AN ABSTRACT OF THE DISSERTATION OF

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Port-Orford-cedar (*Chamaecyparis lawsoniana*) is a forest tree native to a small area of Oregon and California. A root disease caused by *Phytophthora lateralis* causes widespread mortality of Port-Orford-cedar. This dissertation examines three important elements of the Port-Orford-cedar *P. lateralis* pathosystem related to breeding for disease resistance: use of resistant rootstocks to maintain genotypes of Port-Orford-cedar for breeding; the heritability and genetic basis of disease resistance; and variability in virulence and DNA fingerprint among a sample of *P. lateralis* isolates.

Port-Orford-cedar was reciprocally grafted to western redcedar (*Thuja plicata*), incense cedar (*Calocedrus decurrens*), and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Port-Orford-cedar scion graft success was moderate with western redcedar and incense cedar, but extreme overgrowth of the rootstock by the scion indicated incompatibility. Xylem union was good, but phloem union was incomplete or lacking. Nearly all Port-Orford-cedar rootstocks and seedlings exposed to *P. lateralis* died of

root disease. Four percent of the Alaska yellow-cedar exposed also died, confirming this tree as a host for *P. lateralis*.

Resistance of Port-Orford-cedar to *P. lateralis* is rare. A small number of trees have been identified exhibiting resistance. A number of families were tested to determine the genetic basis for resistance. Estimates of narrow-sense and family mean heritability of resistance, as exhibited by restriction of lesion length after inoculation, were determined. Both narrow-sense and family mean heritabilities were between 0.61 and 0.98 in most tests. Between 21% and 32% of the variance was due to differences among families.

Thirteen isolates of *P. lateralis* were collected from three hosts throughout the geographic range of the fungus. Variation in growth rate on artificial media at three temperatures, virulence when used to inoculate Port-Orford-cedar, and DNA fingerprint were compared. There were significant differences in growth rate among isolates at 24C, but fewer differences at lower temperatures and on a rich medium. One isolate produced significantly shorter lesions in three different inoculation tests. Isolates differed at only two of 189 bands produced by Inter Simple Sequence Repeat (ISSR) DNA primers, indicating very little genetic variation among isolates.

Port-Orford-cedar and *Phytophthora lateralis*: Grafting and Heritability of Resistance in the Host, and Variation in the Pathogen

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Michael G. McWilliams, Author

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Chapter 1. Introduction and Literature Review

Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) is an ecologically and economically important forest tree native to a small area of southwest Oregon and northwest California. A root disease caused by the fungus *Phytophthora lateralis* Tucker and Milbrath causes widespread mortality of Port-Orford-cedar in both forest and landscape plantings. Many efforts have been initiated to restrict the movement of the disease and to find and develop fungal resistance in the tree. This dissertation documents elements of the Port-Orford-cedar *P. lateralis* pathosystem related to maintaining and breeding trees with fungal resistance, and understanding variability present in the fungus.

Port-Orford-cedar currently has a limited geographical range in southwest Oregon and northwest California. It grows and competes well on ultramafic parent materials as well as in riparian areas; both are areas with limited competition from Douglas-fir and western redcedar (Zobel and Hawk 1980). Fossil records indicate a much larger geographic range of Port-Orford-cedar in the Tertiary Period than it has presently; it extended both farther north to near the Columbia River (west of the Cascades), and farther east into Idaho, Nevada, and Colorado (Edwards 1983). The present range is limited to relatively equitable climates with moderate rainfall and mild temperatures. Soil temperatures on sites supporting Port-Orford-cedar are generally cold. Mean annual soil temperature was below 12C on nine sites measured (Zobel and Hawk 1980). On drier sites in California, the tree is limited primarily to riparian areas, but it can be found

at any topographic position on sites with underground water or summer fogs. The present range is apparently limited by competition with Douglas-fir to the north, and lack of moisture to the south and east (Zobel et al. 1985, Zobel and Hawk 1980).

There has been relatively little published work done on the genetic structure or mating system of Port-Orford-cedar. A common garden study to examine growth characteristics has been installed by the U.S. Forest Service on four sites in Oregon and California, and includes trees from 320 families collected from throughout the range of Port-Orford-cedar (Richard Sniezko and Jay Kitzmiller, personal communication). Millar and Marshall (1991) examined allozymes representing 32 loci in nine populations of Port-Orford-cedar in California, representing approximately half the geographic range of the tree, and including two disjunct populations. Analysis of variation of allozyme frequencies within and among populations is one of the best and most widely used methods to infer the genetic structure of plant populations. Because allozymes are considered neutral markers (non-adaptive and not of selective advantage), they are an excellent way to compare populations over wide geographical ranges.

All loci varied in at least one population, although seven loci were fixed in most populations (Millar and Marshall 1991). The inland, disjunct populations had fewer polymorphic loci, and lower observed heterozygosity than the coastal populations. One of the two inland populations had very low heterozygosity (F=0.54), and a low ratio of observed to expected heterozygosity, indicating inbreeding in that population. Seven of the nine populations contained alleles not found in other populations, even though percent polymorphic loci and observed heterozygosity were moderately high and inbreeding coefficients were near zero. This indicates that, although there is relatively high outbreeding potential in these populations, the mixing of genetic information among populations is only moderate

(Millar and Marshall 1991). Genetic distances among populations are relatively low; the genetic distance between the inland and coastal populations is about two to three times greater than the genetic distance among any pair of the coastal populations. Apparently the inland populations have become reproductively isolated, and are not effectively interbreeding with the coastal populations. The high diversity within stands, and relatively low proportion of total diversity among stands, would be expected in a species with a formerly larger range that has been forced into a smaller range represented by refugia on ultramafic soils and in riparian areas (Millar and Marshall 1991).

Although Millar and Marshall (1991) sampled throughout the geographic range of Port-Orford-cedar in California, all the stands were on ultramafic soils. The number of individuals as well as the range of sites occupied is greater in Oregon than in California. It is not known whether their conclusions of high variability within populations and relatively little variation among populations would apply to the Oregon populations of Port-Orford-cedar.

Conservation of Port-Orford-cedar is a concern of many government agencies, environmental groups, and individuals. A disease caused by the fungus *Phytophthora lateralis* Tucker and Milbrath (1942) causes mortality throughout the native range of Port-Orford-cedar. A program of breeding for resistance to *P. lateralis* is currently under way through a cooperative effort of the U.S. Forest Service, Bureau of Land Management, and Oregon State University. Management methods that seek to limit the spread of *P. lateralis* have been implemented by the U.S. Forest Service; these include limiting access to uninfested stands and drainages through road closures and other methods designed to reduce transportation of soil infested with propagules of the fungus (Harvey et al. 1985, Hansen et al. 2000). A number of reserves and uninfested populations of Port-Orford-cedar still

exist. The reserves, low risk areas, and management initiatives, combined with the relatively low amount of among population variability at isozyme loci, lessen the risk that significant genetic variability will be lost. Even if some reserves become infested, some trees will probably survive, although some unique and rare alleles, as well as some of the variation not detectable with isozymes, may be lost.

Research is needed to determine the mechanisms of resistance, more accurately demonstrate the genetic basis of resistance, estimate the heritability of this trait, and develop more efficient ways to breed Port-Orford-cedar. To carry out successful breeding and produce sufficient quantities of seed for reforestation, trees with varying levels of resistance must be maintained in seed orchards or similar sites. The hazard of maintaining Port-Orford-cedar in seed orchards is that accidental introduction of *P. lateralis* could cause significant mortality and loss of important genetic resources. If selected trees were grafted and grown on rootstocks resistant or immune to *P. lateralis* it would mitigate this hazard.

The genus *Phytophthora* is among the most destructive pathogens in modern agriculture. The greatest damage results when a species of the fungus is introduced to a new environment. The Irish potato famine of the 1840's (de Bary 1876, Large 1940, Hampson 1992), and the current mortality of a large number of plant species in Australia (Shearer and Tippet 1989, Shearer and Dillon 1995), are examples of the destruction fungi in this genus can cause. In both cases, non-native species of *Phytophthora* were introduced to new continents. It is likely that the current mortality caused by *P. lateralis* is another example of introduction, although the origin of the fungus is unknown.

P. lateralis is a serious threat to natural and cultivated stands of Port-Orford-cedar. The fungus was first reported in nurseries near Seattle in the 1920's (Hunt 1957). In the original report it was noted that "...The disease

prevails throughout the whole Northwest, killing cypress in the private gardens as well as in all nurseries, causing vast loss, as this Lawson cypress is used very much here...". The disease was reported in the Willamette valley in 1937 (Milbrath and McWhorter 1938). In 1952 the fungus was found within the native range of Port-Orford-cedar (Roth et al. 1957), and by 1957 it was found in the heart of the native range in Oregon, south of Powers (Hunt 1959a). The disease has since spread to nearly the entire native range of Port-Orford-cedar (Hansen et al. 2000).

Although the fungus has been reported twice outside North America, neither location is likely to be the origin of *P. lateralis*. *P. lateralis* was reported associated with root, crown, and collar rots on kiwifruit (*Actinidia chinensis*) in New Zealand (Robertson 1982), but this report remains unconfirmed because no voucher collections exist and the fungus has not been reisolated in New Zealand. Zoospores were difficult to produce with the New Zealand isolates, which contrasts sharply with the relatively easy induction of zoosporangia and zoospores possible with *P. lateralis*. More recently, *P. lateralis* was reported from France (Hansen et al. 1999), where it had been isolated from symptomatic *C. lawsoniana* growing in nurseries. The two French isolates and North American isolates have identical ITS-1 sequences and colony morphology in culture. It is likely that these isolates are the result of a recent introduction (Everett Hansen, personal communication).

Within North America, *P. lateralis* has been reported twice outside the Pacific Northwest, in North Carolina (Abad et al. 1994), and Ohio and Pennsylvania (Hoitink and Schmitthenner 1974), but these reports remain unconfirmed. Voucher specimens received from North Carolina were not *P. lateralis*, and isolates came from a number of plants that are not considered hosts of the fungus. Cultural characteristics reported for the fungus from Ohio and Pennsylvania differ markedly from the cultural

characteristics observed in isolates of *P. lateralis* from the Pacific Northwest.

Other species of *Phytophthora* have been reported killing Port-Orford-cedar. Clancy and Kavanaugh (1978) report *P. eriugena* causes stem and collar rot of Port-Orford-cedar in seedbeds in Ireland. This species has a few characteristics in common with *P. lateralis* such as homothallism, but differs in that it is amphigynous. Chlamydospores, which are abundant in cultures of *P. lateralis*, were not observed. *P. eriugena* has been isolated only once (Erwin and Ribeiro 1996), and the isolate is no longer available. *P. cinnamomi* has been reported causing mortality of Port-Orford-cedar in Louisiana (Campbell and Verrall 1963), South Arfrica (Von Broembsen 1984) and has also been isolated from this host in the Pacific Northwest (Atkinson 1965, Hansen et al. 1989, Torgeson et al. 1954, Torgeson 1954,).

Relatively few studies have focused exclusively on *P. lateralis*. Limited variation has been found in previous studies of *P. lateralis*. In a comparison of sporangia production among ten isolates from Oregon, nine isolates were similar and one isolate produced fewer sporangia when grown on pea broth and then rinsed and flooded with distilled water (Trione 1957, Trione 1959). Chlamydospore production varied greatly among isolates, and all isolates produced oospores equally well. Isozyme banding patterns of 11 isolates from Oregon and California were identical (Mills et al. 1991).

Hansen and Hamm (1986) reported little variability detected among 23 isolates of *P. lateralis* collected from throughout the range of the fungus. One isolate (627, from Winchester, OR) grew more slowly than other isolates. There were significant, but unrepeatable, differences in zoospore production, and no differences among total protein bands or isozyme bands. Isolate 627 caused significantly shorter lesions than the other

isolates tested. The authors suggest that a simple difference in growth rate could produce differences in zoospore production and pathogenicity.

P. lateralis can survive for long periods in infected root systems. The fungus can be recovered more readily from organic matter separated from the soil than from the soil itself (Ostrofsky et al. 1977). Structures resembling chlamydospores that were forming sporangia have been observed in organic matter from infested cedar stands (Ostrofsky 1976), and chlamydospores and oospores were reported in naturally infected cedar foliage as well as in foliage that had fallen to the surface of infested soil (Trione and Roth 1957). Although infectivity of soils stored for five months at 5C to 20C was not reduced, soils stored at 25C or above, or soils stored at very low or high moisture contents, had reduced infectivity (Ostrofsky 1976, Ostrofsky et al. 1977). Hansen and Hamm (1996) buried root systems from seedlings that had been killed by *P. lateralis* in a forested area without other Port-Orford-cedar nearby. *P. lateralis* could be isolated from the root systems by baiting for seven years, although the rate of recovery dropped off quickly after two or three years. Another study used infected root fragments in soil bags placed on the soil surface. The fungus could not be recovered after 7 days from bags placed in the sun (Hansen and Hamm, 1996). The fungus could not be recovered after 36 months by baiting soil collected from a previously infested site in California (Kliejunas 1992).

Long distance spread of *P. lateralis* is primarily through transport of infested soil and organic matter associated with logging, recreation, and road construction and maintenance (Hansen et al. 2000, Hunt 1957, Nelson 1964, Roth et al. 1972). Local spread can occur through overland or underground flow of water containing zoospores (Hunt 1959b) or through Port-Orford-cedar root grafts (Gordon 1974, Gordon and Roth 1976). Sampling of streams to detect infestation has been successful using Port-

Orford-cedar seedling root systems placed directly in streams, and also by filtering stream water through a Millipore™ filter and then culturing the filter and attached propagules on a medium selective for *P. lateralis* (Hansen and McWilliams, unpublished data). Refinement of the filtering technique to detect stream infestation, either by using more effective selective media or by testing the filter for propagules with a *P. lateralis*-specific DNA probe, is needed before detection can be considered reliable.

P. lateralis has a very limited host range. In addition to Port-Orford-cedar, *Chamaecyparis obtusa* was reported as susceptible to the disease (Torgeson et al. 1954), although this host has not been systematically retested since the initial report. Several *C. obtusa* have died at an infested site at the Oregon State University Botany Farm (Everett Hansen, personal communication), but isolation of the fungus responsible for the mortality has either not been attempted or has not yielded *Phytophthora*. *Chamaecyparis nootkatensis* was reported as a host (Salisbury 1954) although subsequent inoculation tests of *C. nootkatensis* did not result in mortality (Hunt and O'Reilly 1984). *C. nootkatensis* was confirmed as a host when artificially inoculated (Hansen et al. 1989). *Taxus brevifolia* is also a host for *P. lateralis* (DeNitto 1991), and mortality in this species is concentrated in areas of seasonal high water supporting stands of diseased Port-Orford-cedar (Murray 1996, Murray and Hansen 1997).

The question of variation among isolates of *P. lateralis* is important for a number of reasons. First, it is necessary to know the range of variability in virulence among isolates so that appropriate resistance can be incorporated in the ongoing Port-Orford-cedar breeding program. Second, it is necessary to know the variation in virulence so that appropriate isolates can be used in testing for resistance. Third, the amount of genetic variation among isolates is important information for determining population structure

and whether the fungus exhibits a simple structure compatible with the idea of introduction.

Chapter 2 Reciprocal Grafting Port-Orford-cedar with Species in the Cupressaceae: Graft Union Formation, Growth, and Susceptibility to *Phytophthora lateralis*

Tree grafting, or the union of one genotype to another, is a common practice in horticulture, forestry, and the orchard industry. There are numerous reasons for grafting trees. These include decreasing the vigor or growth rate of the scion by grafting on a dwarfing or semi-dwarfing rootstock, using rootstocks adapted to heavier and wetter soils or with other stress tolerance mechanisms, and promoting more vigorous growth in order to obtain a marketable plant earlier. In forestry it is common to collect scion wood from selected trees in the forest and graft them to rootstocks in a seed orchard in order to preserve these genotypes, enhance pollination or breeding among the selected trees, and promote earlier seed production through the grafting of physiologically mature scions.

Grafting is sometimes done to manage disease. In avocados, rootstocks resistant to *Phytophthora cinnamomi* allow production in groves infested by this disease (Dolan and Coffey 1986). Some citrus cultivars are resistant to *Phytophthora*s and are used as rootstocks for commercial production of oranges (Graham 1995, Widmer et al. 1998). *Pinus koraiensis* is used as a rootstock for *Pinus strobus* because it is less susceptible to *P. cinnamomi* than *P. strobus* (Beaver and Mathers 2000). In apples, the scion can affect expression of resistance in the rootstock, with some scion cultivars causing increased resistance to *Phytophthora cactorum* (Utkhede and Smith 1993).

Many conifers sold by the commercial nursery industry are grafted. Cultivars are grafted on rootstocks to promote faster growth and a higher percentage of marketable plants than is possible from rooted cuttings. Rootstocks are usually the same species as the scion, although some

interspecific graft combinations are successful in *Abies, Cedrus, Picea, Pinus,* and *Juniperus* (Beaver and Mathers 2000, Jayawickrama et al. 1991).

Chamaecyparis cultivars are popular in landscape plantings. *C. nootkatensis* cultivars are usually grown on *C. nootkatensis* rootstock, but *C. lawsoniana* and *C. obtusa* are often grown on *Thuja occidentalis* rootstock (Buley 1983, Beaver and Mathers 2000, Talon Buchholz, personal communication). *T. orientalis* may be used as a rootstock for *C. nootkatensis* (Lamb et al. 1975). The primary reason for grafting these species is that some cultivars are difficult to root, and grafting results in faster growth and production of a 'liner' that is marketable sooner than would be possible with rooted cuttings. Although early growth of *Chamaecyparis* on *Thuja* rootstock is good, incompatibility expressed as scion overgrowth of the rootstock eventually results. This necessitates grafting low on the rootstock, and planting the tree so the graft union is below ground level. The scion then forms adventitious roots on the buried stem of the scion (Talon Buchholz, personal communication). Hunt and O'Reilly (1984) report excellent results using *C. formosensis* and *C. thyoides* as rootstocks for *C. lawsoniana* over two years, with 20 of 24 grafts on *C. formosensis,* and 70 of 94 grafts on *C. thyoides* reported as compatible. Torgeson et al. (1954) grafted *C. lawsoniana* var. *allumi* to three varieties of *C. pisifera*, *Juniperus procumbens,* two varieties of *T. occidentalis*, and *T. plicata*. They found that "good unions occurred, and the young scions grew very rapidly" (page 14), but that by the end of five years "various stages of compatibility" (page 15) between scion and rootstock occurred. Most graft unions exhibited scion overgrowth, and grafted stems had smaller caliper than ungrafted plants. Their figures show severe swelling at the graft union, scion overgrowth of the rootstock, and bark discontinuity on *T. occidentalis* rootstocks.

One objective of this study was to test three species of Cupressaceae as rootstocks for *Chamaecyparis lawsoniana* (Port-Orford-cedar = POC). The three species (*C. nootkatensis, Calocedrus decurrens, and Thuja plicata*) are native to the Pacific Northwest and thought to be resistant or immune to *P. lateralis*. If rootstocks more compatible than *Thuja occidentalis* were identified, it might allow commercial production of POC on forest sites infested by the fungus. Compatible rootstocks could also be used in grafted seed orchards and horticultural production facilities, where mortality of POC would otherwise cause significant financial or genetic loss. I also tested POC with reciprocal grafts in which *C. lawsoniana* was the rootstock and the other three species were scions to determine if the scion confers some resistance to the susceptible rootstock.

A second objective of this study was to test rootstocks of the other three species, especially *C. nootkatensis*, for susceptibility to *P. lateralis*, as there have been conflicting reports whether *C. nootkatensis* is a host for the fungus. Salisbury (1954) reported that one of two *C. nootkatensis* inoculated with the fungus died. Hunt and O'Reilly (1984) reported no mortality when 35 trees were inoculated with two different methods. Hansen et al. (1989) reported that of 12 *C. nootkatensis* seedlings inoculated by incorporating *P. lateralis* in a potting medium, all were girdled by disease. It is not known whether *C. nootkatensis* might be susceptible when outplanted in naturally infested sites, and confirmation of susceptibility when artificially inoculated is needed.

MATERIALS AND METHODS

I selected three genera in the Cupressaceae for use as rootstocks and scions with Port-Orford-cedar. In addition I tested homoplastic (same species of scion and rootstock) grafts of all species (Table 2.1). The three species were *Calocedrus decurrens* (Incense cedar = IC), *Chamaecyparis nootkatensis* (Alaska yellow-cedar = AYC), and *Thuja plicata* (western redcedar = WRC). The IC and WRC seedlings were grown for the U.S. Forest Service by the Reed Creek Nursery near Reedsport, Oregon. The AYC seedlings were grown by the Cowician Lake Research Station, B.C. Ministry of Forests, on Vancouver Island, British Columbia, Canada. Open pollinated seedlings from two seed zones were used for IC, from two elevations within one seed zone for WRC and POC, and from seven seed sources in five locations for AYC (Table 2.2).

Treatment	Rootstock	Scion	Grafted
1	POC	POC	yes
2	POC	IC	yes
3	POC	WRC	yes
4	POC	AYC	yes
5	POC	-	no
6	IC	POC	yes
7	IC	IC	yes
8	IC	-	no
9	WRC	POC	yes
10	WRC	WRC	yes
11	WRC	-	no
12	AYC	POC	yes
13	AYC	AYC	yes
14	AYC	-	no

Table 2.1. List of treatments. Treatments consisted of grafting the scion onto the rootstock, or ungrafted plants. POC = Port-Orford-cedar, IC = incense cedar, WRC = western redcedar, and AYC = Alaska yellow cedar.

Species	U.S.F.S. Tree Source Identification	Elevation (feet)	U.S.F.S. Seed Zone	Location
Calocedrus decurrens	081-11114-500-85 SB S4.1	4100	11114	
Calocedrus decurrens	081-11093-500-85 SB S3.5	3500	11093	
Chamaecyparis lawsoniana	041-11-072-05004 2.0 78SIA S5.1	2000	072	
Chamaecyparis lawsoniana	041-11-072-05004 2.5 78SIA S5.2	2500	072	
Thuja plicata	242-18-482-08000-3.0 82SIA	3000	482	
Thuja plicata	242-18-482-08000-2.0 82SIA	2000	482	
			Cowician Collection Number	
Chamaecyparis nootkatensis		5741	31	Mt. Angeles, WA
Chamaecyparis nootkatensis		4429	32	Whitepass, WA
Chamaecyparis nootkatensis		4429	33	Huckleberry, WA
Chamaecyparis nootkatensis		3773	39	Camp, OR
Chamaecyparis nootkatensis		3937	families: 42, 43, 44)	Humbug Ck, OR

Table 2.2. Seedling species and seed source zone and elevation.

