher mortality in the raised bed trials (table 2). In contrast, families involving relatively susceptible female parents 118463, D-70013, D-70103, and D-70119 have low survival across all tests. The remaining six families generally showed intermediate survival across the tests (table 2).

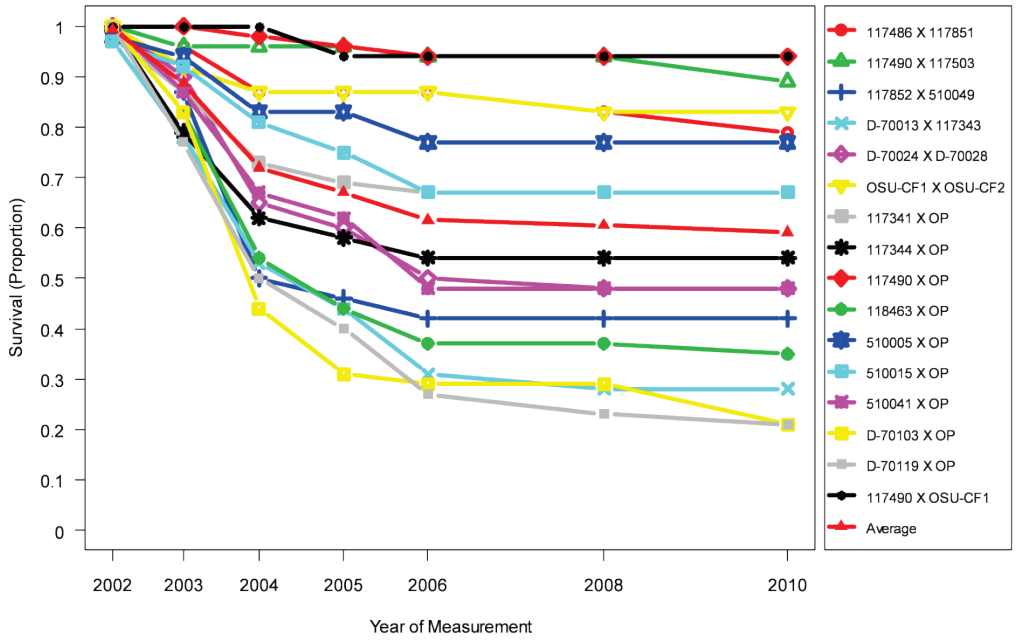

Figure 4—Survival from 2002 to 2010 for 16 Port-Orford-cedar families in Foggy Eden 2002 (FE-02) *Phytophthora lateralis* resistance planting.

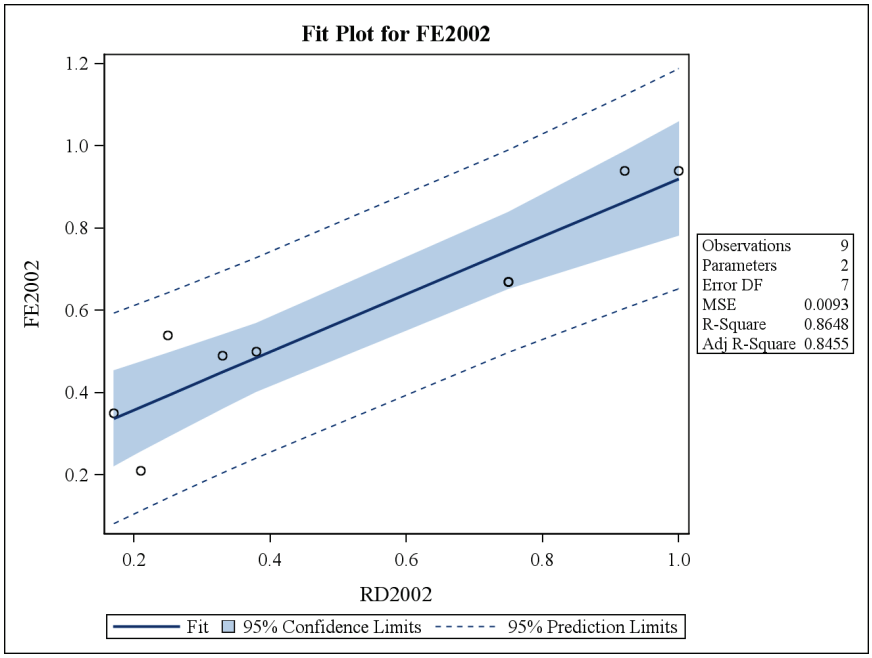

Figure 5—Regression plot between family mean survival in Foggy Eden field test (FE2002) and survival in 2002 root dip test (RD2002) for *Phytophthora lateralis* resistance (using eight common families).