All seedlings were grown in tube containers for one year by the nurseries, and then transplanted in February 1992 to 5 1/4 inch (2.6 L) square plastic pots. Trees were potted in a soilless mix recommended by Cowician Lake Experiment Station for use with *Chamaecyparis* (Table 2.3).

Cowician Soilless Potting Mix	
Ingredient	Amount
perlite	8 cubic ft.
peat	8 cubic ft.
hemlock bark	~14 cubic ft.
18-7-10 Osmocote™ fertilizer	5 kg.
dolomite lime	2 kg.
Micromax™ micronutrients	1 kg
agricultural gypsum	1 kg.
0-25-0 (10) super-phosphate with sulfur	1 kg.

Table 2.3. Soilless potting mix ingredients.

Ingredients were mixed in a horticultural soil mixer. Eight pots containing trees from the same seed source and elevation (or family for AYC) were placed in a plastic flat for ease of handling. Flats were placed on landscape cloth over gravel west of the Oregon State University east greenhouse complex, near Cordley Hall. Trees were watered regularly by an automatic irrigation system. In October 1992 trees were treated with a solution of nematodes parasitic to root weevil larvae. In February 1993 *Botrytis cinerea* was detected in the dense stand of cedars, and all trees were treated with Chipco™ fungicide to prevent further infection. Half of the trees were then moved to a second location on the OSU campus near the Poultry Science building adjacent to an abandoned chicken house (Figure 2.1). Landscape cloth was placed over the soil at the Poultry Science site. Because of continuing concern about root weevil damage, all trees at both sites were treated with Ornalin™ insecticide applied by hand sprayer to the pot soil surface.

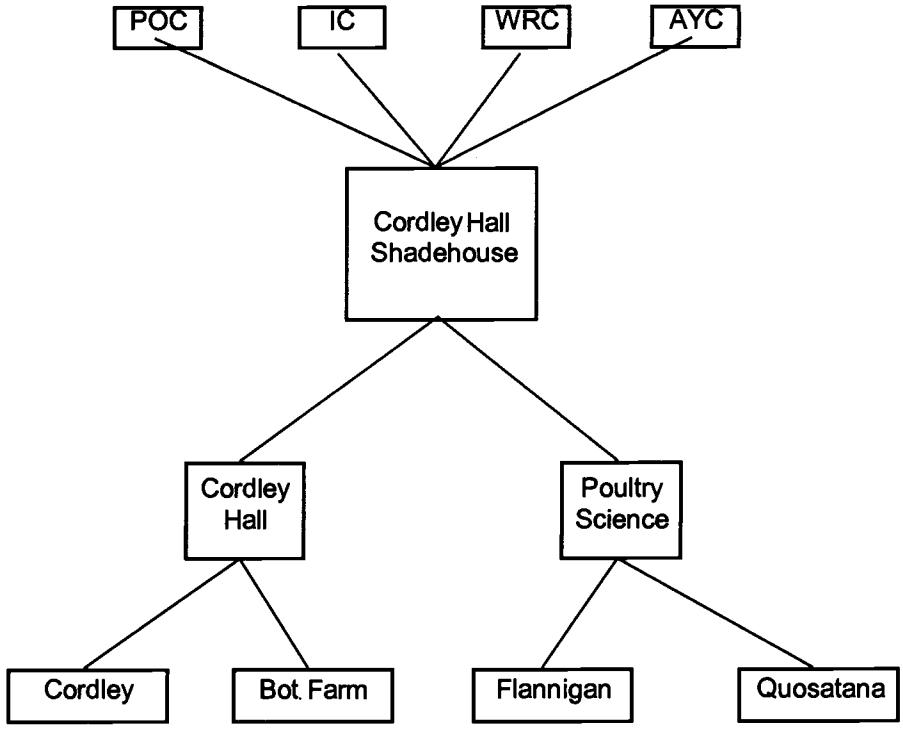

Figure 2.1. Growing location and movement of seedlings. POC = Port-Orford-cedar, IC = incense cedar, WRC = western redcedar, and AYC = Alaska yellow cedar. Top row indicates seedlings obtained from nurseries, second row indicates potted seedlings, third row indicates location of potted and grafted seedlings, and bottom row indicates outplanted or potted seedlings.

In early February 1993, scions were collected from all potted trees by cutting off the top 15-35 cm of each seedling. Scions were wrapped in damp newspaper, placed in plastic bags, and stored in a cooler at 4C until used.

Grafting was done using a veneer or side graft. A long, shallow, tangential cut was made on the rootstock to just under the bark surface. A cut of approximately the same length and width was made on the base of the scion. The scion was placed against the rootstock with cambial regions

aligned on one or both sides. The base of the scion was inserted in a small V shaped notch at the base of the rootstock cut. The rootstock and scion were held together with a rubber grafting band, and the top of the union above the band was sealed with a small piece of grafting wax to prevent water entry into the graft union. Grafting was done from March 1 to April 3. Selection of rootstock and scion for grafting was random with a randomized complete block design, and grafting was alternated each week between the Cordley site and Poultry Science site until all trees were grafted. At time of grafting the following information was recorded for each tree: flat number, rootstock species, rootstock source (seed zone, elevation, or family), scion species, scion source, height of tree, height to graft, length of graft union, length of scion, caliper of rootstock 1 cm above soil surface, whether rootstock had *Botrytis* infection, the day grafting was done, and the location of the rootstock (Cordley or Poultry Science). From March 1 until May 14 watering was controlled by an "artificial leaf" in an effort to maintain a high humidity environment and free moisture on scion surfaces at all times to prevent drying before the graft union formed a functional vascular union. After union formation the trees were watered regularly by an automatic watering system.

In June 1993, tops of rootstocks above the unions were pruned so that the scion was the dominant apical part of the tree. In June trees were sprayed with Talstar™ insecticide to prevent root weevil infestation.

In October 1993 trees at Cordley were randomly assigned either to be outplanted at a disease free site at the O.S.U. Botany Farm just east if Corvallis, or to remain at the Cordley site (Table 2.4). Trees assigned to the Botany Farm were originally intended to be used for a study of rootstock effect on phenology of scion, but because of graft incompatibility indicated by dead scions this portion of the study was discontinued, and the trees were used only for the analysis of graft success and growth. Trees at

the Poultry Science site were randomly assigned to either the Flannigan or Quosatana outplanting site on the Siskiyou National Forest. The condition of each graft was recorded at the time of assignment to growing location, and grafts were recorded as successful if the scion had remained green and exhibited height growth.

Rootstock-Scion Combination	Botany Farm	Cordley: Inoculation	Outplant: half at each of of two field sites
POC-POC	25	50	100
POC-WRC	25	50	100
POC-IC	25	50	100
POC-AYC	25	50	100
POC	25	50	100
WRC-POC	25	50	100
WRC-WRC	25	50	100
WRC	25	50	100
IC-POC	25	50	100
IC-IC	25	50	100
IC	25	50	100
AYC-POC	25	50	100
AYC-AYC	25	50	100
AYC	25	50	100

Table 2.4. Number and growing location of grafted and ungrafted seedlings.

The two outplanting field sites are naturally infested with *P. lateralis*. The Flannigan site is on the Powers Ranger District near Johnson Mountain in section 10, T32S, R12W, at 1300 ft. elevation. This is an area

within a Douglas-fir progeny test site that had been previously rejected as unplantable by the contractors that installed the progeny test site. The area has serpentine influenced soils, is very rocky, and contains some naturally regenerated POC and many large POC stumps from the previous stand. The drainage is very poor even though the site is rocky. During planting there was visible surface water and deep puddles on some areas. The area had been fenced to exclude deer and elk.

The second outplanting site, Quosatana, is in a low lying area near Quosatana Butte on the Gold Beach Ranger District in section 33, T36S, R13W, at 1400 ft. elevation. The serpentine influenced soils are deep and loamy, but the area has a high water table. During planting many planting holes that were filled with water were not used. This site supports small natural POC, but no large stumps are present. Some trees in the Quosatana site were killed after being trampled by wild horses.

Trees were randomized and planted in a row-column two meter grid. Holes for planting were excavated with a soil auger powered by a chainsaw motor. If a hole was unplantable because of rocks or poor drainage its position was skipped. Field sites were planted in December 1993. Mortality and graft condition were recorded in June 1994, October 1994, and March 1995 (except at Quosatana, which had ~2 feet of snow on the site). Tree height, graft condition, and mortality were recorded in October 1995. Cause of mortality was determined by observation of necrotic areas on the stem, and direct isolation from the margin of necrotic tissue. Graft condition was determined from observations of the scion growth and foliage color.

Trees remaining at Cordley were repotted in March 1994 into 3 gallon round plastic pots using the same soilless mix described previously (Figure 2.3). In May, Cordley trees were again treated with nematodes parasitic to root weevil larvae. In September *P. lateralis* infection was found in three

areas at the Cordley site. The fungus had apparently been introduced into the potted trees from adjacent infested areas, possibly by domestic cat digging or birds burying and moving acorns.

Trees at Cordley were artificially inoculated with *P. lateralis* on three occasions, each time with an increasing amount of inoculum. In October 1994 each tree was inoculated with 6 ml of a suspension containing ~25 chlamydospores/ml. The inoculum consisted of approximately equal numbers of chlamydospores from three isolates that had been collected from dead trees at the two field outplanting sites. Inoculations were done by inserting three Pasteur pipets half way between the stem and the edge of the pot. The pipets were spaced evenly around the stem. Two ml of inoculum was inserted into each pipet. In June 1995 the trees were inoculated a second time by removing each tree from the pot, spraying 8 ml of a suspension containing approximately 75 chlamydospore/ml on the exposed roots at the edge of the soil mass, and then reinserting the tree in the pot. Trees had roots throughout the soil in the pots, so the soil and roots held together when removed from the pots. The inoculum consisted of approximately equal numbers of spores from the three isolates used previously. Trees were inoculated a third and final time in October 1995 by injecting 5 ml of an inoculum suspension into each of two holes in the soil near the stem. The holes were created by inserting a large nail into the soil just before inoculation. Inoculum consisted of approximately equal numbers of chlamydospores from the three isolates used previously at a concentration of 100 spores/ml. Increasing inoculum densities were used at each attempt because mortality due to the earlier attempts was not evident. Mortality was recorded approximately every three months for trees at Cordley, with a final recording in December 1996.

Condition of the graft union was determined by observing the presence or absence of new growth of the scion and the color of the branchlets of the

scion at the end of 31 months. Success combined both initial graft take and compatibility over this time period. If the scion had bright green foliage, it was recorded as condition 1 (healthy), if the scion was olive green or yellowish green it was recorded as condition 2 (failing), and if the scion was brown or red, or appeared dry, it was recorded as condition 3 (dead). In the analysis of grafts condition 2 and 3 were combined, because scions previously recorded as condition 2 had usually progressed to condition 3 by the next measurement, and condition 2 and 3 scions usually had no stem elongation. Data were summarized and evaluated using several SAS procedures (SAS Institute Inc., 1985): FREQ for summarization of number and condition of grafts and rootstocks, GLM for analysis of continuous variables (scion growth), and CATMOD for analysis of categorical variables (graft union success for the 31 months of the study, mortality).

When analyzing graft success, data from all sites were combined. In the analysis of scion growth, data from all sites were combined but included data only from trees that were alive 31 months after grafting. In the analysis of susceptibility to *P. lateralis* only data from the two outplanting sites and the Cordley site were used.

Five graft unions of each rootstock/scion combination were collected in October 1995 from the trees at the Cordley site that had exhibited the greatest scion elongation. The unions were cut with a microtome into cross-sections, stained, and evaluated microscopically for union of xylem and phloem cells and tissue organization (Gnose and Copes 1975, Copes 1970, Copes 1980a, Copes 1980b). Evidence of anatomical incompatibility such as atypical cell types, irregular or atypical cell orientation, and the inclusion of suberized cells was evaluated. In addition, a stem form factor (ratio of stem caliper above union to stem caliper below union) was calculated (Copes 1989) to express degree of swelling at the graft union. Swelling of graft unions is often an early sign of incompatibility, or of

translocation problems through the phloem of the union. Measurements were not recorded for AYC and WRC homoplastic grafts.

RESULTS

Homoplastic grafts on all four species were very successful. Survival ranged from 69% for POC-POC to 96% for AYC-AYC. The grafts showed healthy scion color and good shoot vigor after 31 months (Figure 2.2). Microscopic examination of the graft union showed normal xylem connections at the union, with successful reestablishment of normal rays and few or no suberized inclusions in the zone where rootstock and scion cells merged. The phloem connections in graft unions were more difficult to examine microscopically than xylem, because the thin bark tissues tended to tear off when unembedded unions were sectioned with a sliding microtome. Homoplastic graft unions showed little swelling, and no root initials were observed on the scion.

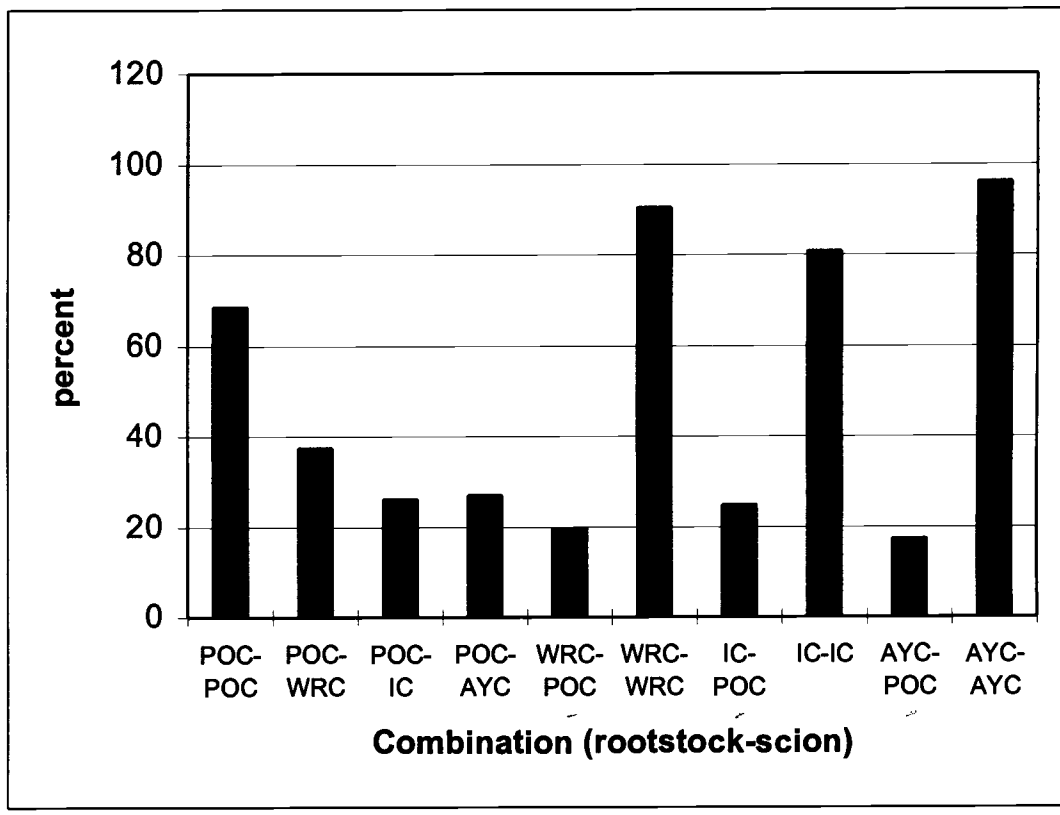

Figure 2.2. Percent graft success for graft combinations.

Heteroplastic (scion of a different species than the rootstock) grafts were much less successful. Only 26% to 38% of the heteroplastic grafts on POC rootstock had good color, and their shoot growth was less than that of homoplastic grafts (Figure 2.3). Stem diameters of POC scions were larger than the rootstock when measured 33 months after grafting (Figure 2.4), whereas the heteroplastic grafts with WRC, IC, and AYC as scions had smaller stem diameters than the POC rootstocks. POC scions were equally successful on the different rootstock species in heteroplastic grafts.

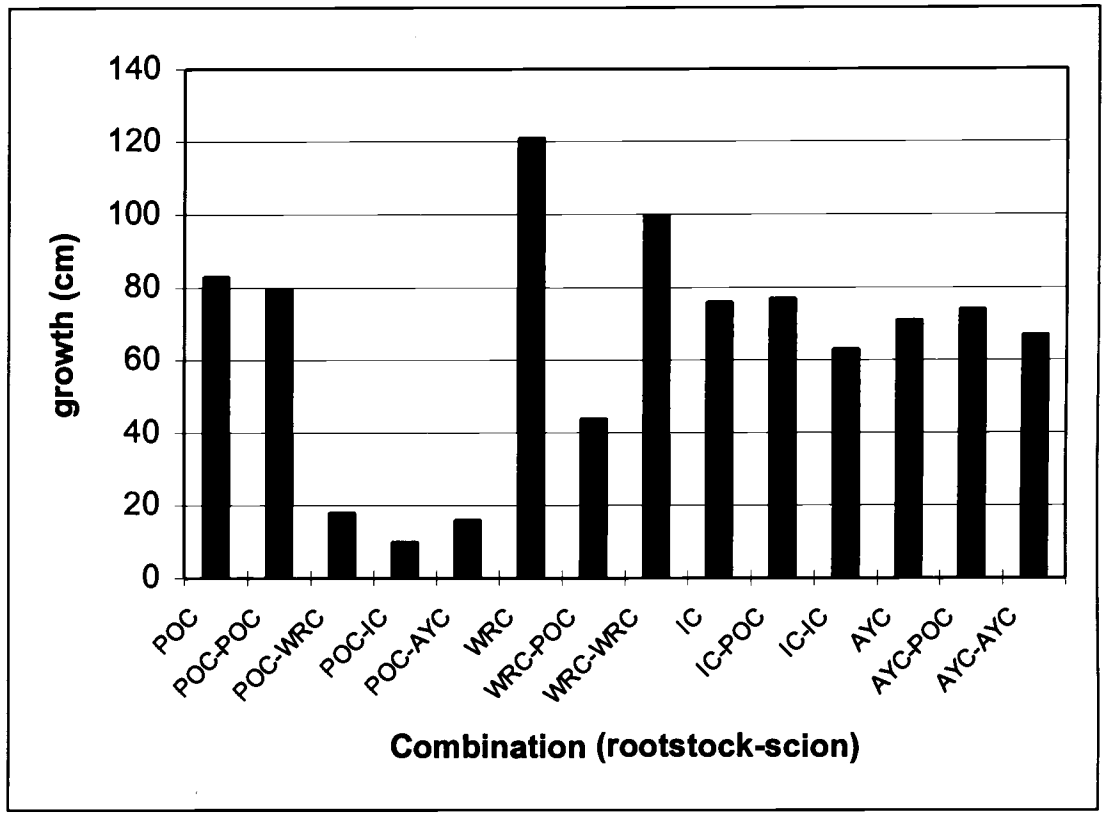

Figure 2.3. Mean growth of trees of given graft combinations that survived over the 31 months of the study at all locations.

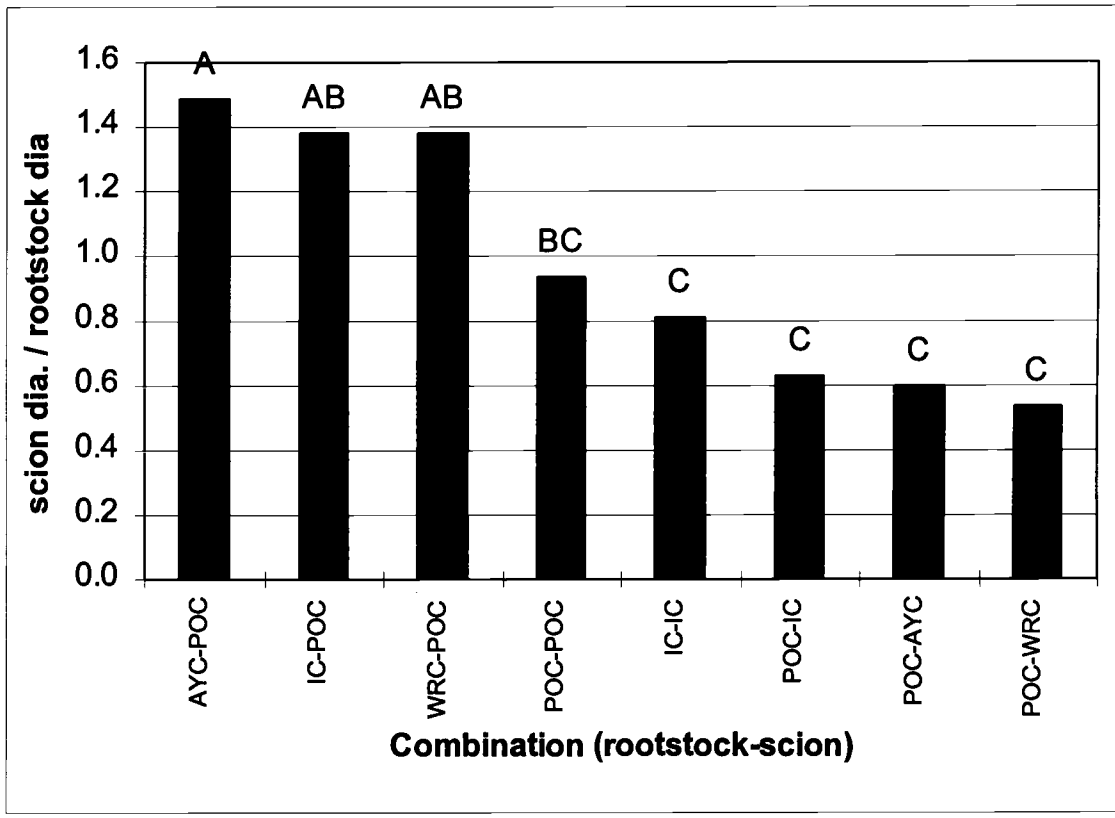

Figure 2.4. Form factor (ratio of scion diameter just above union to rootstock diameter just below union) for five trees of each combination that had the most height growth at Cordley. AYC and WRC homoplastic grafts were not measured. Letters above bars indicate form factors that were significantly different (p=0.05).

There was a significant (p=0.037) difference in susceptibility to *P. lateralis* between the two POC rootstock sources used. The rootstock source from the higher elevation had a higher mortality rate than the rootstock from the lower elevation (Figure 2.5).

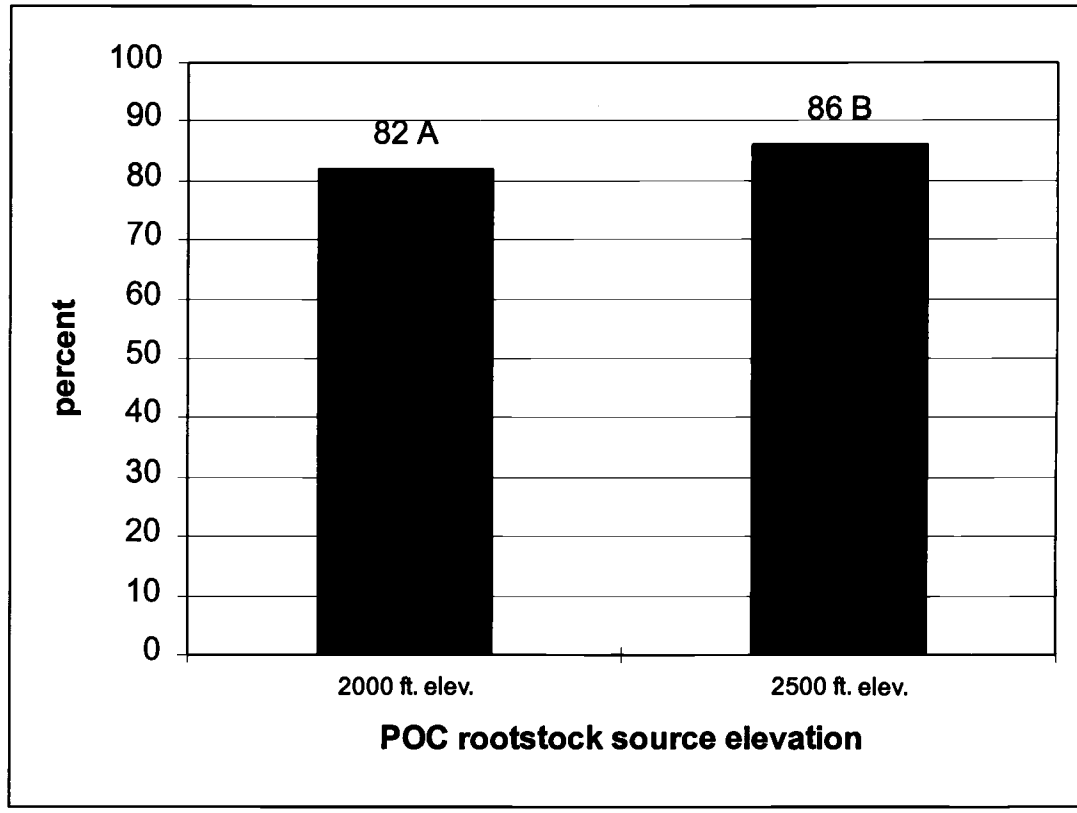

Figure 2.5. Difference in percentage of POC trees killed by *P. lateralis* from the two elevation zones. Percentages were significantly (p=0.05) different.

WRC was a vigorous rootstock, and promoted greater scion growth in homoplastic grafts than homoplastic grafts of the other rootstock species (Figure 2.3). WRC as a rootstock for POC was only successful in 20% of the grafts (Figure 2.2), and promoted the least POC scion growth (Figure 2.3). Visible swelling was present in the graft union zone and indicated a lack of, or poorly functioning, phloem union. The swelling probably resulted from the buildup of photosynthetic products just above the defective area.

IC promoted the greatest heteroplastic POC scion growth (Figure 2.3) and had the highest success rate of any heteroplastic rootstock tested with POC scions (Figure 2.2). Although visible swelling was present in many

graft unions, a few had nearly no swelling and excellent scion growth. Sectioned graft unions showed fewer suberized inclusions in the xylem than other rootstocks, and the overall structure and cell alignment in the xylem union was excellent in three of the five unions examined.

AYC had the lowest success rate as a rootstock for POC (Figure 2.2), but promoted scion growth nearly equal to IC(Figure 2.3). Noticeable swelling at the union was present in every union examined. The form factor for AYC-POC grafts showed the greatest scion-stock overgrowth (Figure 2.4).

The length of the scion grafted significantly influenced the success of the graft (Figure 2.6). A higher percentage of grafts with scions under 10 cm long formed successful graft unions. None of the 30-40 cm long scions formed successful unions. Graft success was negatively correlated with length of scion.

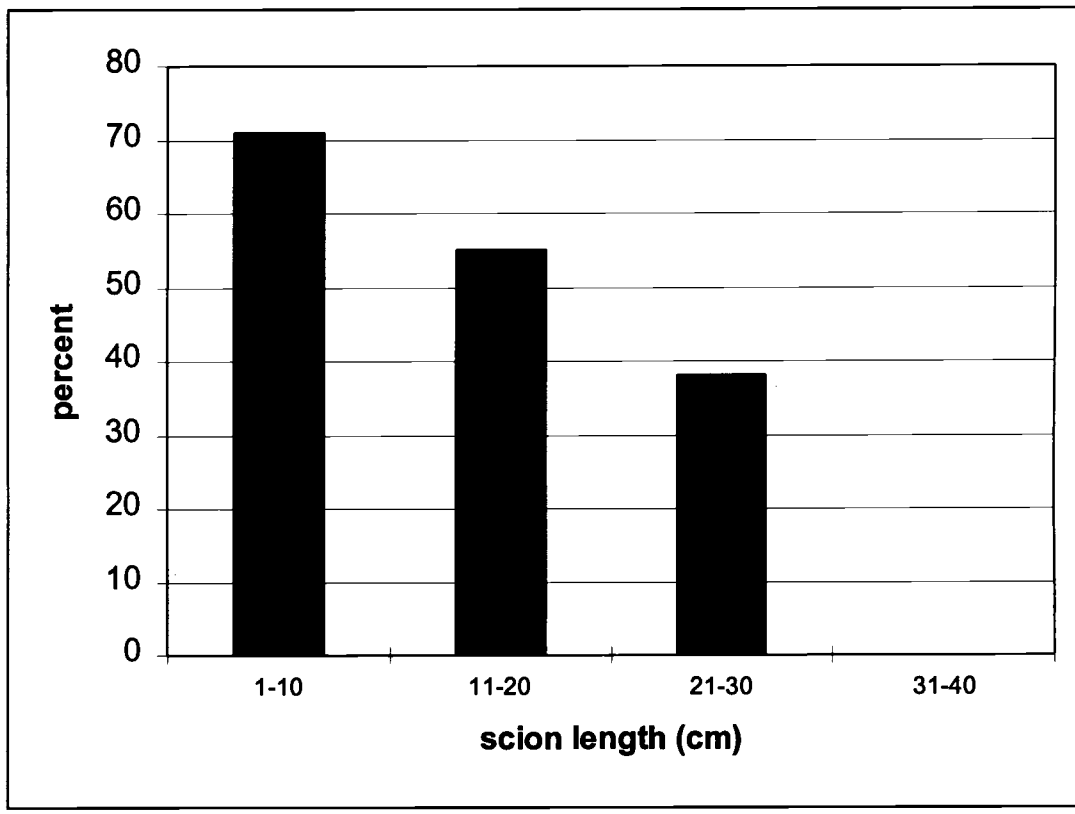

Figure 2.6. Percent graft success over 31 months by scion length class. All percentages are significantly different (p=0.05).

Mortality from *P. lateralis* was high for POC both at the two infested outplanting sites and at the Cordley site. Between 78% and 90% of the POC rootstocks exposed to the fungus died. The percentage surviving was not significantly different for homoplastic, heteroplastic in which POC was the rootstock, or ungrafted POC rootstocks (Figure 2.7), nor was it significantly different among infested sites (Figure 2.8). A few trees died from *P. lateralis* at the Botany Farm, probably from infections originating before the trees were moved from Cordley.

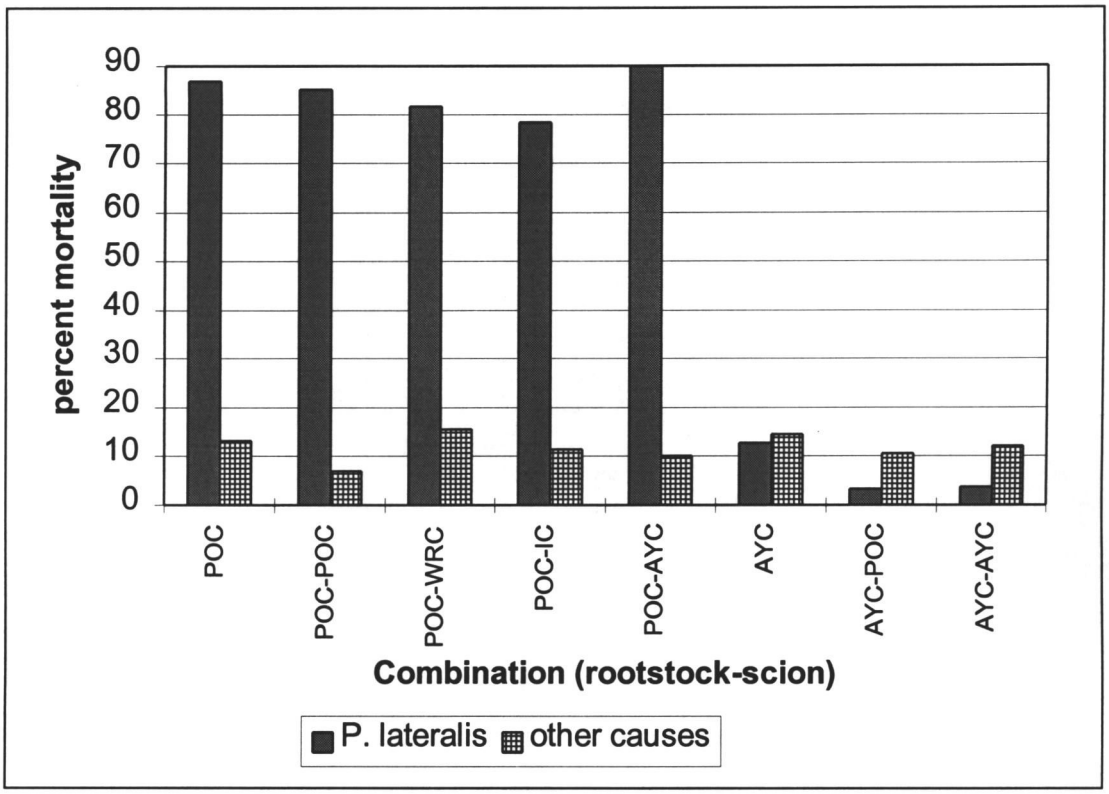

Figure 2.7. Percent of grafted and ungrafted trees at Cordley, Flannigan, and Quosatana killed by *P. lateralis* or other causes (animal damage or unknown cause).

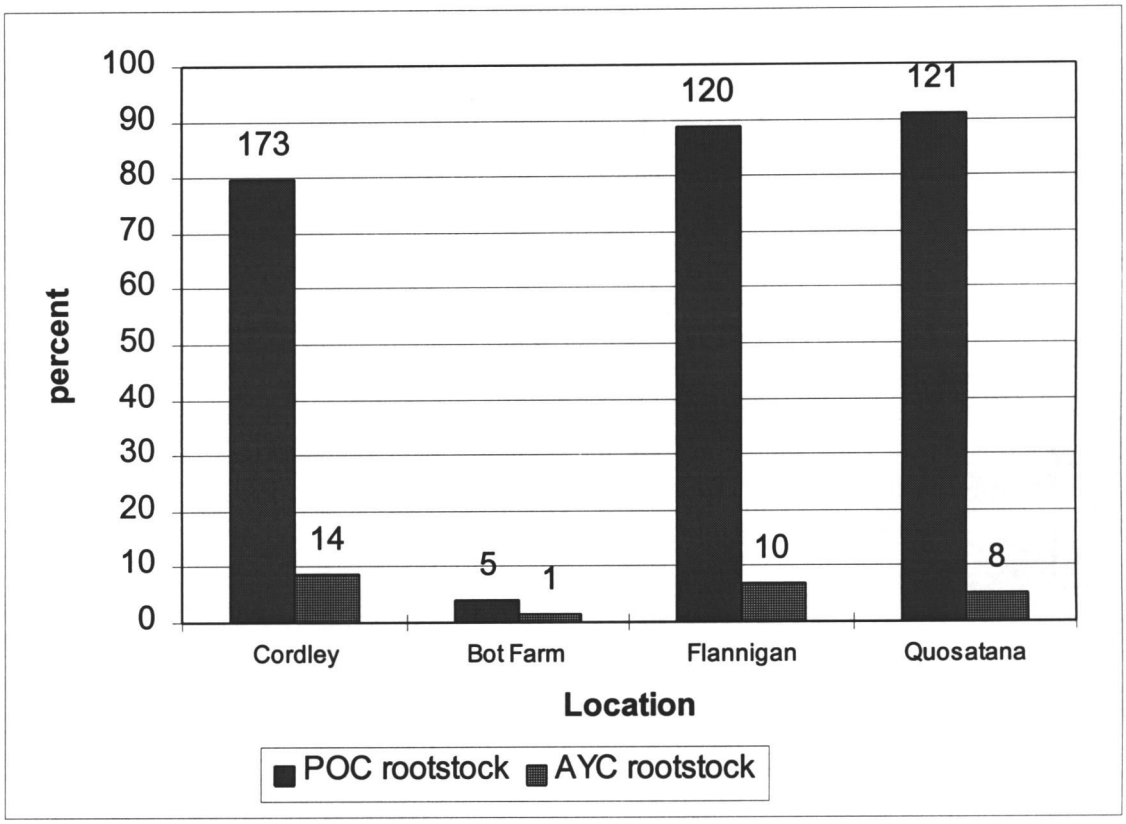

Figure 2.8. Percent of POC and AYC rootstocks killed by *P. lateralis* by location. Numbers above bars indicate number of trees killed.

A small number of AYC were killed by *P. lateralis* at all three infested sites (Figure 2.8). Direct isolation from stems was attempted from the 33 AYC killed by disease, and 9 were successful, resulting in a confirmation rate of 27%. There were no significant differences in mortality from *P. lateralis* among rootstock sources of AYC (Figure 2.9). *P. lateralis* was never recovered from dead IC or WRC, and the stain characteristic of *P. lateralis* infection was not observed on dead trees of these species.

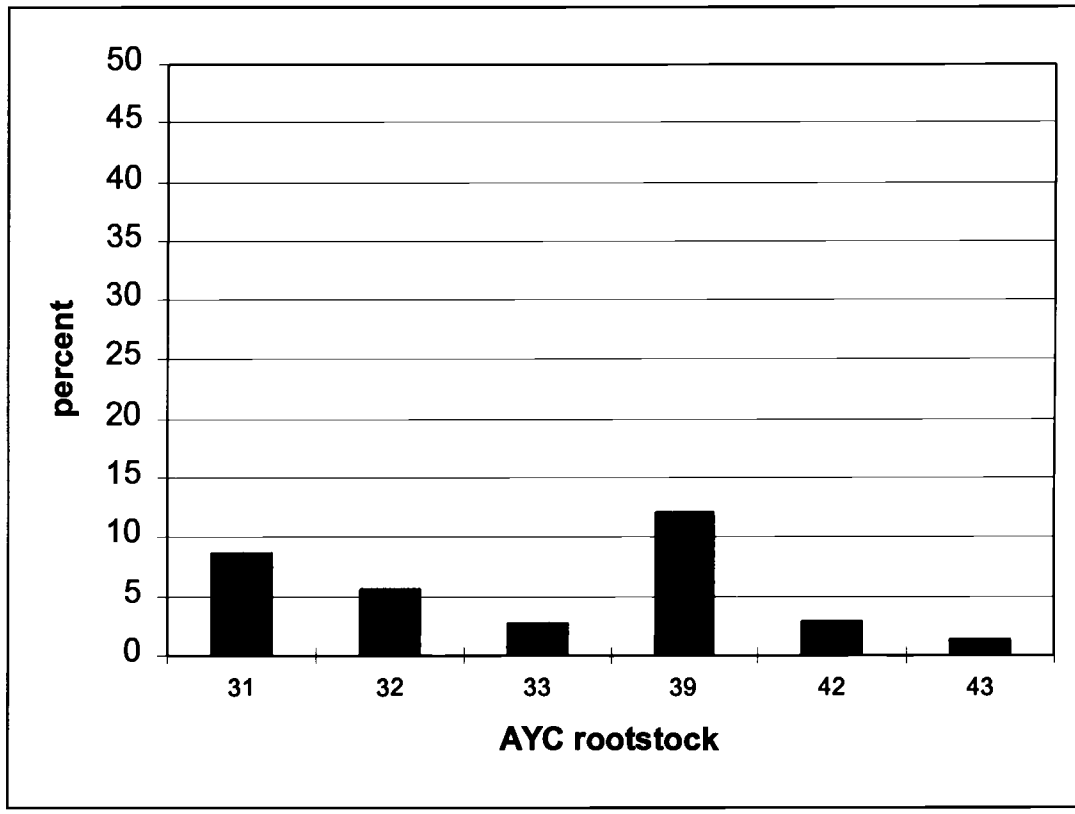

Figure 2.9. Mortality percentage for AYC seed sources. Differences among sources were not significantly (p=0.05) different.

DISCUSSION

This study demonstrated a high graft take and compatibility for homoplastic grafts of the four species tested. Veneer grafting is a good graft type to use for grafting these species in the Cupressaceae. The success of heteroplastic grafts of all four species combinations was much lower. With few exceptions, intergeneric grafts in conifers are not successful in the long term (Jayawickrama et al. 1991). The initial graft take and continued healthy growth of some heteroplastic grafts for 31 months suggests that some intergeneric combinations may be of use in the short term for preserving valuable genotypes of POC. If the graft unions

are made high on the rootstock and adequate foliage is left on the rootstock to maintain root and stem growth of the rootstock, many scions without good phloem connections can continue to live and grow for a number of years. In most heteroplastic grafts in this study, functional xylem connections existed and scion growth rate was adequate. The fact that most successful grafts showed visible swelling at the graft union from scion overgrowth parallels the experience of the nursery industry with intergeneric grafts with POC scions.

There was a moderately high graft success (Figure 2.2) and good growth (Figure 2.3) with POC rootstock and scion (Figure 2.2). There are several individuals and families of POC that are highly resistant to *P. lateralis*, and these should be tested for suitability as rootstocks for less resistant POC. Homoplastic grafting onto resistant rootstocks could be used to help preserve genotypes of POC exhibiting varying levels of resistance for use in breeding programs, without the high hazard of *P. lateralis* introduction and subsequent mortality inherent in growing susceptible POC in a seed orchard setting.

Based on graft success, incense cedar appeared to be the most compatible rootstock for POC of the three species tested. It promoted the greatest scion elongation. Although rare, several unions showed no swelling and had what appeared to be normal phloem unions. This suggests that some highly compatible families or individuals of incense cedar might exist, and that further study of compatibility between IC and POC might prove fruitful.

Port-Orford-cedars studied were nearly universally susceptible to mortality caused by *P. lateralis*. There were no significant differences among ungrafted, homoplastic grafted, or heteroplastic grafted trees in which POC was the rootstock. Grafting other species of scion on POC rootstock did not confer any significant resistance to the otherwise

susceptible rootstock. The slight but significant difference in susceptibility between the two POC rootstock source elevations suggests there may be differences in susceptibility over elevation or latitude gradients. Work is currently under way at the U.S. Forest Service Dorena Tree Improvement Center to further elucidate susceptibility trends among elevation zones and seed zones for POC.

The susceptibility of *C. nootkatensis* to *P. lateralis* confirms work by Salisbury (1954) and Hansen et al. (1988). Although AYC is much less susceptible to mortality caused by the fungus, the fact that AYC can support growth of the fungus suggests another possible reservoir or source of the fungus when POC and AYC are growing on the same site. Further study on the susceptibility of AYC, and resistance mechanisms that allow growth of the tree in infested sites without suffering mortality, may show approaches that would be useful in examining resistance mechanisms in POC.

Chapter 3 Genetically Based Resistance of *Chamaecyparis lawsoniana* to *Phytophthora lateralis*

Port-Orford-cedar (*Chamaecyparis lawsoniana*) (POC) is a forest tree endemic to southwest Oregon and northern California. Although it can be found in pure stands, it most often grows in mixed stands with other conifers (Zobel et al. 1985). It grows on a wide range of soil types and slope positions, but is particularly important on ultramafic soils and in riparian areas. It is also grown throughout the temperate regions of the world as an ornamental.

In the 1920's a previously unreported disease began to cause mortality in nurseries growing POC as an ornamental (Zobel et al. 1985, Hunt 1957). Mortality was first reported from a nursery near Seattle in 1923, and the initial report stated that mortality was occurring in other nurseries growing POC as well as in urban plantings. By the 1940's, urban plantings of POC were being killed throughout western Washington and Oregon (Roth et al. 1957). In 1942 the causal agent was identified as *Phytophthora lateralis* (Tucker and Milbrath 1942). In 1952 the disease was reported in the native range of POC at Coos Bay, Oregon, and it may have been present in the native range as early as 1944 at Charleston, Oregon (Roth et al. 1957).

The origin of the fungus causing the original reported infections is unknown. From the original focus, the disease was probably spread in infested soil associated with movement of nursery stock. The first infestations in the native range occured in parks and farms, and along major roads (Roth et al., 1957). Current long distance spread is by movement of infested soil on vehicles or the feet of ungulates (Zobel et al. 1985), as well as streams of water containing zoospores, detached sporangia, or infested organic matter.

Resistance to *P. lateralis* in POC is rare. Nearly all POC trees exposed to the fungus die, often rapidly. Several researchers at Oregon State University (L. Roth, L. Englander, E. Hansen and P.Hamm) have collected and rooted cuttings from trees that survived in root diseased areas, and a few individuals continue to survive in heavily infested soil in cold frames at Oregon State University (Hansen et al. 1989). One of these trees (coldframe1 = CF1) is among the most tolerant trees tested, and is routinely used as a tolerant control in testing for resistance. More recently, the U.S. Forest Service has collected seed from individuals surviving in mortality areas as well as seed from throughout the range of Port-Orford-cedar. They are currently testing seedling families from those seed for resistance to *P. lateralis* and possible inclusion in a program of breeding for resistance (Hansen et al. 2000) Many tests are ongoing at Oregon State University to retest families and individuals using various inoculation methods (mycelium insertion under bark, zoospore dip inoculation, outplanting in naturally and artificially infested seed beds and field plots) and to determine the correlation among these inoculation methods and survival of outplanted trees.