Discussion

After 9 years in the field, we found very large differences in survival among the 16 families in the FE-02 trial. Visual examination or PCR testing confirmed the likelihood of *P. lateralis* as the causative agent of mortality and no other apparent cause of mortality was noted at this site. Little mortality has occurred in the last 5 years, suggesting that the genetic resistance present may be

Table 2—Mean survival proportions (and number of seedlings tested) by family for five trials: field test (FE-02), two raised bed tests (RB-00, RB-02), and two root dip tests (RD-00, RD-02)

	Test				
Family	**FE-02**	**RB-00**	**RB-02**	**RD-00**	**RD-02**
117490 x CF1	0.94 (16)	nt	0.77 (47)	nt	1.00 (48)
117490 x w	0.94 (48)	0.33 (6)	0.50 (48)	1.00 (6)	0.92 (24)
117490 x 117503	0.90 (48)	0.72 (18)	nt	1.00 (18)	nt
CF1 x CF2	0.83 (24)	0.67 (12)	nt	0.83 (12)	nt
117486 x 117851	0.79 (24)	0.92 (12)	nt	0.92 (12)	nt
510005 x w	0.77 (48)	0.67 (12)	nt	0.75 (12)	nt
510015 x w	0.67 (36)	0.29 (24)	0.21 (18)	0.58 (12)	0.75 (48)
117341 x w	0.67 (48)	0.44 (18)	0.33 (48)	0.50 (18)	0.75 (48)
117344 x w	0.54 (24)	0.25 (12)	0.23 (48)	0.50 (12)	0.25 (24)
510041 x w	0.50 (48)	0.33 (6)	0.17 (48)	0.60 (6)	0.38 (48)
70024 x 70028	0.49 (49)	0.33 (6)	0 (48)	0 (6)	0.33 (48)
117852 x 510049	0.42 (24)	0.39 (22)	nt	0.58 (24)	nt
118463 x w	0.35 (48)	0 (12)	0.02 (48)	0 (12)	0.17 (48)
70013 x 117343	0.28 (36)	0.12 (24)	nt	0.08(24)	nt
70103 x w	0.21 (47)	0 (12)	0.04 (24)	0 (12)	0.21 (24)
70119 x w	0.21 (48)	0 (12)	nt	0 (12)	nt

Note: nt = not tested; w= wind-pollinated.

Table 3—Family mean correlations (number of families; significance value) for survival between five tests for *Phytophthora lateralis* resistance: Foggy Eden field trial (FE-02), two raised bed trials (RB-00, RB-02), and two root dip trials (RD-00, RD-02)

	RB-00	**RB-02**	**RD-00**	**RD-02**
FE-02	0.81 (15; <0.001)	0.89 (9; 0.001)	0.91 (15; 0.001)	0.93 (9; <0.001)
RB-00		0.60 (8; 0.118)	0.82 (15; <0.001)	0.68 (8; 0.066)
RB-02			0.93 (8; <0.001)	0.87 (9; 0.002)
RD-00				0.79 (8; 0.019)

durable or that the environmental conditions present now are less conducive to further infection and impact by *P. lateralis*. None of the families, including those with 100 percent mortality in some of the earlier seedling screening trials, have reached 100 percent mortality at FE-02 suggesting that micro-site or other factors may at least temporarily reduce the impacts by *P. lateralis* in this area. This site will continue to be monitored for changes in status of the remaining trees, and especially the more resistant families.

In many resistance programs, short-term testing of seedlings is a common procedure for evaluating resistance of parent trees or their progenies. However, verification of seedling test results in field trials is essential to confirm the relationship with seedling tests and to examine durability of resistance. In this series of tests examining genetic variation in *P. lateralis* resistance, there is generally good correspondence between survivals in the four short-term tests with that in the FE-02 field trial through the 2010 assessment. Similar results have also been found in early assessments from several other field trials and these seedling trials (Sniezko et al. 2006). The somewhat higher than expected mortality of a few resistant families in the two raised bed trials warrants further examination. These families were selected from among the first tests of *P. lateralis* resistance, and

more recent trials will be examined to further evaluate resistance in the different trial types. Future updates from FE-02 and the earlier reported trials (Sniezko et al. 2006) will also be of interest.

The seedlings planted at FE-02 are in the understory of an existing forest stand and are growing slowly. Although this may be somewhat typical for some stands with POC, and the survival results are very encouraging, data from additional field sites are needed to more fully examine the effectiveness of *P. lateralis* resistance under a wider range of growing conditions. Several other field trials were planted in Oregon in 2002 using a subset of these 16 families, and they show little or no mortality from *P. lateralis* at this point (Harrington et al. 2012), indicating that many sites may be free of *P. lateralis*, at least initially. Classification of sites for *P. lateralis* hazard (low, moderate, high, and extremely high) would be helpful in establishing any further locations or interpreting results from current field trials as well as in making recommendations to land managers on the use of resistant POC seed. Additional field trials have been established in areas where *P. lateralis* is known to be active, and results from these trials should greatly expand the scope of reference regarding effectiveness of *P. lateralis* resistance.

Resistant POC seed is now available from seed orchards for several breeding zones and is in use by several organizations. Resistant seed lots are generally bulk collections and would be expected to give some intermediate level of field survival in the presence of *P. lateralis* as opposed to the high survival shown by the top families in the FE-02 trial. Further testing of these bulk open-pollinated seed orchard lots at multiple sites is needed to define the level of survival to be expected for general restoration and reforestation. Breeding work in POC has begun and further increases in resistance are expected in future seed orchard populations.

A genetic resistance program offers a natural method of coping with major pathogen and insect problems in forest trees. The POC program is one of the few resistance programs for non-native pathogens or insects in forest trees to reach the level of producing seed for operational use (FAO 2008, Sniezko 2006). However, as discussed at this conference, there is more need and interest than ever in using this approach. Existing programs such as the one for POC can provide insights to help increase the efficiency of efforts in other species. Regional centers of expertise and facilities can also facilitate efficient development of resistance in multiple species. Sustained support is essential to help ensure success in resistance programs.

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