To detect and quantify levels of resistance, trees can be artificially inoculated and the resulting response measured. Methods to test for resistance to *Phytophthora* have included artificial inoculation of leaves (Dorrance and Inglis 1997), roots (Bolkan 1985) or stems (Dolan and Coffey 1986) with mycelium or zoospores. Resistance has also been evaluated by outplanting in naturally or artificially infested soil in the greenhouse (Butcher and Stukely 1997b) or the field (Butcher and Stukely 1997a, Harris et al. 1983). Resistance has been measured by differences in lesion length resulting from inoculation of roots or stems, or by recording time until death of test plants.

In many cases, greenhouse tests for resistance to *Phytophthora* were accurate predictors of field performance in infested sites. In a test of avocado resistance to *P. cinnamomi*, lesion length resulting from stem inoculation was positively correlated with lesion length resulting from root inoculation, and ranking of cultivars was the same with both tests (Dolan and Coffey 1986). *Pinus radiata* families, determined to be resistant in glasshouse tests, had greater survival and better growth when outplanted on infested sites (Butcher et al. 1984, Butcher and Stukely 1997a). Families of *Eucalyptus marginata* that show short lesion length when the stem is inoculated with *P. cinnamomi* also have lower mortality when planted in artificially infested soil, and greater survival after planting in an infested field site (Stukely and Crane 1994). In tomato and potato, field resistance to infection by *Phytophthora* is generally correlated with resistance demonstrated in greenhouse tests (Dorrance and Inglis 1997, Bolkan 1985).

Hansen et al. (1989) tested resistance in POC, measured by lesion length, resulting from stem inoculation, zoospore inoculation, and inoculation by planting trees in infested soil. Results from the stem wound inoculation method agreed well with results from zoospore and infested soil inoculation methods. Trees that showed resistance with one inoculation method did so with other inoculation methods.

Resistance to disease in plants results from a combination of environmental and genetic factors. Heritability is that portion of the total variance in a trait due to genetic factors (Wright 1976). Estimates of the heritability of resistance to *Phytophthora*s are often high. Heritability of resistance to *P. cinnamomi* in *Pinus radiata* was near 1 when families from the native range of the tree were tested, and 0.79 when Australian families were tested. Genetic variance accounted for 85-90% of the total variance (Butcher et al. 1984) Family mean heritability estimates ranged from 0.74

to 0.85 in different experiments when *E. marginata* was tested for resistance to *P. cinnamomi*, and the narrow sense heritability was calculated as 0.43 (Stukely and Crane 1994).

The genetic basis for resistance or tolerance to *P. lateralis* has not been demonstrated. The genetic basis and heritability of resistance in seedlings, and sources of variation in inoculation experiments, are the subjects of this paper.

The approach used to determine the genetic component of resistance was to conduct a series of inoculation experiments on half-sib (wind - pollinated) seedling families and on rooted cuttings from individual trees. An initial test of two hundred trees in four families involved inoculating them with mycelium under the stem bark and measuring resulting lesions (experiment 1, Table 3.1). A second test involved inoculating both excised stems and trees with intact root systems with a zoospore suspension (experiment 2). The third test involved inoculating multiple rooted cuttings (ramets) from single trees with mycelium under the stem bark, and measuring resulting lesions (experiment 3).

The first test with four half-sib-families was designed to test differences in family mean lesion length, and obtain an estimate of heritability of resistance. The second test was part of a larger test of common garden families conducted for the U.S. Forest Service. It included families that had previously been tested and was done to compare family rankings with different inoculation methods. The third test with rooted cuttings was undertaken to obtain more precise estimates of the contribution of genetics to resistance, as well as to compare lesion length resulting from inoculation among different isolates of *P. lateralis*.

MATERIALS AND METHODS

Seed for the trees used in these tests was collected by personnel of the U.S. Forest Service. Two types of collections were made. Seed was collected from trees throughout the range of POC without regard to possible disease exposure or tolerance ("common garden" families). Seed was also collected from individuals that had been previously tested for resistance to *P. lateralis* by excised branch inoculation ("control" families). All seed used was open (wind) pollinated; seedlings grown from seed of a single parent tree are half-sibs and are referred to as a family. The seedlings and cuttings were grown either at the U.S. Forest Service Dorena Tree Improvement Center or Chico Tree Improvement Center. Plants were grown using standard practices for forest tree container nurseries.

Experiment 1: stem inoculation.

For experiment 1, four open pollinated POC families from previously tested seed parents were chosen: one with short lesion length, one with long lesion length, and two with moderate lesion length (Table 3.1, Table 3.2). These parents had been located by U.S. Forest Service employees, and were trees surviving in areas that had experienced severe mortality from *P. lateralis.* Fifty seedlings from each familiy were grown for one year in 4 cm wide by 20 cm long plastic containers. One isolate of *P. lateralis* (366, isolated from *C. lawsoniana* near Shelly Creek, CA) was grown for one week on corn meal agar; 3 mm plugs were cut from the advancing edge of the colony and grown for one week in split pea broth (150 g dry split peas autoclaved for 3 min at 121C in 1 L deionized water, liquid

decanted through a double layer of cheesecloth, and autoclaved for 15 min at 121C, then decanted to 100 mm plastic petri plates). Inoculations were performed by cutting 1 cm vertical slits through the bark of seedlings, inserting a small weft of mycelium from the pea broth cultures under the bark, and then sealing the cut with petroleum jelly. After 3 weeks the maximum length of the resulting lesion was measured by slicing through the bark to expose the disease caused discoloration, and then measuring the distance between the advancing edges of cambial discoloration.

Summary of experiments			
experiment	number of families	number of trees per family	inoculation method
Experiment 1	4	50	stem inoculation under bark with mycelium
Experiment 2 (common garden families)	337	15	root inoculation with zoospore suspension
Experiment 2 (control families)	7	45	root inoculation with zoospore suspension
Experiment 2 (common garden families)	339	15	excised stem inoculation with zoospore suspension
Experiment 2 control families	7	45	excised stem inoculation with zoospore suspension
	number of trees	number of cuttings per tree	
Experiment 3 (rooted cuttings)	11	30 to 36	stem inoculation under bark with mycelium

Table 3.1. Summary of number of families, number of trees, and inoculation methods used.

Abbre-viation	U.S.F.S. Tree No.	Location of Parent
Experiment 1		
15	510015	Gasquet RD, Six Rivers NF
18	510018	Gasquet RD, Six Rivers NF
49	510049	Gasquet RD, Six Rivers NF
501	11093501	Powers RD, Siskiyou NF
Experiment 2 Control Families		
15	510015	Gasquet RD, Six Rivers NF
01	118801	Powers RD, Siskiyou NF
34	118834	Powers RD, Siskiyou NF
70	118570	Powers RD, Siskiyou NF
16	510016	Gasquet RD, Six Rivers NF
23	SAC 1-10	Mt. Shasta RD, Shasta-Trinity NF
24	DUN 2-11	Oregon Dunes NRA, Jordan Lake
Experiment 3		
015A	510015A	Gasquet RD, Six Rivers NF
346	117346	Gold Beach RD, Siskiyou NF
570	118570	Powers RD, Siskiyou NF
015B	510015B	Gasquet RD, Six Rivers NF
568	118568	Powers RD, Siskiyou NF
822	118822	Powers RD, Siskiyou NF
497	117497	Gold Beach RD, Siskiyou NF
567	118567	Powers RD, Siskiyou NF
018	510018	Gasquet RD, Six Rivers NF
016	510016	Gasquet RD, Six Rivers NF
489	117489	Gold Beach RD, Siskiyou NF

Table 3.2. Family number, abbreviation, and geographic source of families used in experiments 1, 2, and 3.

Experiment 2: stem and root inoculation.

For experiment 2, seedlings in the "control" half-sib families (Table 3.2) were grown from seed of seven previously tested parents. Parent selection was done to encompass maximum, minimum, and moderate lesion lengths

in previous tests. Seedlings in the "common garden" families were grown by the U.S. Forest Service from seed collected as part of a U.S. Forest Service common garden study. These families had not been previously tested. All seeds were open (wind) pollinated.

Ninety seedlings from each "control" family were grown in 4 X 20 cm plastic containers for one year in a 1:1 peat:perlite potting medium. Thirty seedlings from each of the 337 "common garden" families were grown in the same way. Separate seedlings from the same "control" and "common garden" families were used for the stem and root inoculation procedures, and were sown and grown together. Seedlings were randomly assigned to either the stem or root inoculation treatment.

Inoculum for the test was prepared by growing two isolates (366, 368) for 10 days at ~24C on corn meal agar containing 20 ppm *B*-sitosterol, and then transferring 3 X 3 mm plugs to split pea broth for an additional 7 days. Medium was removed and colonies were rinsed three times under a stream of deionized water. Plates were then filled with non-sterile water from a local creek that had been filtered through Whatman #2 filter paper. This creek water had previously been found effective in inducing zoosporangium formation, and is much easier to prepare than the more standard soil extract water. One day later, water containing zoospores was poured from plates and placed in 1 quart glass jars. Water from one plate of each isolate was combined in each jar with an additional 40 ml deionized water, resulting in a total volume of ~60 ml and a depth of ~1 cm.

Seedlings used for stem inoculations were excised at the ground line, wrapped in plastic bags, and stored in a styrofoam box for transportation from Dorena Tree Improvement Center to Corvallis, Oregon. On the day following excision, 10 randomly chosen excised seedlings were placed in each inoculation jar containing freshly prepared zoospore suspension ~1 cm deep for 24 hours, and then were placed in 4 X 20 cm plastic tree tubes

containing moist perlite. The excised seedlings were held in a greenhouse and watered every other day for 20 or 21 days. Lesion length was recorded by measuring the distance between the excised end and the upper limit of discoloration inside the bark, and length (mm) of lesion extension per day was calculated. All culture preparation, inoculations, and measurements were done by Wendy Sutton at Oregon State University through a contract with the U.S. Forest Service.

Inoculum for root inoculation was prepared in the same manner as for stem inoculation. For the root inoculations, intact seedlings in 4 X 20 cm growing tubes had their projecting roots trimmed at the bottom of the container. The growth medium was fully hydrated before inoculation. The distal 1 cm of the growing tube containing the root system was immersed in zoospore suspension in a large plastic cups for 24 hours. Seven randomly chosen seedlings were inoculated in each cup. Following inoculation containers were replaced in plastic growing racks, held in a greenhouse, and watered every other day until measurements were made. The time until measurement varied from 30 to 125 days (mean 92 days). Lesion length was recorded by measuring the distance between the bottom of the root mass and the upper extent of discolored roots. The mm lesion extension per day was calculated for this analysis. Five seedlings of each common garden family (each seedling was analyzed as a replicate of the family) were inoculated on each of three days (each day was analyzed as a block). Fifteen seedlings of each control family were inoculated on each of three days (blocks), and groups of three seedlings that were inoculated together were analyzed as a replicate.

Experiment 3: stem inoculation of rooted cuttings.

For experiment 3, stem cuttings of eleven four-year-old POC seedlings (clones or ortets) from ten different families were made and rooted under mist (Table 3.2). The seed parents of the seedlings had been located by U.S. Forest Service employees, and were survivors in areas that had experienced severe mortality from *P. lateralis*. After adventitious roots formed, the ramets were potted in 3 gallon round plastic pots in a standard soilless mix containing approximately 2 parts Douglas-fir bark, 1 part peat, and 1 part pumice. The ramets were grown for an additional year with periodic fertilization with an encapsulated time release fertilizer (Nutricote™ 18:6:8 with minor elements, 6 month formulation, 1.5 Tbs per pot).

Ten isolates of *P. lateralis* were grown for one week on CMAS (corn meal agar (Difco) amended with 20 ppm *B*-sitosterol). Plugs cut from the advancing edge of colonies were then transferred to split pea broth. Colonies were grown for an addtional 7 days, rinsed in deionized water, and used for inoculation.

Ramets were inoculated by cutting a longitudinal 1.5 cm slit in the bark, and placing a small weft of mycelium (approx. 1 mm^3) under the bark. Slits were then sealed with sterile petroleum jelly. Ramet number, ortet, isolate, height of inoculation, and caliper of stem at inoculation site were recorded for each inoculation. Mean height of inoculation was 32 cm (range 13-57), and mean caliper at inoculation site was 7 mm (range 2.5-12). Each ramet was inoculated with one isolate, and there were three replications per isolate per ortet. Eleven ortets were used, but most ortets did not have sufficient numbers of ramets to be inoculated by all 10 isolates, so one or two isolates were randomly excluded. Total lesion length was measured after 7 weeks.

Lesion length was analyzed using the General Linear Model procedure (SAS Institute Inc., 1985) rather than ANOVA because of unequal cell sizes (Table 3.3). Duncan's multiple range test was used to test for differences among ortet means (level p=.05).

Experiment	Source	df	ms	F value	Prob > F
Experiment 1 stem inoculation	family	3	10404	19.54	0.0001
	error	188	532		
Experiment 2 root inoculation control families	family	6	10.5	22.13	0.0001
	block	2	2.0	4.25	0.0150
	rep	4	1.0	2.08	0.0840
	error	267	0.5		
Experiment 2 root inoculation common garden families	family	336	2.0	5.26	0.0001
	block	2	5.1	13.58	0.0001
	rep	4	5.0	13.34	0.0001
	error	4582	0.4		
Experiment 2 stem inoculation control families	family	6	3.9	14.02	0.0001
	block	2	3.1	11.08	0.0001
	rep	4	0.6	2.03	0.0897
	error	299	0.3		
Experiment 2 stem inoculation common garden families	family	338	0.4	1.53	0.0001
	block	4	50.4	191.6	0.0001
	rep	2	1.4	5.31	0.0003
	error	4683	0.3		
Experiment 3 stem inoculation rooted cuttings	ortet	10	6551	87.9	0.0001
	error	309	74		

Table 3.3. Summary of statistical analysis of lesion length resulting from inoculation of individual trees.

The model used for analysis of lesion length for experiment 1 was:

lesion length = f (family)

The model used for analysis of lesion length for experiment 2 was:

lesion length per day = f (family, block, replicate)

The model used for analysis of lesion length for experiment 3 was:

lesion length = f (ortet)

Estimates of the narrow-sense single-tree heritability (ht2) of resistance to lesion extension caused by *P. lateralis* were calculated using the formula:

$$ht2 = 3sf2/(sf2+sb2+se2)$$

where sf2 is the variance due to families (or ortet), sb2 is the variance due to block, and se is the error variance (Wright, 1976). There were no blocks in experiment 1, so the block term was dropped. For this analysis the additive genetic variance was calculated as three times the family variance, a more conservative calculation than using four times the family variance. This calculation is favored by some workers (Frank Sorensen, personal communication) because of the possibility of self-pollinations and because pollen parents in a location are likely to be related. Standard errors for narrow sense tree heritability were calculated according to Swigger et al. (1964).

Estimates of family mean heritability were calculated using the formula:

$$hf2=sf2/(sf2+(sb2/b)+(se2/nb))$$

where b is the number of blocks, and n is the number of trees in a block (Wright, 1976). There were no blocks in experiment 1 so the block term was dropped.

Proportion of variance explained by the different elements in the models was determined using the variance component estimation procedure of SAS.

RESULTS

Experiment 1.

Seedlings exhibited a wide range of within and among family variation in lesion length resulting from inoculation. Family means for lesion length were significantly (p=.05) different (Table 3.3, Fig 3.1, Fig. 3.2), and lesion length for individual trees ranged from near 0 mm to over 100 mm. Family 15 had a significantly shorter necrotic area extending from the site of inoculation than other families. The individual tree heritability of resistance was high, but the standard error of the estimate was large because of the small number of families measured (Table 3.4). Family heritability of resistance was also high, but relates only to the families included in this test.

Twenty eight percent of the total variability was accounted for by family, showing a strong genetic component involved in determining resistance (Table 3.5).

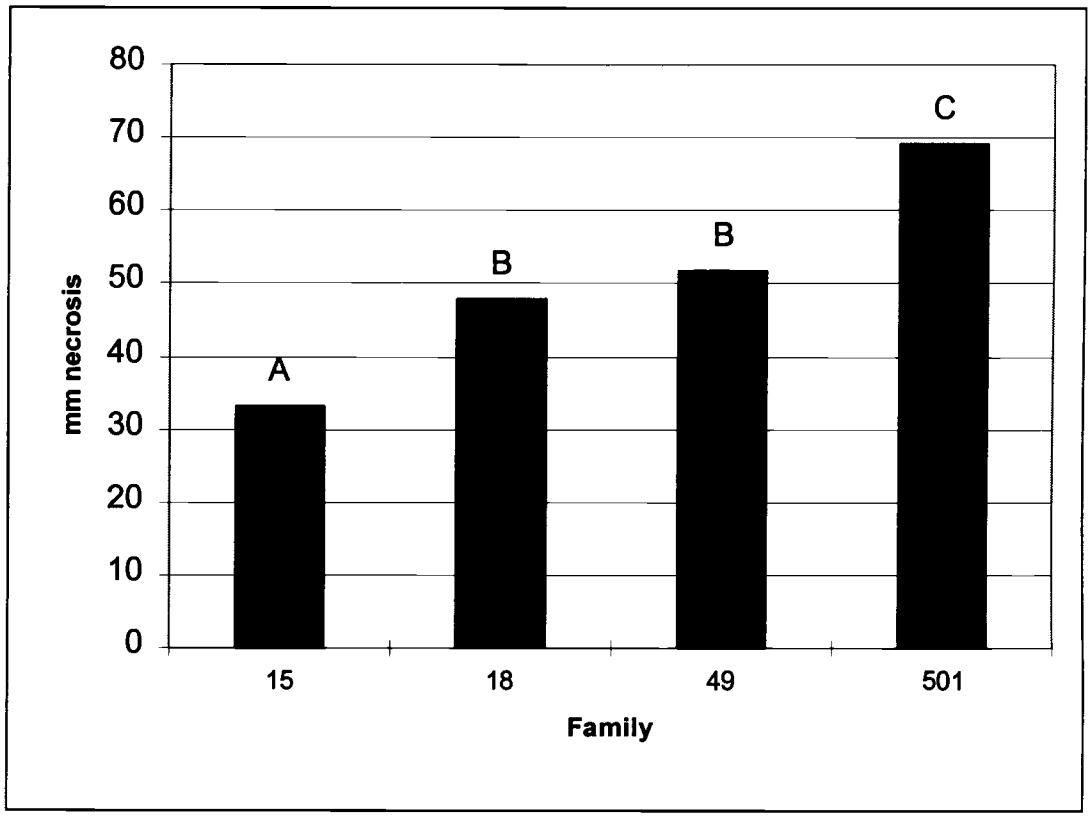

Figure 3.1. Mean lesion length of four families in experiment 1. Different letters above bars indicate families that were significantly (p=0.05) different.

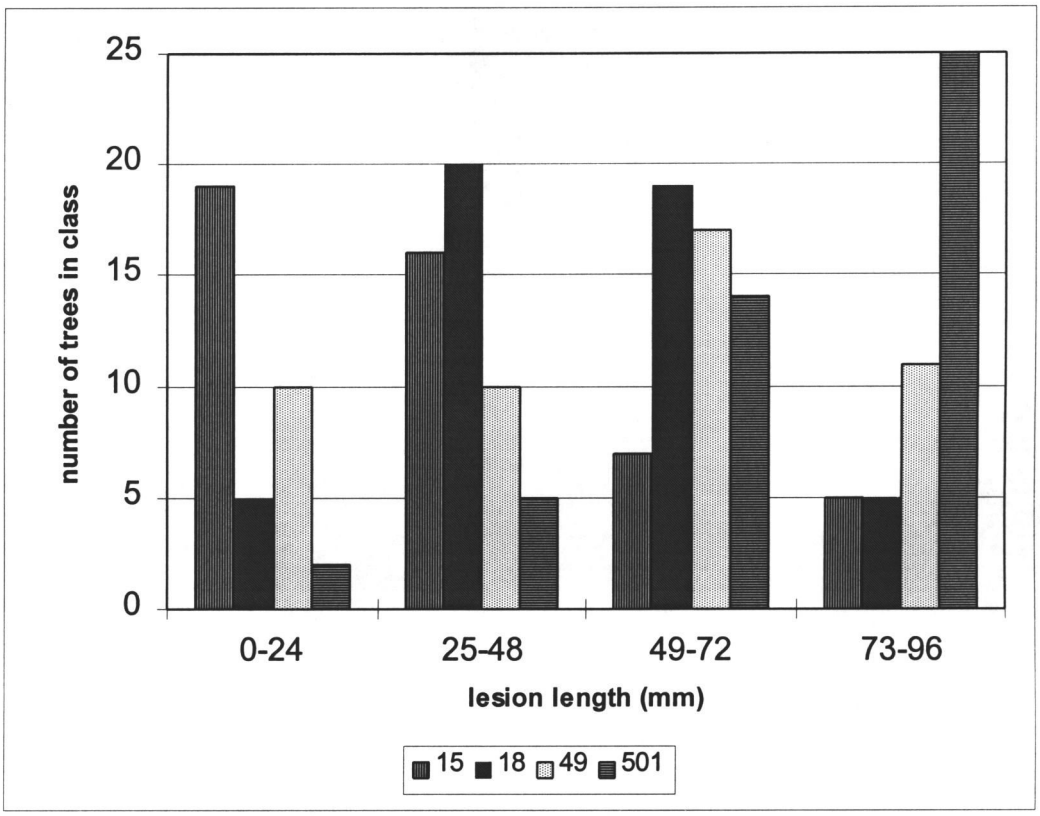

Figure 3.2. Distribution of lesion length of four families in experiment 1.

Data Set	Individual Tree Heritability	Std. error of est.	Family Heritability
Experiment 1	0.836	0.699	0.949
Experiment 2 common garden families, root inoculation	0.664	0.068	0.918
Experiment 2 control families, root inoculation	0.979	0.540	0.953
Experiment 2 common garden families, stem inoculation	0.096	0.029	0.536
Experiment 2 control families, stem inoculation	0.609	0.411	0.882

Table 3.4. Narrow sense individual tree heritabilities, standard error of estimate, and family mean heritabilities for experiments. Standard error is inversely related to the number of families tested.

data set	family	block	error
Experiment 1	28	0	72
Experiment 2 common garden families, root inoculation	22	1	77
Experiment 2 control families, root inoculation	32	1	66
Experiment 2 common garden families, stem inoculation	3	10	87
Experiment 2 control families, stem inoculation	21	7	72
Experiment 3 Rooted Cuttings all isolates	74	9	17
Experiment 3 Rooted Cuttings without isolate Yew2	82	0	17

Table 3.5. Percentage of variance explained by components of model. The SAS variance components procedure model was lesion length = *f*(family, block).

Experiment 2.

Family means for lesion length were significantly (p=0.05) different for both stem and root inoculations among the "control" families (Table 3.3, Figure 3.3, Figure 3.4). Family 15 had a significantly shorter lesion length after inoculation than other "control" families. The narrow sense single-tree heritability of resistance was high, but the standard error of the estimate

was large because of the limited number of families measured (Table 3.4). Family mean heritability of resistance was also high.

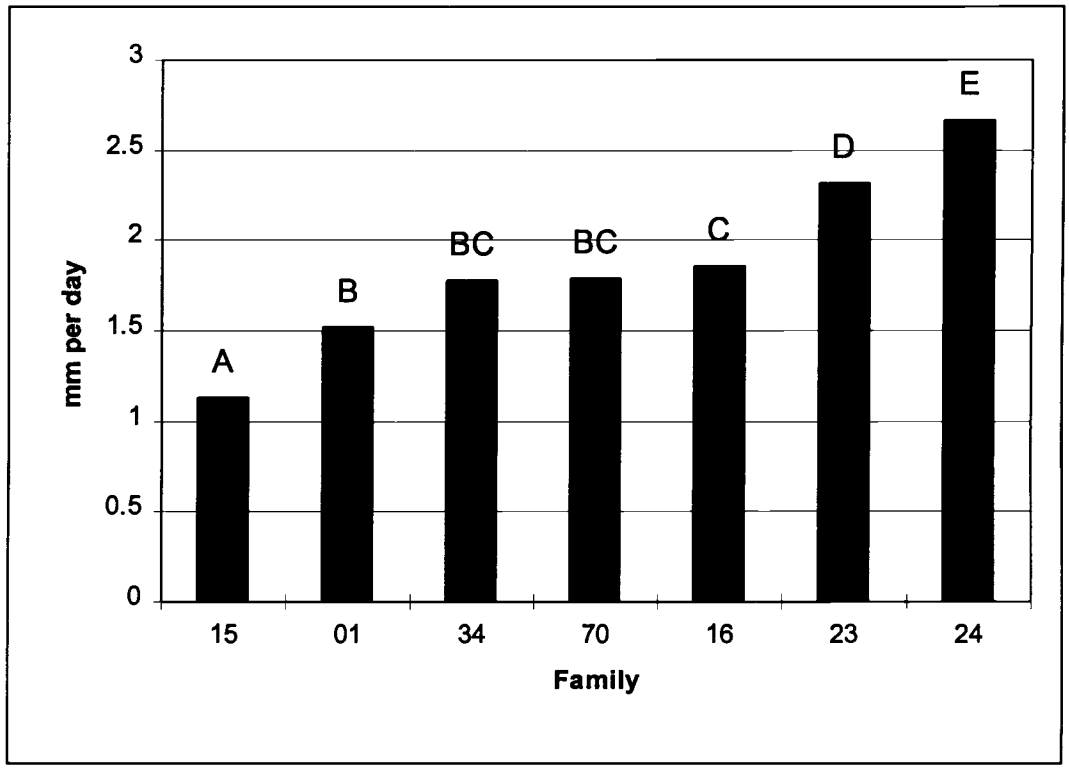

Figure 3.3. Family mean root lesion length per day for "control" families in experiment 2. Different letters above bars indicate significant (p=0.05) differences among family means.

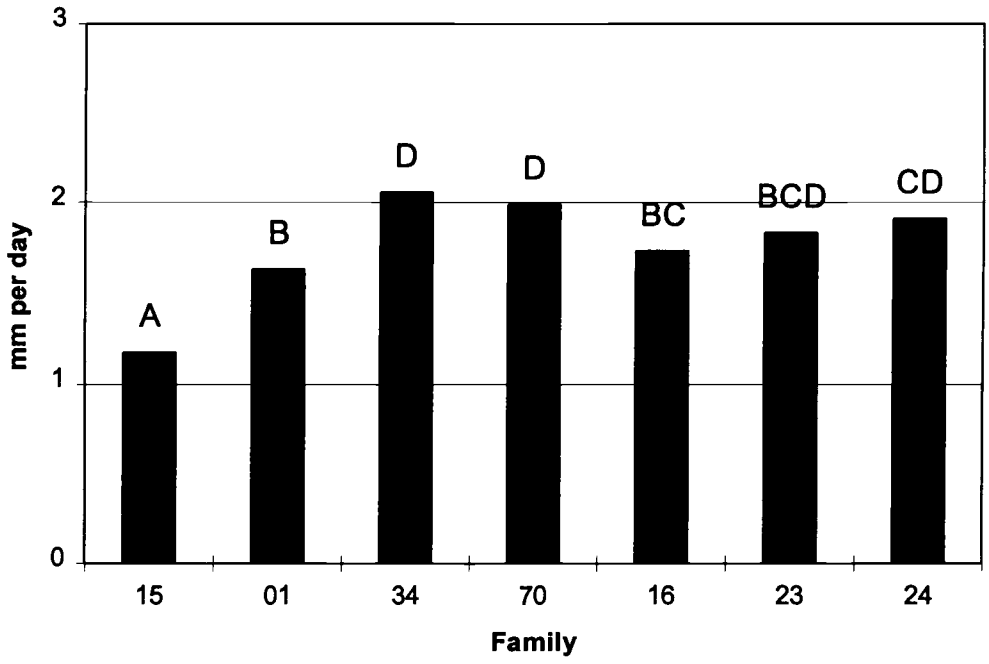

Figure 3.4. Family mean stem lesion length per day for "control" families experiment 2. Different letters above bars indicate significant (p=0.05) differences among family means.

Family means for lesion length were significantly different (p=0.0001) for both inoculation methods with the "common garden" families (Table 3.3). Family means showed continuous variation over the range of values. The narrow-sense heritability of rate of lesion expansion was high in the "common garden" families when measured by root inoculation, but low when measured by stem inoculation (Table 3.4). Heritabilities are lower than those reported by Sniezko, possibly because he included both "common garden" and "control" families together in the same analysis (Hansen et al. 2000) The standard error of the estimate of narrow-sense heritability was low due to the large number of families tested.

The correlation between family mean lesion length for "control" families using the different inoculation methods in experiment 2 is moderate but not significant (r=0.66, p=0.11), and correlation between rankings based on family means is also moderate but not significant (r=0.43, p=0.34).

The range of lesion extension for the "control" families was less than the "common garden" families with both inoculation methods. Mean lesion extension for the control families with root inoculation was 1.13 to 2.66 mm/day, and with stem inoculation was 1.18 to 2.06 mm/day. The common garden families ranged from 1.14 to 3.28 mm/day with root inoculation, and 1.54 to 3.36 mm/day with stem inoculation. Many "common garden" families were more susceptible than the most susceptible "control" family. The rates of lesion extension for many families exceeded the growth rate of *P. lateralis* in culture (Fig 4.1).

The "common garden" families showed no correlation between the two inoculation methods for family means (r=0.09), or ranking based on family means (r=0.09).

Experiment 3

There were significant (p=0.05) differences among mean lesion length calculated for individual clones. The mean lesion length of the most susceptible clone was nearly four times the lesion length of the most resistant clone (Figure 3.5).

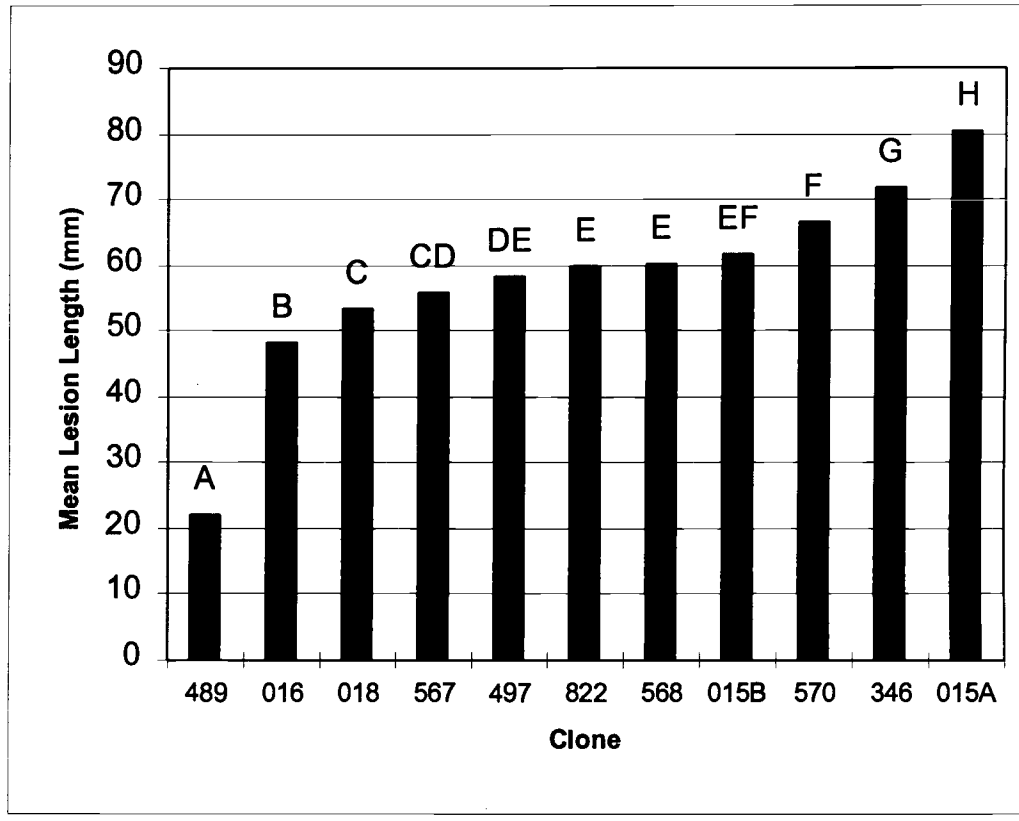

Figure 3.5. Clone mean stem lesion length for experiment 3. Different letters above bars indicate significant (p=0.05) differences among clone means.

In this test there were multiple replications of single genotypes, because cuttings came from individual trees. The variance attributable to differences in host genetics was 74% (Table 3.4). One isolate of *P. lateralis*, Yew2, produced significantly shorter lesions on all families than other isolates tested (Chapter 4). When this isolate was removed from the analysis, individual clone genetics accounted for 82% of the total variance. There was no tree by isolate interaction. Only 17% of the total variance was due to experimental error, which included such factors as differences

in effectiveness of inoculation, inaccuracy in lesion measurement, and environmental variation.

DISCUSSION

The 4 families included in experiment 1 exhibited a wide range of mean lesion lengths caused by *P. lateralis*. The range of lesion lengths exhibited by the "common garden" families in experiment 2 showed a normally distributed range of values. No families were immune to necrosis caused by inoculation. The continuous range of lesion lengths among families suggests that the resistance to necrosis involves multiple genes rather than a gene-for-gene interaction (Vanderplank 1982). Resistance in *P. radiata* to *P. cinnamomi* follows a similar pattern of a continuous range of lesion lengths within a family (Stukely and Crane 1994).

The heritability of resistance to *P. lateralis* was remarkably high at both the family and individual tree levels in most tests. Heritability of a trait calculated on a small number of non-randomly chosen families (the four families in experiment 1 and the "control" group in experiment 2) applies strictly only to those families; however, heritability calculated on the "common garden" families is valid because these families were collected from throughout the range of POC without regard to disease resistance characteristics. I conclude that resistance as exhibited by restriction of necrosis following inoculation is under strong genetic control. There were significant differences among families in the two experiments where families were treated. The heritability of resistance as measured by root inoculation in experiment 2 was high in both "control" and "common garden" families (Table 3.4). Heritability of traits such as height and diameter growth in forest trees is often in the range of 0.15 to 0.3, and for disease

resistance traits is seldom higher than 0.3 (Carson and Carson 1989, Wu and Ying 1997). However, family mean heritability of resistance to *P. cinnamomi* in forest trees has been reported at between 0.74 and 0.90 (Butcher et al. 1984, Stukely and Crane 1994), and the family mean heritabilities reported here for most experiments are near or within that range.

Families that showed the shortest mean lesion lengths (15 and 801) were also the families that had the lowest mortality when planted at two infested field sites (Everett Hansen, unpublished data). Studies of resistance in *P. radiata* have shown that families exhibiting resistance in artificial inoculation tests also had better survival when outplanted to infested sites (Butcher and Stukely 1997).

Correlation of family rankings from the stem and root inoculation family means in experiment 2 was moderate for the "control" families, but there was no correlation among the "common garden" families. This could be because the stem and root inoculation methods may actually measure different and unrelated mechanisms of resistance. Family (15) showed the most resistance both in experiment one and experiment two with both inoculation methods. It also had the lowest mortality when planted in a naturally infested field site (Everett Hansen, unpublished data).

More research is needed to define the relationship between the various inoculation methods, and how these measurements relate to field survival. One approach would be to use multiple rooted cuttings from individual trees to compare inoculation of stems, roots, and outplanting survival. Resistance testing using seedlings from open pollinated families could lead to error in selection of resistant families, if low numbers of trees per family are tested. It is also important to quantify and standardize inoculum when using zoospores. This was not done in experiment 2 and some of the

experimental variation in that test could be due to significant differences in inoculum densities.

The ranking of the two clones from family 15 in experiment 3 is of special interest. The two trees that were chosen for producing rooted cuttings from this family were apparently more susceptible, and inoculation produced longer lesions, than might be expected from other tests of members of this family. In fact, one clone (15A) was the most susceptible tree in this test (Fig. 3.5), even though this family as a whole was the most resistant in experiments 1 and 2. This indirectly highlights the continuous within family variation in resistance and susceptibility, and suggests that selection for a breeding program needs to be done on an individual tree basis from within generally resistant families, because even resistant families can contain very susceptible trees.

Clone 486 in experiment 3, which had the lowest mean lesion length, had atypical lesions. The lesions did not have sharply defined edges, and had lighter color than the lesions on other clones. This clone also had several ramets with lesions that were measured as 0 mm, and may possess a unique mechanism for restricting fungal growth.

Large amounts of genetic gain can be expected from a breeding program for highly heritable traits. The heritabilities calculated from these data demonstrate that a breeding program for resistance to *P. lateralis* in *C. lawsoniana* would be expected to yield large gains in resistance in very few breeding cycles. However, even trees showing high resistance may not survive when outplanted in very infested sites, if the number of individual infections overcomes the resistance. Deployment of resistant individuals should focus on sites with low disease risk until the mechanisms and long-term durability of resistance is demonstrated.

Chapter 4. Variation in *Phytophthora lateralis*

Phytophthora lateralis (Tucker and Milbrath) is a soilborne fungal pathogen that causes a serious root disease and mortality of Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl) (POC) in the Pacific Northwest. The disease was first reported in a letter from a commercial ornamentals nursery near Seattle, Washington, in 1923. In the original report it was noted that "...The disease prevails throughout the whole Northwest, killing cypress in the private gardens as well as in all nurseries, causing vast loss, as this Lawson cypress is used very much here..." (Hunt, 1957). Although perhaps overstated in this communication, it is clear that the fungus was present in the Pacific Northwest for some time before 1923.

The fungus has been reported twice outside North America. *P. lateralis* and three other *Phytophthora* species were reported associated with root, crown, and collar rots on Kiwifruit (*Actinidia chinensis*) in New Zealand (Robertson 1982). Inoculations using macerated mycelium mixed with soil were attempted, but none of the *Phytophthora* species produced "significant levels of infection" although some vines showed root rot symptoms. Inoculation of POC was not reported and no voucher collection of the isolate is available, hence the report is impossible to confirm. Zoospores were reported to be difficult to produce, which contrasts with the relatively easy induction of zoosporangia and zoospores possible with *P. lateralis*. More recently, *P. lateralis* was reported from France where it had been isolated from symptomatic POC growing in nurseries. The two French isolates and North American isolates have identical ITS-1 nucleotide sequences and colony morphology in culture. Isolates from Germany identified as "similar to" *P. lateralis* proved to be *P. gonapodyides* (Hansen et al. 1999).

Within North America, *P. lateralis* has been reported twice outside the Pacific Northwest. In North Carolina, a fungus identified as *P. lateralis* was isolated from diseased creeping juniper (*Juniperus horizontalis*), azalea (*Rhododendron* spp.), Fraser photinia (*Photinia* X *fraseri*), mountain laurel (*Kalmia latifolia*), and Madagascar periwinkle (*Vinca rosea*) (Abad et al. 1994). Isolates from these collections received at Oregon State University were apparently contaminated and were found not to be *P. lateralis* (McWilliams, unpublished data). A *Phytophthora* was isolated from *Rhododendron* crowns in Ohio and Pennsylvania (Hoitink and Schmitthenner 1974) and identified as *P. lateralis* on the basis of laterally attached, thin walled chlamydospores. These isolates differed from the original description of *P. lateralis* in that they grew well at high temperatures and did not form sporangia when flooded with nonsterile soil extract water. Isolates from the Pacific Northwest generally form sporangia readily and abundantly. Inoculations on *Rhododendron* caused "only slight root damage". Voucher collections are not available. None of these reports can be confirmed because of the lack of availability of voucher collections. It is likely that only the French isolates were actually *P. lateralis*. There are other *Phytophthora* species that resemble *P. lateralis* in having laterally borne chlamydospores. The fact that the New Zealand and *Rhododendron* isolates did not readily produce sporangia makes them quite different from Pacific Northwest *P. lateralis* isolates, which readily produce sporangia. The *Rhododendron* isolates grew at higher temperatures than most Pacific Northwest isolates. The North Carolina report is suspect because of the large number of hosts reported, which would be very different from the limited host range exhibited by Pacific Northwest isolates of *P. lateralis*.

The question of variation in the population of *P. lateralis* is very important for a number of reasons. It is necessary to know the range of variability in virulence among isolates so that appropriate resistance can be

incorporated in the ongoing POC breeding program, and so that appropriate isolates can be used in testing for resistance. The amount of genetic variation among isolates will offer important data for determining population structure and whether the fungus exhibits low variation, which is compatible with the idea of recent introduction. If genetic information is consistent with the idea that this fungus was introduced to North America, then it will support efforts to determine the origin of the fungus, and give a basis for comparison if other populations of *P. lateralis* are found.

Several techniques are available to detect variation among isolates of a fungus. Differences in morphological characters among isolates, such as spore ornamentation, colony morphology, or pigmentation, are often useful in distinguishing species. Growth rate on various agar media or natural substrates is useful in distinguishing among isolates within a species. Differences in pathogenicity and virulence on a range of hosts may distinguish isolates both within and among species. Isozyme banding pattern and the presence of unique isozyme loci aid in determining population structure in a group of isolates, distinguishing between closely related species, and determining whether an isolate is haploid or dikaryotic. The recent development of techniques to examine the DNA complement of a fungus directly, including RAPDs (Randomly Amplified Polymorphic DNA), RFLPs (Restriction Fragment Length Polymorphism), and direct sequencing, offer powerful tools to examine a level of variability that was previously undetectable.

Since the development of the polymerase chain reaction, several techniques have been developed to analyze differences in DNA complements among isolates of fungi. The most widely used technique uses RAPD bands to produce 'fingerprints' that can be compared among isolates. A more recently developed technique uses primers that amplify specific repetitive sequences present in most organisms to produce a

'fingerprint'. This technique is variously called RAMS (Randomly Amplified Microsatellites) (Hantula et al. 1996) and ISSR (Inter Sequence Simple Repeat). The primers for these repetitive sequences consist of a three nucleotide motif repeated four or five times, with a nonspecific base at the 3' or 5' end. The entire primer is 16 to 22 nucleotides long. Fingerprints based on these ISSR primers tend to be more repeatable than RAPDs, because they target specific repetitive sequences with longer primers.

DNA fingerprinting to determine relationship among isolates and species have been used in a number of studies of *Phytophthora*. Cooke et al. (1999) used RAPDs to compare *P. quercina* to other *Phytophthoras*, and showed that it was not only a separate species, but had a very simple population structure indicative of a newly introduced species (Brasier and Hansen 1992). RAPDs were used by Zheng and Ward (1998) to determine species and population structure among isolates of six *Phytophthora* species on rubber and citrus in China. Hantula et al. (1997) used ISSR primers to examine population structure of *P. cactorum* in Europe, and showed that isolates from strawberry had identical fingerprints; this was cited as evidence that isolates infecting strawberry may be from a single clone. ISSR primers have been used to examine genetic variation in other fungi. In *Phlebiopsis gigantea*, ISSR primers produced five bands that were common to all isolates, and 21 that were variable (Vainio et al. 1998). ISSRs were also used with *Gremmeniella abietina*, and fingerprints corresponded with previously identified races, although there was some variation within each race (Hantula and Müller 1997). RAPDs and ISSRs have also been used to determine relationships among a wide range of agricultural plants including brassicas, citrus, and grapes. Usually there is a high degree of variability among cultivars, while individual plants of a single cultivar or germ line show low or no variation.

Variation in pathogenicity and virulence among isolates of *Phytophthora* has been investigated in a number of pathosystems. Most comparisons involve inoculation of roots or stems with mycelium or zoospores, and measurement of resulting lesion length or disease index. Lilja et al. (1998) compared isolates of *P. cactorum* from birch and strawberry, and found that only birch isolates caused lesions on birch, and birch isolates did not cause disease in strawberry. Among strawberry isolates, one isolate was more pathogenic (resulted in a higher disease index) than the others, although there was no difference in RAPD fingerprints. In inoculation tests on two cultivars of *Rhododendron* (Hoitink and Schmitthenner, 1974), isolates of *P. cinnamomi* from ericacious plants were equally virulent, but some isolates from non-ericaceous plants were significantly less virulent. Linde et al. (1999) compared growth rate and pathogenicity of *P. cinnamomi* isolates from South Africa and Australia. They found significant differences in lesion length among isolates, related in part to the length of time the isolate had been in culture. The host from which the isolates were obtained did not affect level of virulence.

Limited variation has been found in previous studies of *P. lateralis*. In a comparison of sporangia production in ten isolates from Oregon, nine isolates were similar and one isolate produced fewer sporangia when grown on pea broth and then rinsed and flooded with distilled water (Trione 1957). Chlamydospore production varied greatly among isolates, and all isolates produced few oospores. Isozyme banding patterns of 11 isolates from Oregon and California were identical (Mills et al. 1991).

Hansen et al. (1986) reported on the lack of variability detected among 23 isolates of *P. lateralis* collected from throughout the range of the fungus. One isolate (627, from Winchester, OR) grew more slowly than other isolates. There were significant, but unrepeatable, differences in zoospore production, and no difference among total protein bands or isozyme bands.

Isolate #627 caused significantly shorter lesions than the other isolates tested. The authors suggest that a simple difference in growth rate could produce differences in zoospore production and virulence.

This report addresses variation among isolates of *P. lateralis*. I compared growth rate at three temperatures, virulence when inoculated on POC, and DNA fingerprint among isolates collected from Oregon, Washington, California, and British Columbia (Table 4.1). Isolates were from 3 hosts: POC, *C. nootkatensis* (Alaska yellow-cedar), and *Taxus brevifolia* (Pacific yew).

MATERIALS AND METHODS

Isolates used in this study were collected from throughout the known range of *P. lateralis* (Table 4.1). Isolates collected by McWilliams were obtained by direct isolation from the margin of necrotic tissue on symptomatic trees. Isolates from *C. nootkatensis* were from individuals growing outside the native range of the tree that had been planted in sites known to be infested with *P. lateralis*. The method of isolation for isolate 366, the French isolates, and the isolate of *P. cryptogea* is not known. These isolates were obtained from the collection housed at Oregon State University.

Isolate	Host	Origin	Date Isolated	Isolated by
366	C. lawsoniana	Shelly Cr., CA	?	Kleijunas
POC-2	C. lawsoniana	Kalmiopsis Wild., OR	Jun-94	McWilliams
YEW-2	Taxus brevifolia	Kalmiopsis Wild., OR	Jun-94	McWilliams
1-1	C. lawsoniana	Vancouver, B.C.	Oct-95	McWilliams
1-10	C. lawsoniana	Vancouver, B.C.	Oct-95	McWilliams
20-1	C. lawsoniana	Bellingham, WA	Oct-95	McWilliams
21-2	C. lawsoniana	Bellingham, WA	Oct-95	McWilliams
6187	C. lawsoniana	Johnson Mt., OR	Jun-94	McWilliams
6248	C. nootkatensis	Quosatana Cr., OR	Jun-94	McWilliams
6900	C. nootkatensis	Quosatana Cr., OR	Jun-94	McWilliams
7144	C. lawsoniana	Johnson Mt., OR	Jun-94	McWilliams
7354	C. nootkatensis	Quosatana Cr., OR	Jun-94	McWilliams
8041	C. nootkatensis	Quosatana Cr., OR	Jun-94	McWilliams
Plat1	C. lawsoniana	France	1998	Streito
980093.1	C. lawsoniana	France	1998	Streito
P. crypto.	C. lawsoniana	OR	?	?
P. cinn.	C. lawsoniana	Cottage Grove, OR	Jun-94	McWilliams

Table 4.1. Isolate number, host, origin, date isolated, and person performing isolation for *Phytophthora* isolates used in this study. P. crypto = *P. cryptogea*, P. cinn. = *P. cinnamomi.* Other isolates listed are *P. lateralis*.

Comparison of Growth Rate

Fifteen isolates of *P. lateralis* , including two from France, were transferred from storage tubes to 100 mm petri plates containing corn meal agar (Difco) amended with 20 ppm *B*-sitosterol (CMAS). After 9 days of growth at 18C, plugs were cut with a #2 cork borer and placed on 100 mm petri plates containing 20 ml of CMAS. Four isolates were also grown on clarified V8 agar to compare growth rates on different media. After 3 days of growth at 20C, the edge of the advancing colony margin was marked on 4 radii per plate, and 2 plates of each isolate were placed in each of 3

covered plastic boxes. Boxes were placed in Sorvall constant temperature incubators held at average temperatures of 12C, 18C, and 24C. The edge of the advancing colony was marked at 4, 7 and 11 days, and a growth rate per day was calculated from these measurements. The experiment was repeated once, and small electronic temperature loggers were included in each box during the second growth period. The measured average temperature in each incubator was 12.2C, 18.8C, and 24.8C during the second growth period.

Growth rates were analyzed using ANOVA (SAS Institute Inc., 1985), and differences among isolates were determined using Duncan's multiple range test.

Virulence Comparisons Using Stem Inoculation with Mycelium

Virulence of isolates of *P. lateralis* was compared in 3 tests. In the first test stem cuttings of individual four-year-old POC trees were made and rooted under mist by the U.S. Forest Service Dorena Tree Improvement Center. After root formation, cuttings were potted in 3 gallon round plastic pots in a standard soilless mix containing approximately 2 parts Douglas-fir bark, 1 part peat, and 1 part pumice and grown for an additional year with periodic fertilization with an encapsulated time release fertilizer (Nutricote™ 18:6:8 with minor elements, 6 month formulation, 1.5 Tbs per pot). Trees were moved to Corvallis, Oregon and inoculated on January 29 and 30, 1997. For analysis purposes, all cuttings from a single tree were treated as a genet.

Ten isolates of *P. lateralis* and one isolate of *P. cinnamomi* were grown for one week on CMAS. Of the isolates listed in table 4.1, the following isolates were not used: 1-10, 7144, 8041, Plat1, 980093.1, and *P.*

cryptogea. Plugs cut from the advancing edge of colonies were then transferred to pea broth (150 g dry split peas per liter, autoclaved for 3 min and strained through a double layer of cheesecloth, then autoclaved for 20 min at 121C). Colonies were grown for an addtional 7 days (5 days for *P. cinnamomi*), rinsed in deionized water, and used for inoculation.

Ramets were inoculated by cutting a longitudinal 1.5 cm slit in the bark, and placing a small weft of mycelium (approx. 1 mm^3) under the bark. Slits were then sealed with sterile petroleum jelly. Ramet number, ortet, isolate, height of inoculation, and caliper of stem at inoculation site were recorded for each tree inoculated. Mean height of inoculation was 32 cm (range 13-57), and mean caliper at inoculation site was 7 mm (range 2.5-12) Each ramet was inoculated with one isolate, and there were three replications per isolate per ortet. Eleven ortets were used, but most ortets did not have sufficient numbers of ramets to be inoculated by all 10 isolates, so one or two isolates were randomly excluded. There were a total of 341 inoculations. Total extent of necrosis was measured after 7 weeks. Lesion length was analyzed using the General Linear Model procedure (SAS Institute Inc., 1985) rather than ANOVA, because of unequal cell sizes.

Virulence Comparison Using Zoospore Inoculum

Two additional tests were carried out to compare virulence among isolates using zoospore inoculation of stems and roots. Five isolates of *P. lateralis* (366, Yew2, 1-1, 6187, and 6248) were grown for one week on CMAS. Three X 3 mm plugs cut from the advancing edge of colonies were then transferred to pea broth and grown for 7 days. Medium was then removed, plates were rinsed with deionized water, and sporangium formation and zoospore release were induced by filling plates with filtered

water from a local creek. One day later, the water containing the colonies and zoospores was poured from plates and placed in 1 quart glass jars. Water from one plate of each isolate along with an additional 40 ml deionized water were combined in each jar to an approximate depth of 1 cm.

Trees used to compare virulence of isolates were POC seedlings that had been grown for one year by the U.S. Forest Service Dorena Tree Improvement Center from open-pollinated seed collected from trees with potential resistance to *P. lateralis.*

For stem inoculation, seedlings were excised at the ground line, wrapped in plastic bags, and stored in a styrofoam box for transportation to Oregon State University. On the day following excision, 10 excised seedlings were placed in each inoculation jar containing freshly prepared zoospore suspension for 24 hours, and then were placed in 4 X 20 cm plastic tree growing tubes filled with moist perlite. The excised seedlings were held in a greenhouse and watered every other day for 20 or 21 days. Lesion length was recorded by measuring the distance between the excised end and upper limit of discoloration inside bark, and rate of lesion extension (mm/day) was calculated for the analysis. Twenty seven seedling families were used for a total of 386 inoculations.

For root inoculation, intact seedlings in 4 X 20 cm plastic tree growing tubes had projecting roots trimmed at the bottom of the container. The distal 1 cm of the seedling container was immersed in zoospore suspension, prepared as outlined above, in plastic cups for 24 hours. Seven seedlings were inoculated in each cup. Tree containers were replaced in plastic racks, and were held in a greenhouse and watered every other day until measurements were made. The time until measurement was 65 or 66 days. Lesion length was recorded by measuring the distance between the bottom of the root mass and the upper extent of discolored

roots, and rate of lesion extension (mm/day) was calculated . Twenty six tree families were used for a total of 339 inoculations. Lesion length was analyzed using the General Linear Models procedure (SAS Institute Inc., 1985).

DNA Fingerprint Analysis

DNA fingerprints were obtained from 13 isolates of *P. lateralis*, and one isolate each of *P. cinnamomi* and *P. cryptogea* (table 4.1). DNA extraction was done twice using different methods, from isolates grown in different media.

For the first extraction, isolates were grown for 10 days on CMAS. Three X 3 mm plugs were cut from the advancing edge of colonies and placed in 125 ml Erlenmeyer flasks containing approximately 50 ml of clarified V-8 juice broth (350 ml V-8 juice, mixed with 5 g$CaCO_3$, centrifuged at 2000 rpm for 20 min, 200 ml supernatant removed and brought to 1 L with deionized water and autoclaved for 20 minutes at 121C). Cultures were grown at room temperature for 15 days. The contents of each flask was then poured in a Buchner funnel with a Miracloth™ filter, vacuum was applied and colonies were rinsed with sterile deionized water. The Miracloth™ filter was removed, blotted dry on paper towels, and the mycelium scraped off and placed in an Eppendorf tube. Each tube contained 0.1g to 0.17g fresh weight of mycelium. Tubes were then lyophyllized.

DNA was extracted by placing lyophillized mycelium in a mortar with about 5 ml of liquid N_2, grinding, then adding a small amount of autoclaved sand and grinding in 7 ml of an extraction buffer containing 3% CTAB (cetyltrimethylammonium bromide), 2% NaCl, and 1% PVP (polyvinyl

propylene), 100mM Tris-HCl (tris[Hydroxymethyl]aminomethane-HCl), 20mM EDTA (ethylenediaminetetracetic acid) pH 8, and 1% 2-mercaptoethanol. Ground mycelium and buffer was placed in a 15 ml plastic conical bottom tube to a final volume of 7 ml and incubated at 37C for 3.5 hours. An equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed, then centrifuged at 7000 rpm for 5 min. The aqueous layer was removed, an equal volume of chloroform:isoamyl alcohol was added, mixed, centrifuged at 7000 rpm for 5 min, and the aqueous layer removed. This sequence was repeated one additional time. After the removal of the aqueous layer, 2/3 volume of isopropanol was added, and tubes were stored in a refrigerator for 9.5 hours. Tubes were then centrifuged at 7000 rpm for 10 min, the supernatant was removed, and 8 ml 80% ethanol added. After 5 min tubes were centrifuged at 7000 rpm for 5 min, liquid was removed, and the resulting pellet was dried in a laminar flow hood. After all ethanol had evaporated, the pellet was redissolved in 100*ul* of TE (10mM Tris-HCl, 5mM EDTA) buffer, placed in an Eppendorf tube, and stored in a -20C freezer until used for reactions.

For the second extraction, isolates were grown for 10 days on CMAS. Three X 3 mm plugs were cut from the advancing edge of colonies and placed in 125 ml Erlenmeyer flasks containing approximately 50 ml of glucose-yeast-peptone-broth. Cultures were grown at room temperature for 21 days. Mycelium was harvested as stated above.

Mycelium was placed in a 15 ml conical bottom plastic tube with three 6 mm glass beads and about 5 ml liquid N_2, and vortexed about 90 sec to grind mycelium. Seven ml of JEB buffer (100mM Tris-HCl pH 7.5, 12.5mM xanthogenate, 10 mM EDTA, 700 mM NaCl) was added and tubes were incubated at 65C for 40 min. Then 2/3 volume chloroform:isoamyl alcohol (24:1) was added, tubes were inverted to mix, and then centrifuged at 7000 rpm for 15 min. Supernatant was removed, and 70*ul* RNAse (10mg/ml)

was added and incubated at 35C for 15 min. An equal volume of chlorform:isoamyl alcohol was added, and tubes were centrifuged at 7000 rpm for 10 min. Supernatant was removed and 3.25 ml isopropanol was added, and tubes were incubated at room temperature for 19.5 hours. Tubes were centrifuged at 5000 rpm for 5 min, then 7000 rpm for an additional 5 min. Supernatant was removed, and 6 ml ice cold 70% ethanol was added, incubated for 5 min at room temperature, centrifuged for 5 min at 7000 rpm, supernatant was removed and the pellet was dried for 12 hours. The pellet was then resuspended in 60 *u*l TE buffer, and stored in a -20C freezer until used for reactions.

PCR primers for DNA fingerprints were based upon inter-simple-sequence-repeats (ISSR) and obtained from the University of British Columbia (UBC Primer Set 9) One hundred primers were screened for ability to amplify sequences and produce readable bands with *P. lateralis* DNA. Forty five primers were chosen and used to amplify DNA from 13 isolates of *P. lateralis* and one isolate each of *P. cinnamomi* and *P. cryptogea*. Amplifications were performed in 28 *u*l reaction volumes containing 1.4mM $MgCl_2$, 0.7X amplification buffer, 112*u*M dNTPs, 0.04% DMSO (dimethylsufoxide), 18 *u*g BSA (bovine serum albumin), 0.38 unit Tfl DNA polymerase (Epicentre Technologies), 0.6*u*M primer, and approximately 80 ng DNA in 2 *u*l TE buffer. Amplifications were run on an MJ Research PTC-100 thermocycler with a 1 minute initial denaturation at 94C, 40 cycles of 45 second denaturation at 94C, 30 sec annealing at 52C, and 1 min extension at 72C, followed by a final extension step of 7 minutes at 72C. Products were run on 20 X 20 cm 2% agarose gels containing 0.57*u*g ethidium bromide for approximately 1 hour at 148 volts. Gels were then visualized on an ultraviolet transilluminator, photographed, and bands were read from photographs.

Molecular weight of bands was estimated by comparison to a 100 kilobase DNA ladder, and scored for presence or absence of each band. A total of 189 different bands were scored. A distance matrix was constructed using Jaccard's similarity coefficient which only takes positive matches into account. A dendrogram was constructed from the distance matrix using the neighbor-joining algorhithm in PAUP (Swofford 1991). A genetic distance (Nei and Li, 1979) was also calculated based on the number of shared and unique bands among isolates according to the formula $D_{xy} = 1 - 2 X P_{xy}/(B_x+B_y)$, where D_{xy} is the distance between isolate X and Y (value = 0 to 1), P_{xy} is the number bands shared by X and Y, and B_x and B_y are the total number of bands corresponding to isolate X and Y, respectively.

RESULTS

Comparison of Growth Rates

There were significant differences in growth rates among isolates grown on cornmeal agar (Figure 4.1). For each specific temperature or time period there were at least two and as many as four groups of isolates that differed significantly from the other groups. Although relative growth rates of most isolates differed among temperatures and growth period, two isolates (980093.1 and 20-1) were always among the slowest growing, and Yew2 was usually among the fastest. Some isolates seemed able to tolerate 24C, while other isolates had reduced growth rate at that temperature. Most isolates grew faster at 24C than 18C during the 0 to 4 day time period, and slower at 24C during the 4 to 7 day time period. This was probably due to drying of the medium or nutrient depletion as the test progressed.

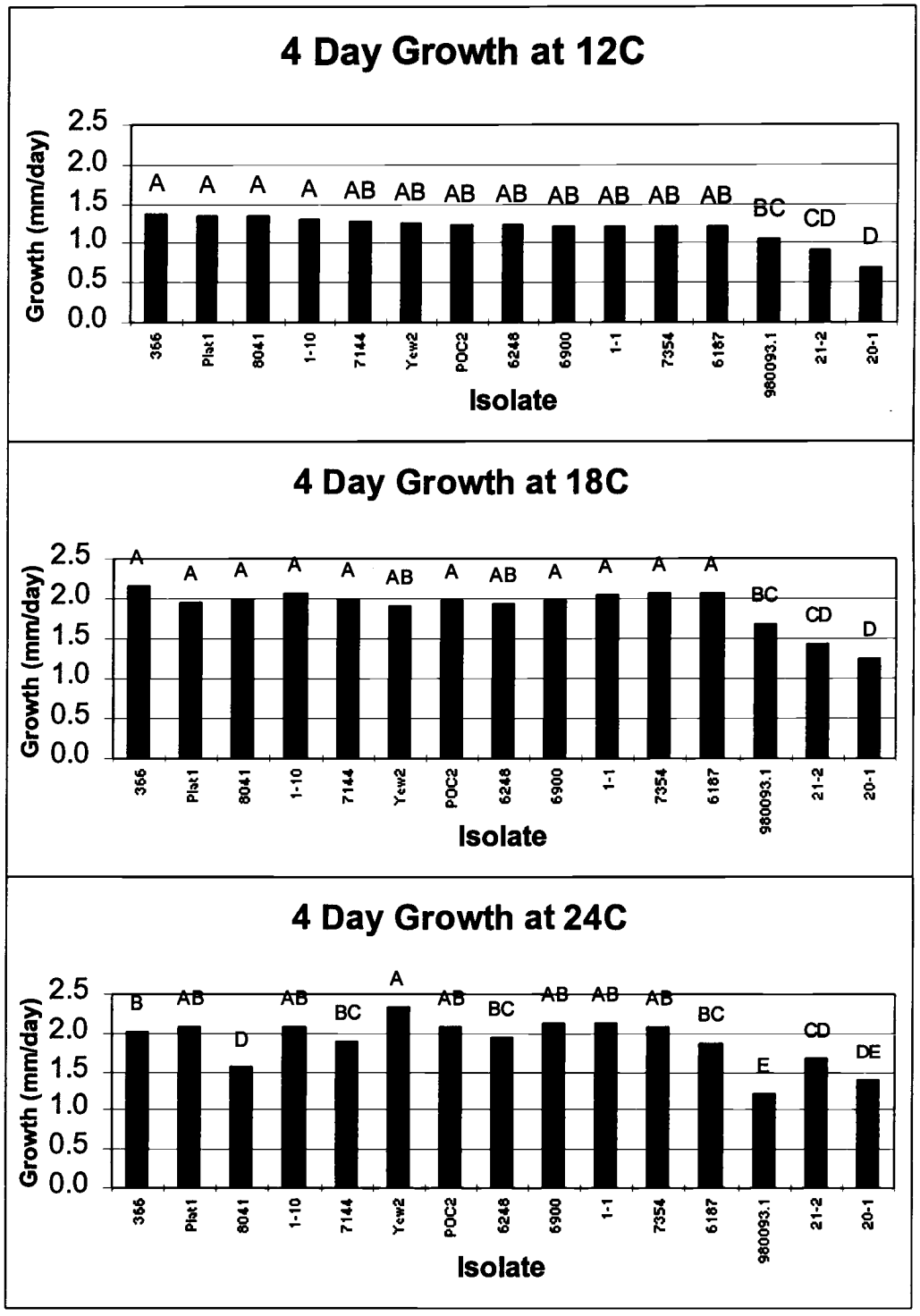

Figure 4.1. Comparison of growth rates of 15 isolates of *P. lateralis* at three temperatures on cornmeal agar. Different letters above bars indicate significant (p=0.05) differences between growth rates.

There was less variation among growth rates for four isolates when grown on V8 agar than when grown on CMAS (Figure 4.2). Growth rate of Yew2 was significantly slower at 12C on both media, no isolates were significantly different at 18C, and Yew2 was significantly faster at 24C. All isolates maintained their relative rank on both media, but growth rate on V8 was nearly twice as fast as on CMAS.

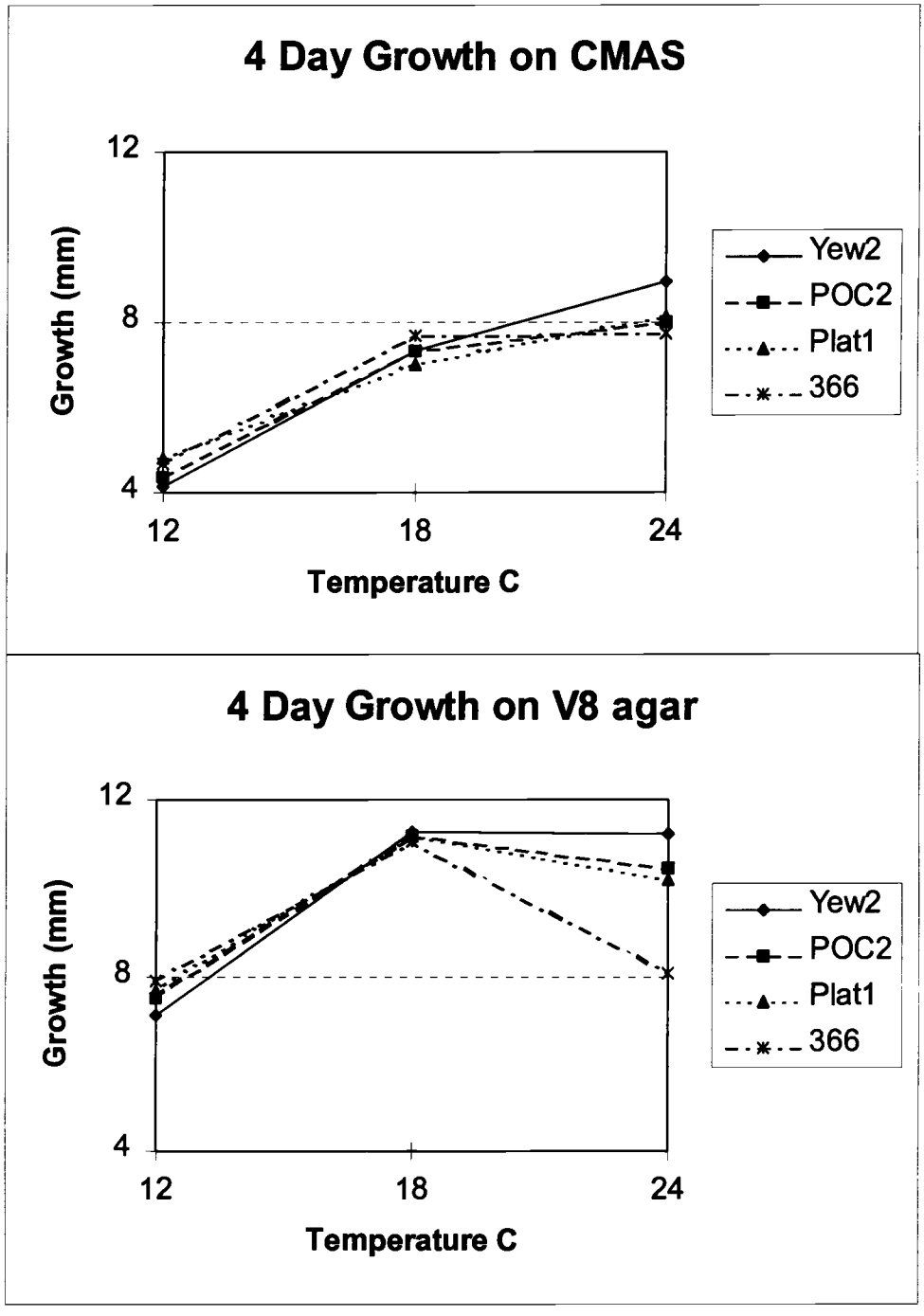

Figure 4.2. Comparison of growth rate of four isolates on two media.

Comparisons of virulence among isolates

Lesion length differed significantly among isolates in both inoculation tests (Figure 4.3). In the test using stem inoculation with mycelium, one isolate(Yew2) caused significantly shorter lesions than other isolates (Figure 4.3). All *P. lateralis* isolates caused significantly longer lesions than the one isolate of *P. cinnamomi* tested. In the tests using zoospore inoculum on roots, Yew2 caused significantly shorter lesions than other isolates tested (Figure 4.4). On excised stems Yew2 caused the shortest lesions, although these were not significantly different from 2 other isolates. The differences among other isolates, although statistically significant, were small. There was no evidence of isolate by family or isolate by ortet interactions. There were more marked differences among isolates in the root inoculation than in either of the stem inoculation tests.

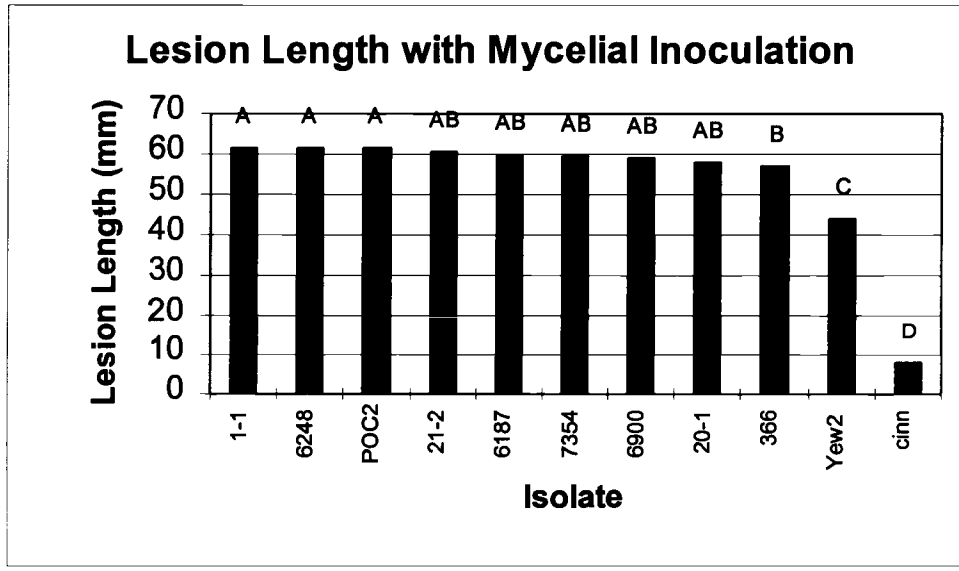

Figure 4.3. Comparison of lesion length among isolates of *P. lateralis* and *P. cinnamomi* with mycelial inoculation of stems. Different letters above bars indicate significant (p=0.05) differences between lesion lengths.

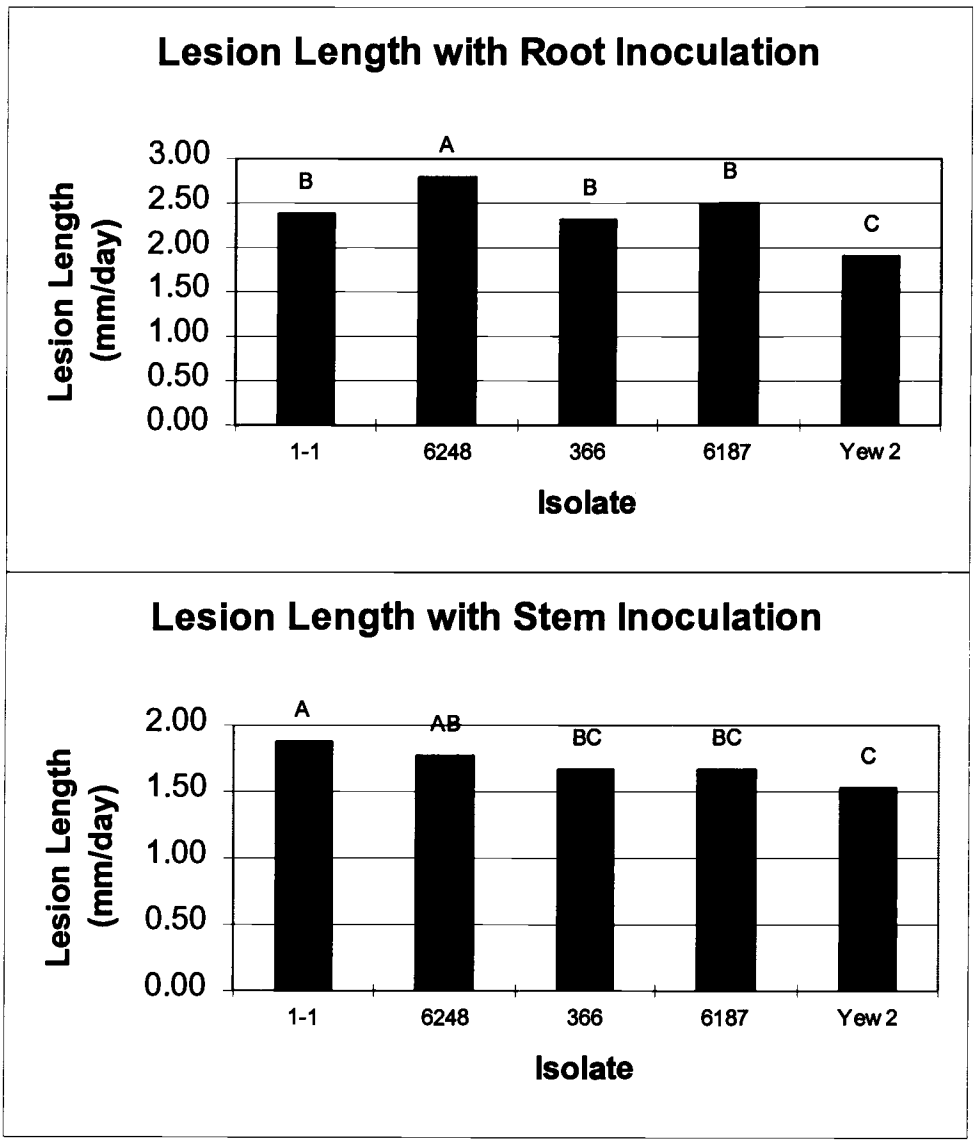

Figure 4.4. Comparison of lesion length with stem and root inoculation using zoospores. Different letters above bars indicate significant (p=0.05) differences between lesion lengths.

Comparison of DNA fingerprints among isolates

All *P. lateralis* isolates had nearly identical banding patterns. There were only two polymorphic bands. The isolates formed two groups: all those that had a missing band with primer 808 also had a missing band with primer 835. These two primers have similar sequences (808 = AGA GAG AGA GAG AGA GC, and 835 = AGA GAG AGA GAG AGA GYC with Y being either C or T), and may serve as primers for the same repeated motif. There was no association between latitude of host or host species and banding pattern. Isolates from adjacent trees in a location (1-1 and 1-10, 20-1 and 21-2, POC-2 and Yew2) had identical banding patterns, indicating the grouping was not an artifact. Isolates from Flannigan and Quosatana had some members in each group. The *P. lateralis* isolates had no bands in common with the *P. cinnamomi* or *P. cryptogea* isolates. This caused all *P. lateralis* isolates to group together very closely when analyzed by the neighbor joining algorithm of PAUP (Figure 4.5). DNA from the two French isolates was amplified with 8 primers and had fingerprints identical to the North American isolates, with one of the French isolates in each group (data not shown). Genetic distance between the two groups of North American *P. lateralis* is 0.0103, based on 96 ISSR bands in common, and two that were polymorphic.

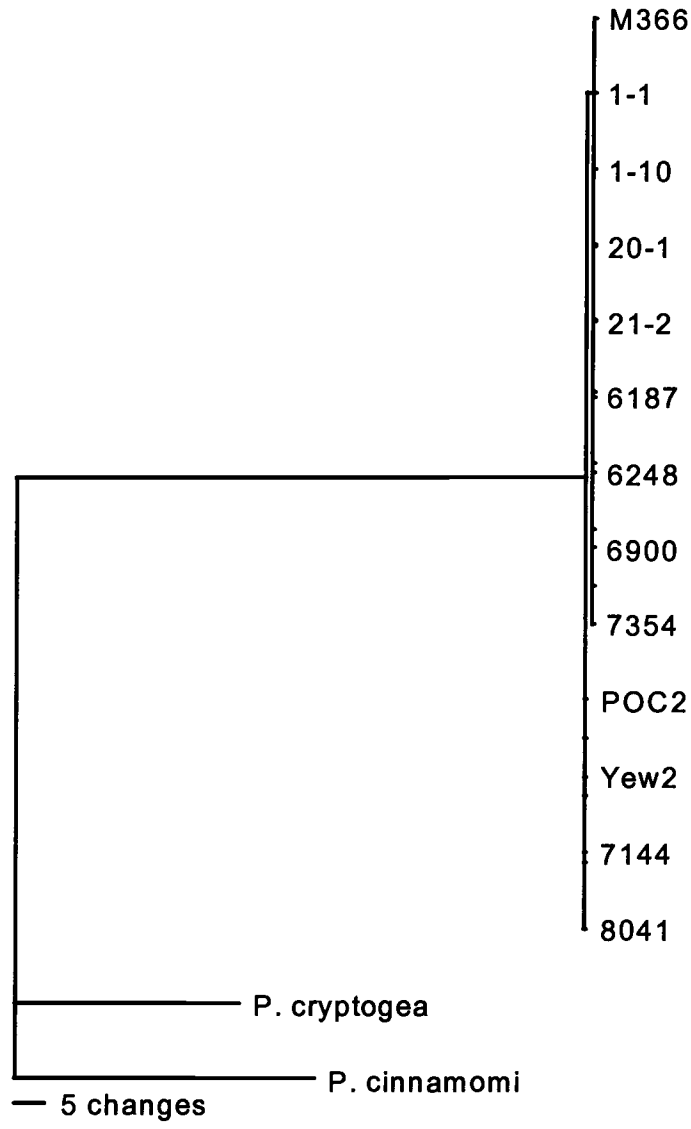

Figure 4.5. PAUP neighbor joining dendrogram showing relationship among isolates of *P. lateralis* based on ISSR polymorphisms. Isolates POC2, Yew2 7144 and 8041 form the group missing a band.

DISCUSSION

The homoogeneity among isolates of *P. lateralis* is remarkable. Although some differences in growth rate on a weak artificial medium (CMAS) were detected, the importance of growth rate on artificial media is questionable. Such differences may be due to such factors as age of culture, storage conditions, and stochastic factors as much as genetic factors affecting potential growth rate. There was much more variation in growth rate on cornmeal agar than on V-8 agar. In *P. cinnamomi,* growth rate on PDA was positively correlated with virulence in field inoculation tests (Linde et al. 1999). Isolates that had been in culture for longer periods had slower growth rates and caused shorter lesions than isolates that had been more recently isolated. In contrast, Robin and Desprez-Loustau (1998) found no relationship between lesion length and age of culture. This correlation between growth rate *in vitro* and lesion length is not the case with *P. lateralis*. The isolate that caused the shortest lesions (Yew2) in three inoculation tests was among the fastest growing isolates at all temperatures.

The near uniformity of ISSR profiles among all isolates indicates an extremely low level of genetic variation. These data, combined with the previously published uniformity of isozyme profiles (Mills et al. 1991), and the complete homology of ITS1 sequences among four isolates (Hansen et al. 1999), suggest very limited genetic variability in *P. lateralis*. Other studies using ISSR or RAMS amplification have shown considerable variation among isolates of *P. cactorum* in Europe (Hantula et al. 1997) as well as among isolates of other fungi (Hantula and Muller 1997, Vainio et al. 1998). When lack of variation was found among isolates of *P. cactorum* from strawberry, it was said to suggest a single clone on that host (Hantula et al. 1997).

Uniformity in genetic structure is characteristic of an introduced pathogen. Cooke et al. (1999) suggest that the uniformity of RAPD patterns among isolates of *P. quercina* from Europe is indicative of a fungus exhibiting clonal spread, or a recent introduction from outside Europe. Linde et al. (1999) found limited genotypic diversity in isolates of *P. cinnamomi* in South Africa and Australia when compared to genotype diversity in Papua New Guinea, and took this as evidence that the fungus had been introduced to both Africa and Australia. Furnier et al. (1999) found uniformity in RAPD fragments among isolates of *Sirococcus clavigignenti-juglandacearum* in North America, and suggested this was consistent with the fungus having been introduced as a single isolate. The genetic uniformity found in *P. lateralis*, combined with the extreme susceptibility of the host, is further evidence that this fungus has been introduced to the range of Port-Orford-cedar.

Given the extreme genetic uniformity of this fungus, it is interesting that there are significant differences in virulence among isolates on the primary host. In well controlled experiments only one isolate shows decreased virulence, even though this particular isolate was among those with the fastest growth rates at most temperatures tested. This difference in virulence may be due to attenuation because of storage conditions or other factors, or some change caused by growing in Pacific yew. One study showed low levels of Taxol inhibited growth of several *Phytophthora* species, but when isolates were transferred to Taxol-free medium normal growth resumed (Wagner and Flores, 1994). Whether Taxol or some other substance in Pacific yew might have an irreversible effect on isolate virulence is unknown. More isolates from yew should be tested for pathogenic ability to determine whether this phenomenon is common.

The existence of variation in virulence suggests that isolates of known pathogenic ability should be chosen when performing tests to detect

resistance in Port-Orford-cedar. The differences among isolates, when roots and shoots are exposed to zoospore inoculum, may be due to differences in the susceptibility of roots and stems, differences in host mechanisms to limit growth in the different plant tissues, variations in the inoculation technique, or variations in the number of zoospores in the inoculum. In testing *Eucalyptus* for resistance to *P. cinnamomi,* family rankings based on survival in field plantings and lesion length with stem inoculation tests were equal, and stem tests were recommended for screening for resistance (Stukely and Crane, 1994). Given the greater variation among isolates using a root inoculation, and the difficulty of measuring lesions on root systems, stem inoculation using isolates of known pathogenic ability is most likely to yield reproducible and reliable results. However, the correlation between stem lesion length and survival in infested sites must be determined.

Chapter 5 Discussion, Conclusions, and Recommendations

Forest disease management requires the use of a wide array of techniques. Approaches used to manage native and introduced diseases are often different, but both require knowledge of the biology of the pathogen and the host. This dissertation provides information concerning key areas of both host and pathogen biology, as well as techniques for manipulation of the host, that is needed for the successful management of the introduced root disease caused by *P. lateralis*.

Management for native diseases often centers on modifying silvicultural treatments to avoid growing susceptible species in areas likely to result in high losses to disease. Native diseases have undergone coevolution with their hosts over long periods of time, and this has often resulted in a degree of tolerance or resistance on the part of the host (Mack et al. 2000, Brasier 1986) or relatively low levels of virulence in the pathogen (Brasier 1986, Harlan 1976). Not all individuals of the host species are killed, but rather the host survives in areas of lower disease risk, or some factor restricts the movement of disease to all susceptible hosts. Silvicultural treatments can capitalize on these disease avoidance and tolerance mechanisms to reduce losses. For example, in the Pacific northwest losses to *Phellinus weirii* are often reduced by first conducting surveys to determine where the disease is present, and then planting resistant species in these areas (Thies 1984). Douglas-fir and the true firs are very susceptible to *P. weiriii*, but other species such as western redcedar or western white pine often grow well, provide adequate economic return, and maintain conifer cover in disease centers. This approach is successful with *P. weirii* because spread of the fungus is primarily vegetative, areas infested by the disease are fairly easy to detect, and susceptibility of conifer hosts is well established (Nelson and Sturrock 1993). Another native disease successfully managed

through silvicultural modifications is the root disease caused by *Heterobasidion annosum* (Otrosina and Cobb 1989). This disease is of greatest concern in drier pine or mixed conifer stands in the Pacific Northwest and California. Although the disease does spread vegetatively, spore spread is much more important. Silvicultural management stresses both detection of infested areas, and reduction of infection courts for the spores of the fungus. Fresh stumps and wounds near the bases of trees are important infection courts. Logging and thinning in stands at risk for the disease seeks to minimize bark damage on residual trees, and stump tops can be effectively protected by an application of borate or an antagonistic fungus.

Management of introduced disease often requires different strategies. Development of disease resistance is often important. Extreme susceptibility of the host and extreme virulence of the pathogen may occur when a host and pathogen are introduced for the first time (Harlan 1976). The mortality of native trees when chestnut blight and white pine blister rust were introduced to North America illustrate the destructive capacity of introduced forest diseases. The efforts to reduce losses to white pine blister rust, caused by *Cronartium ribicola*, are illustrative. This fungus was introduced to the east coast of the U.S. in the early 1900's, and was also introduced to Vancouver B.C. in 1910. This fungus has airborne spores that infect the *Ribes* alternate host. Spores produced on *Ribes* then infect pine. Early control effort focused on eradication of the *Ribes* alternate host, but this ultimately proved unsuccesful (Benedict 1981). Breeding for white pine resistance has met with greater success, and F2 resistant white pines are now available for outplanting (Bingham 1983). Although there is strong single-gene resistance available, the emphasis has been on developing horizontal or polygenic resistance. Because the fungus has a sexual cycle,

it is constantly evolving, and there is concern that resistance may be overcome.

Development of white pine resistance has included a number of separate steps, all of which contribute to an effective breeding program, and that are applicable to a breeding program for resistance to *P. lateralis*. Collection of phenotypically resistant white pine in the field continues, and this plant material is grafted to rootstocks to enable breeding and seed production in seed orchards. Heritability of the various resistance mechanisms in pine has been established. Inoculation and testing of white pine continues with each new generation produced in the seed orchard. Monitoring of genotypes of the fungus present in the field has enabled resistance testing with new fungal genotypes as they appear.

Development of disease resistance requires a method to preserve and maintain phenotypically resistant individuals. The genetic basis of resistance, and heritability of the trait, must be demonstrated to make breeding successful. Reliable techniques to test for resistance to the fungus need to be available to screen selections for inclusion in a breeding program. Knowledge of the genetics and variation in virulence of *P. lateralis* is needed to choose appropriate isolates for screening, as well as to plan deployment and predict the durability of resistant Port-Orford-cedar. These elements have been investigated in the work documented in this dissertation.

To enable breeding for resistance, and produce sufficient seed for reforestation, Port-Orford-cedar needs to be grown in a seed orchard. Trees surviving in areas of *P. lateralis*-caused mortality are currently preserved in two ways. Seed has been collected from trees bearing cones, and branches have been collected and cuttings rooted from these. Individual trees from open pollinated seed have an unpredictable level of resistance, and until individual trees are tested from families showing

resistance their utility in a breeding program is not known. Rooted cuttings from previously tested trees have a more predictable level of resistance, but in nearly all cases are still susceptible to *P. lateralis*. The current operational seed orchard is grown in pots and consists of very young (2 to 4 year old) trees at the Dorena Tree Improvement Center. Although seed production can be induced early in Port-Orford-cedar, it seems probable that trees will need to be grown in the ground in a grafted seed orchard. In a seed orchard the hazard of introduction of *P. lateralis* and subsequent loss of genetic resources increases. The work documented here has shown nearly 70% graft success in homoplastic Port-Orford-cedar grafts when done in a fairly uncontrolled, outdoor location. This percentage could probably be increased by maintaining newly grafted trees in a greenhouse with high humidity and lower light levels, as is done in the ornamentals industry. If highly resistant individuals, such as Coldframe 1 from OSU or other resistant clones, were used as rootstock for the grafted orchard, the hazard of *P. lateralis* mortality in the orchard could be mitigated. This work has also shown 25% graft success on incense cedar rootstock, and although signs of incompatibility were usually present the scion did not die during the 31 month test period. Using incense cedar as a rootstock, and a search for more graft compatible families of incense cedar, would enable preservation of Port-Orford-cedar genotypes on a rootstock immune to *P. lateralis*.

To make breeding for resistance a possibility, resistance must have a genetic basis, and be a heritable trait. I have shown that family means for lesion length are significantly different among various Port-Orford-cedar families. This demonstrates that lesion length after inoculation is under genetic control. The variance attributable to family in experiments using different inoculation methods ranged from 3 to 32 percent of the total variance in the experiment, with most inoculation methods showing 21 to

32 percent of the total variance due to family. These variance estimates produce very high heritability estimates, indicating breeding for resistance has a good chance of producing resistant trees if highly resistant parents are selected. The very low variance attributable to family in the experiment using stem inoculation with zoospores is probably due to differences in inoculum density, temperature, and other uncontrolled variables. The fact that with rooted cuttings 74% of the variance is due to clone again shows that most variation in lesion length is due to the genetics of the host.

Initial selection in a breeding program often focuses on resistant families. Port-Orford-cedar seedlings from a single parent possess nearly continuous levels of resistance. In chapter 3 it was shown that a family demonstrating the most resistance on a family mean basis included very susceptible individuals. After selection of resistant families, testing should focus on individual seedlings from those families to confirm resistance before choosing trees to incorporate in a breeding program.

The difference in variance components between stem dip and root inoculation with zoospores needs further clarification, especially if either of these is to become the operational inoculation method for screening. Additionally, the correlation between lesion length and field resistance needs clarification. Both these problems could be addressed with a simple experiment. I propose growing 120 trees from four to six open pollinated families. After one year of growth, seedlings would be randomized, and 30 seedlings of each family would be exposed to *P. lateralis* in four different ways. Root systems and excised stems would be inoculated with zoospores as stated in chapter 3, but with very careful control of inoculum density and environmental variables. Trees should be inoculated on the same day, with quantified inoculum of one *P. lateralis* isolate. If possible, stems and roots should be inoculated in a single large container using zoospores free of mycelium, to eliminate variation among inoculation

vessels. An additional 30 trees from each family should be inoculated using the method of inserting mycelium under the seedling bark. Inoculum could be controlled in this method by growing *P. lateralis* on filters placed on nutrient medium, and then cutting filters into uniform pieces for insertion under the bark, using the same isolate used for producing zoospores. The inoculated seedlings, roots and excised stems should then be held until measurement on adjacent benches of the same greenhouse, to restrict variation in environment during symptom development. Thirty seedlings from each family should also be outplanted to an infested coldframe at Cordley Hall. Lesion length comparisons among the inoculated families would show the relationship between the artificial inoculation methods, and correlations with period of survival in the coldframe would give an indication of the relationship between lesion length and survival time. This experiment should be repeated twice, with a fall and early spring inoculation when greenhouse temperatures can be maintained below the lethal temperature of 25C for *P. lateralis*. The experiment could be strengthened by including a set of trees outplanted to an infested field site, or doing the same experiment with rooted cuttings from individual trees if sufficient cuttings could be produced. Less variation in reaction is present among rooted cuttings, so the numbers of trees inoculated would not have to be as great as when using seedlings, although the variable root morphology of rooted cuttings might be a confounding factor.

The very limited variability in virulence and DNA fingerprints documented in this dissertation is an indication that resistance, once developed, will be durable. Many resistance breeding programs, such as those for stem rust resistance in wheat or blister rust resistance in white pine, are faced with constantly changing fungal genotypes. The fact that variation in *P. lateralis* is so restricted, and that the fungus is homothallic and therefore has limited opportunity for genetic recombination, provide a

stable fungus for resistance development. The only difference in virulence detected was reduced virulence, rather than a more aggressive isolate. A limited number of isolates were thoroughly tested in the work documented here, and more testing of isolates, particularly isolates from *Taxus brevifolia,* to confirm the limited variation in virulence is needed. Additional testing of more isolates, using other DNA fingerprinting techniques, could also confirm the uniformity of genotypes found here. It would be wise to discover the origin of *P. lateralis* and assess the variation present in those populations, so extraordinary measures could be taken to avoid introduction of other genotypes of the fungus that may carry additional virulence capabilities.

The work documented in this dissertation has provided several of the essential pieces of information needed to progress with a successful breeding program. I have demonstrated techniques that could be used to preserve and grow candidate trees in a seed orchard, shown the genetic basis for resistance and heritability estimates, and shown the very limited variation present in the fungus that resistant trees will encounter in the field. This information, combined with ongoing efforts to refine testing techniques and correlate these results with field resistance, should enable efficient selection for resistance.

A breeding program should concentrate on individual tree selection. The efforts of the U.S. Forest Service, Bureau of Land Management, and others to select and preserve surviving trees in areas of mortality should continue. Branch dip inoculation with zoospores or stem inoculation with mycelium enable rapid testing of candidate trees, but inoculum should be quantified and testing should only take place in fall and spring when greenhouse temperatures are moderate. Once promising trees are identified they should be recollected and preserved as rooted cuttings and scions on resistant Port-Orford-cedar or other rootstocks. Seed collected

from resistant trees in the wild is not as useful because of the continuous variation that is present in open pollinated families.

In parallel with the collection of resistant trees, efforts should continue to refine resistance testing techniques. Studies to determine mechanisms of resistance might yield techniques to more efficiently screen for resistance, but if a correlation between an artificial inoculation technique and field resistance can be shown then mechanisms are not as immediately important.

Good progress in being made at the Dorena Tree Improvement Center to develop techniques to induce cone production and effect cross breeding in young trees. Testing of crosses from previously tested individual trees will yield information about segregation of resistance characters, and possibly give an indication of the number of genes involved. However, resistance is likely to be polygenic as suggested by the continuous variation within a family, so an accumulation of resistance genes rather than the selection for one or a few genes is the more likely scenario.

Resistant trees, although tested in high and moderate hazard sites, should probably be deployed operationally to low hazard sites. Very few, if any, Port-Orford-cedar are immune to the disease, and if challenged with sufficient inoculum, will probably become infected. Experience with white pine has shown that when resistant trees are planted at sites with high *Ribes* populations and a moist environment, resistance is often overcome. Even though Port-Orford-cedar is an important riparian species, planting trees near infested streams will probably result in mortality and should be avoided.

The current "common garden" study being conducted by the U.S. Forest Service and others was initiated to examine silvicultural characteristics of the species, such as cold tolerance, seasonal growth patterns, and variation in other growth parameters. Although this effort will

yield useful information on the growth of the species, genetic structure of populations, and seed transfer guidelines, it is unlikely to aid in the search for resistance. Because trees were not selected on the basis of exposure to *P. lateralis* and survival, the level of resistance is generally low in this set of families. Continued selection of phenotypically resistant individuals within each of the areas occupied by Port-Orford-cedar, combined with the results of the common garden study, will yield trees both suited to particular environments as well as resistant to disease. Other growth parameters are relatively unimportant in the face of a disease as deadly, and moved as easily, as that caused by *P. lateralis.*

Bibliography

Abad, Z.G., T. Cresswell, R.K. Jones, and H.D. Shew. 1994. Occurrence of *Phytophthora* species on various hosts in North Carolina. Plant Disease 78:830.

Atkinson, R.G. 1965. *Phytophthora* species inciting root rot of *Chamaecyparis Lawsoniana* and other ornamentals in coastal British Columbia. Canadian Journal of Botany 43:1471-1475.

Beaver, J., and H. Mathers. 2000. Rootstock selection for grafting conifers. The Digger, 44:37-43.

Benedict, W.V. 1981. History of white pine blister rust control- a personal account. U.S.D.A. Forest Service, FS-355. 47p.

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. U.S.D.A. Forest Service, Intermountain Forest and Range Experiment Station, General Technical Report INT-146. 45p.

Bolkan, H.A. 1985. A technique to evaluate tomatoes for resistance to *Phytophthora* root rot in the greenhouse. Plant Disease 69:708-709.

Brasier, C.M. 1986. The population biology of Dutch elm disease: its principal features and some implications for other host-pathogen systems. Advances in Plant Pathology 5:53-118.

Brasier, C.M., and E.M. Hansen. 1992. Evolutionary biology of *Phytophthora*, Part II: Phylogeny, speciation, and population structure. Annual Review of Phytopathology 30:173-200.

Buchholz, T. 1997. Personal communication. June 1995.

Buley, N. 1983. Seven Oregon nurserymen share their grafting and budding techniques. American Nurseryman, Mar 1, 1983.

Butcher, T.B. and M.J.C. Stukely. 1997a. Field response of *Pinus radiata* selected for resistance to *Phytophthora cinnamomi*. Forest Research Institute Bulletin 203:250-251. Rotorua, New Zealand.

Butcher, T.B., and M.J.C. Stukely. 1997b. Variation in natural populations of *Pinus radiata* in resistance to *Phytophthora cinnamomi*. Forest Research Institute Bulletin 203 :105-107. Rotorua, New Zealand.

Butcher, T.B., M.J.C. Stukely, and G.W. Chester. 1984. Genetic variation in resistance of *Pinus radiata* to *Phytophthora cinnamomi*. Forest Ecology and Management 8:197-220.

Campbell, W.A., and A.F. Verrall. 1963. *Phytophthora cinnamomi* associated with Lawson cypress mortality in Louisiana. Plant Disease Reporter 47:808.

Carson, S.D., and M.J. Carson. 1989. Breeding for resistance in forest trees- a quantitative genetic approach. Annual Review of Phytopathology 27:373-395.

Clancy, K.J., and J.A. Kavanagh. 1978. *Phytophthora eriugena* a new pathogen on Lawson cypress. Abstracts, 3rd International Congress of Plant Pathology, Munich, Aug 10-23, 1978.

Cooke, D.E.L., T. Jung, N.A. Williams, R. Schubert, G. Bahnweg, W. Oβwald, and J.M. Duncan. 1999. Molecular evidence supports *Phytophthora quercina* as a distinct species. Mycological Research 103:799-804.

Copes, D. 1970. Initiation and development of graft incompatibility symptons in Douglas-fir. Silvae Genetica 19:101-107.

Copes, D.L. 1980a. Anatomical symptoms of graft incompatibility in *Pinus monticola* and *P. ponderosa*. Silvae Genetica 29:77-82.

Copes, D.L. 1980b. Development of internal graft incompatibility symptoms in *Pinus radiata* D. Don. New Zealand Journal of Forestry Science 10:367-380.

Copes, D.L. 1989. Bark scoring problem grafts in five Douglas-fir seed orchards: a case history. U.S.D.A. Forest Service, Pacific Northwest Research Station Research Note PNW-RN-487.

De Bary, A. 1876. Researches into the nature of the potato-fungus *Phytophthora infestans*. Journal of the Royal Agricultural Society, England. 12:239-269.

DeNitto, G.A. 1991. First report of *Phytophthora lateralis* on Pacific Yew. Plant Disease 75:968.

Dolan, T.E., and M.D. Coffey. 1986. Laboratory screening technique for assessing resistance of four avocado rootstocks to *Phytophthora cinnamomi*. Plant Disease 70:115-118.

Dorrance, A.E., and D.A. Inglis. 1997. Assessment of greenhouse and laboratory screening methods for evaluating potato foliage for resistance to late blight. Plant Disease 81:1206-1213.

Edwards, S.W. 1983. Cenozoic history of Alaskan and Port Orford *Chamaecyparis* cedars. Ph.D Thesis, University of California, Berkeley.

Erwin, D.C., and O.K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, Minnesota. 562p.

Furnier, G.R., A.M. Stolz, R.M. Mustaphi, and M.E. Ostry. 1999. Genetic evidence that butternut canker was recently introduced into North America. Canadian Journal of Botany 77:783-785.

Gnose, C.E., and D.L. Copes. 1975. Improved laboratory methods for testing graft compatibility in Douglas-fir. U.S.D.A. Forest Service, Pacific Northwest Forest and Range Experiment Station Publication PNW-265, Portland OR.

Gordon, D.E. 1974. The Importance of Root Grafting in the Spread of *Phytophthora* Root Rot in an Immature Stand of Port-Orford-cedar. Thesis, Master of Science, Oregon State University, Corvallis, Oregon.

Gordon, D.E., and L.F. Roth. 1976. Root grafting of Port-Orford-cedar- an infection route for root rot. Forest Science 22:276-278.

Graham, J.H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology 85:111-117.

Hampson, M.C. 1992. Some thought on the demography of the great potato famine. Plant Disease 76:1284-1286.

Hansen, E.M., and P.B. Hamm. 1986. Screening Port-Orford-cedar for resistance to *Phytophthora lateralis*. Final Report., P.O. No. 40-04H1-60018N, USDA Forest Service, Region 6.

Hansen, E.M., and P.B. Hamm. 1996. Survival of *Phytophthora lateralis* in infected roots of Port-Orford-cedar. Plant Disease 80:1075-1078.

Hansen, E.M., D.J. Goheen, E.S. Jules, and B. Ullian. 2000. Managing Port-Orford-cedar and the introduced pathogen *Phytophthora lateralis*. Plant Disease 84:4-14.

Hansen, E.M., J.-C. Streito, and C. Delatour. 1999. First confirmation of *Phytophthora lateralis* in Europe. Plant Disease 83:587

Hansen, E.M., P.B. Hamm, and L.F. Roth. 1989. Testing Port-Orford-cedar for resistance to *Phytophthora*. Plant Disease 73:791-794.

Hansen, E.M., P.B. Hamm, C.G. Shaw III, and P.E. Hennon. 1988 *Phytophthora drechsleri* in remote areas of Southeast Alaska. Transactions of the British Mycological Society 91:379-384.

Hantula, J., and M.M. Müller. 1997. Variation within *Gremmeniella abietina* in Finland and other countries as determined by random amplified microsatellites (RAMS). Mycological Research 101:169-175.

Hantula, J., A. Lilja, and P. Parikka. 1997. Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe. Mycological Research 101:565-572.

Hantula, J., M. Dusabenyagasani, and R.C. Hanelin. 1996. Random amplified microsatellites (RAMS)- a novel method for characterizing genetic variation within fungi. European Journal of Forest Pathology 26:159-166.

Harlan, J.R. 1976. Diseases as a factor in plant evolution. Annual Review of Phytopathology 14:31-51.

Harvey, R.D., Jr., J.S. Hadfield, and M. Greenup. 1985. Port-Orford-cedar root rot on the Siskiyou National Forest in Oregon. Office Report, U.S.D.A. Forest Service, Forest Insect and Disease Management, Pacific Northwest Region, Portland, Oregon. February 1985.

Harris, J.A., Kassaby, F.Y., Smith, I.W. and G.C. Marks. 1983. Intra-specific variation in resistance to *Phytophthora cinnamomi* in *Eucalyptus regnans*. Australian Plant Pathology 12:20-22.

Hoitink, H.A.J., and A.F Schmitthenner. 1974. Relative prevalence and virulence of *Phytophthora* species involved in Rhododendron root rot. Phytopathology 64:1371-1374.

Hunt, J. 1957. Early history of Port-Orford-cedar root rot. Office Report, U.S.D.A. Forest Service, Division of Forest Disease Research, Portland, Oregon.

Hunt, J. 1959a. *Phytophthora lateralis* on Port-Orford-cedar. Office Report, U.S.D.A. Forest Service, Division of Forest Disease Research, Portland, Oregon. Jan. 5, 1959.

Hunt, J. 1959b. *Phytophthora lateralis* on Port-Orford-cedar. U.S.D.A. Forest Service, Pacific Northwest Forest and Range Experiment Station Research Note 172, 6 p.

Hunt, R.S., and H.J. O'Reilly. 1984. Evaluation of control of Lawson cypress root rot with resistant root stocks. Canadian Journal of Plant Pathology 6:172-174.

Jayawickrama, K.J.S., J.B. Jett, and S.E. McKeand. 1991. Rootstock effects in grafted conifers: a review. New Forests 5:157-173.

Kliejunas, J. 1992. Soil monitoring for *Phytophthora lateralis* at Cedar Rustic Campground, Gasquet Ranger District, Six Rivers National Forest. U.S.D.A. Forest Service, Pacific Southwest Region, Report No. R92-03.

Lamb, J.G.D., J.C. Kelly, and P. Bowbrick. 1975. Nursery Stock Manual. Grower Books, London.

Large, E.C. 1940. The Advance of the Fungi. Henry Holt and Company, New York.

Lilja, A., R. Karjalainen, P. Parikka, K. Kammiovirta, and H. Nuorteva. 1998. Pathogenicity and genetic variation of *Phytophthora cactorum* from silver birch and strawberry. European Journal of Plant Pathology 104:529-535.

Linde, C., G.H.J. Kemp, and M.J. Wingfield. 1999. Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*. European Journal of Plant Pathology 105:231-239.

Mack, R.N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689-710.

Milbrath, J.A. 1940. A *Phytophthora* disease of *Chamaecyparis*. Phytopathology 30:788.

Milbrath, J.A., and F.P. McWhorter. 1938. Preliminary recommendations for control of the root and crown disease of cypress. Oregon Agricultural Experiment Station, Circular of Information 187. 2p.

Millar, C.I. and K.A. Marshall. 1991. Allozyme variation of Port-Orford-cedar (*Chamaecyparis lawsoniana*): Implications for genetic conservation. Forest Science 37:1060-1077.

Mills, S.D., H. Forster, and M.D. Coffey. 1991. Taxonomic stucture of *Phytophthora cryptogea* and *P. drechsleri* based on isozyme and mitochondrial DNA analyses. Mycological Research 95:31-48.

Murray, M.S. 1996. Susceptibility of Pacific Yew (*Taxus brevifolia* Nutt.) to *Phytophthora lateralis*. Thesis, Master of Science, Oregon State University, Corvallis, Oregon.

Murray, M.S., and E.M. Hansen. 1997. Susceptibility of Pacific yew to *Phytophthora lateralis*. Plant Disease 81:1400-1404.

Nei, M., and W-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, U.S.A. 76:5269-5273.

Nelson, E.E. 1964. *Phytophthora* root disease of Port-Orford-cedar 1964 survey findings. Office Report, U.S.D.A. Forest Service, Pacific Northwest Forest and Range Experiment Station, June 12, 1964.

Nelson, E.E., and R.N. Sturrock. 1993. Susceptibility of western conifers to laminated root rot (*Phellinus weirii*) in Oregon and British Columbia field tests. Western Journal of Applied Forestry 8:67-70.

Ostrofsky, W.D. 1975. Survival of *Phytophthora lateralis* in an organic matter fraction of the soil. Thesis, Master of Science, Oregon State University, Corvallis, Oregon.

Ostrofsky, W.D., R.G. Pratt, and L.F. Roth. 1977. Detection of *Phytophthora lateralis* in soil organic matter and factors that affect its survival. Phytopathology 67:79-84.

Otrosina, W.J., and F.W. Cobb. 1989. Biology, ecology, and epidemiology of *Heterobasidion annosum*. U.S.D.A. Forest Service, Pacific Southwest Forest and Range Experiment Station, General Technical Report PSW-116:26-33.

Robertson, G.I. 1982. Kiwi fruit can tolerate *Phytophthora*, but not 'wet feet'. Orchardist of New Zealand 55:148-152.

Robin, C., and M.-L. Desprez-Loustau. 1998. Testing variability in pathogenicity of *Phytophthora cinnamomi*. European Journal of Plant Pathology 104:465-475.

Roth, L.F., E.J. Trione, and W.H. Ruhmann. 1957. *Phytophthora* induced root rot of native Port-Orford-cedar. Journal of Forestry 55:294-298.

Roth, L.F., H.H. Bynum, and E.E. Nelson. 1972. *Phytophthora* root rot of Port-Orford-cedar. U.S.D.A. Forest Service, Forest Pest Leaflet 131.

Salisbury, P.J. 1954. A study of the *Phytophthora* root rots of Lawson cypress in British Columbia. Mimeographed Report, Forest Biology Division, Science Service, Canada Department of Agriculture, March, 1954.

SAS Institute, Inc. 1985. SAS/STAT guide for personal computers. Cary, North Carolina. 378p.

Shearer, B.L., and M. Dillon. 1995. Susceptibility of plant species in *Eucalyptus marginata* forest to infection by *Phytophthora cinnamomi*. Australian Journal of Botany 43:113-134.

Shearer, B.L., and J.T. Tippet. 1989. Jarrah dieback: the dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin no. 3, Department of Conservation and Land Management, Como, Western Australia.

Stukely M.J.C., and C.E. Crane. 1994. Genetically based resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. Phytopathology 84:650-656.

Swigger, L.A., W.R. Harvey, D.O. Everson, and K.E. Gregory. 1964. The variance of intraclass correlation involving groups with one observation. Biometrics 20:818-826.

Swofford, D.L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Thies, W.G. 1984. Laminated root rot, the quest for control. Journal of Forestry, June 1984:345-356.

Torgeson, D.C. 1954. Root rot of Lawson cypress and other ornamentals caused by *Phytophthora cinnamomi*. Contributions to the Boyce Thompson Institute 17:359-373.

Torgeson, D.C., R.A. Young, and J.A. Milbrath. 1954. *Phytophthora* root rot diseases of Lawson Cypress and other ornamentals. Oregon Agricultural Experiment Station Bulletin 537. 18p.

Trione, E.J. 1957. The physiology and pathology of *Phytophthora lateralis* on native *Chamaecyparis Lawsoniana*. Thesis, Ph.D., Oregon State University, Corvallis, Oregon.

Trione, E.J. 1959. The pathology of *Phytophthora lateralis* on native *Chamaecyparis lawsoniana*. Phytopathology 49:306-310.

Trione, E.J., and L.F. Roth. 1957. Aerial infection of *Chamaecyparis* by *Phytophthora lateralis*. Plant Disease Reporter 41:211-215.

Tucker, D.M,. and J.A. Milbrath. 1942. Root rot of *Chamaecyparis* caused by a species of *Phytophthora*. Mycologia 34:94-103.

Utkhede, R.S., and E.M. Smith. 1993. Response to artificial infection by *Phytophthora cactorum* of four apple scion cultivars on three rootstocks. HortScience 28:717-718.

Vainio, E.J., K. Korhonen, and J. Hantula. 1998. Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. Mycological Research 102:187-192.

Vanderplank, J.E. 1982. Host-Pathogen Interaction in Plant Diseases. Academic Press, New York.

Von Broembsen, S. 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. Phytophylactica 16:221-225.

Wagner, L.J., and H.E. Flores. 1994. Effect of Taxol and related compounds on the growth of plant pathogenic fungi. Phytopathology 84:1173-1178.

Widmer, T.L., J.H. Graham, and D.J. Mitchell. 1998. Histological comparison of fibrous root infection of disease-tolerant and susceptible citrus hosts by Phytophthora nicotianae and P. palmivora. Phytopathology 88:389-395.

Wright. J.W. 1976. Introduction to Forest Genetics. Academic Press, New York.

Wu, H.X., and C.C. Ying. 1997. Genetic parameters and selection efficiencies in resistance to western gall rust, stalactiform blister rust, needle cast, and sequoia pitch moth in lodgepole pine. Forest Science 43:571-581.

Zheng, F.C., and E. Ward. 1998. Variation within and between *Phytophthora* species from rubber and citrus trees in China, determined by polymerase chain reaction using RAPDs. Journal of Phytopathology 146:103-109.

Zobel, D.B., and G.M. Hawk. 1980. The environment of *Chamaecyparis lawsoniana*. American Midland Naturalist 103:280-297.

Zobel, D.B., L.F. Roth, and G.M. Hawk. 1985. Ecology, pathology, and management of Port-Orford-cedar (*Chamaecyparis lawsoniana*). U.S.D.A. Forest Service, Pacific Northwest Forest and Range Experiment Station, General Technical Report PNW-184.