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Range-Wide Genetic Variation in Port-Orford-Cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.): I. Early Height Growth at Coastal and Inland Nurseries

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INTRODUCTION

Public resource managers and scientists in Oregon and California are conducting a species conservation program to protect Port-Orford-

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cedar *(Chamaecyparis lawsoniana)* against a major threat from a highly virulent, exotic root disease fungus, *Phytophthora lateralis*. Port-Orford-cedar (POC) is narrowly restricted to northwestern California and southwestern Oregon where it grows in a variety of habitats, including those with ultramafic soils, from sea level to 1950 m. The genetic phase of this concerted effort includes genetic testing, selection, and breeding to:

a. determine the genetic structure of populations,
b. develop disease resistant strains, and
c. identify adaptive zones for use of resistant stock in restoration plantings.

Genetic conservation of POC is justified by:

a. the species' ecological amplitude and importance,
b. its high commercial value for both timber and ornamental markets,
c. the increasing mortality from root rot in natural stands, and
d. the apparently low disease resistance.

In 1995, POC nursery stock was grown from range-wide seed collections for three types of genetic tests. Root disease resistance testing on a large scale began using 344 wind-pollinated (W-P) families at Oregon State University (Sniezko et al., 1996). A long-term common garden study was outplanted on four forest sites in 1996. A short-term common garden study was established in 1996 at a coastal (Humboldt Nursery) and an inland (Dorena Tree Improvement Center) site using 298 W-P families. This paper presents the two-year height growth results from the short-term "raised bed" study.

Much is known about the allozyme diversity, ecology, and physiology of the species (Millar and Marshall, 1991; Millar et al., 1992, unpublished; Zobel and Lui, 1980; Zobel, 1983; Zobel et al., 1985; Zobel, 1998). These previous studies found relatively low genetic differentiation among populations. However, the genetic structure of growth, survival, and disease resistance traits from range-wide common garden tests is not known.

METHODS

Seed Collection and Sample Populations

Seed was collected from 344 healthy parent trees in 52 native stands during 1991-94 by the United States Forest Service (USFS) and Bureau of Land Management (BLM). Two bulk seed lots from Humboldt County, California were also included. Stands were sampled throughout much of the ca 350 km NW-SE range from the extreme northwestern portion (Oregon Dunes, a few meters from the ocean) to the extreme southeastern stands (Pond Lily Creek, Upper Trinity River at 1630 m elevation and 144 km inland). Sample trees were grouped into 10 regional watersheds: six in Oregon and four in California, and into 52 stands: 36 in Oregon and 16 in California (Table 1). Within stands, most sample trees were located within 1 km distance and 100 m in elevation. However, the distribution of watersheds, stands within watersheds, and trees within stands was uneven.

Raised Bed Study Design

In 1995, 300 seed lots were grown in 4 cm^3 containers at the Simpson Korbel Nursery, Korbel, California. In spring 1996, the 1-0 seedlings were transplanted to two locations: Dorena Tree Improve-

TABLE 1

Regional Watershed	No. Stands	No. Trees	Elev. Range (m)	Latitude Range (deg)	Longitude Range (deg)	2-Yr Ht (cm)
Trinity	2	9	1585-1615	41.0885-.1255	122.4720-.5301	80.0
Sacramento	3	30	1143-1585	41.2200-.2500	122.3959-.4600	85.9
Klamath	3	24	914-1372	41.0000-.8234	123.4651-.9000	99.0
Smith	8	40	402-1585	41.7237-.9657	126.6493-124.0690	99.4
Illinois	2	13	1024-1067	42.0332-.1250	123.3553-.5535	102.2
Applegate	4	29		42.1188-.2073	123.2789-.4057	104.1
Rogue	6	28	664-1097	42.4277-.6917	123.7248-124.2843	105.4
Coquille	18	82	122-838	42.7083-.2600	123.7800-124.1333	115.9
Dunes	4	26	15-59	43.3400-.4500	124.2500-.3400	124.4
Sixes and Elk	2	19	61-246	42.7799-.8333	124.4315-.4833	127.9

ment Center (DTIC), Cottage Grove, Oregon, and Humboldt Nursery (HN), McKinleyville, California. The HN site is only 3.2 km from the ocean at 76 m elevation near the southern end of the range. DTIC is 100 km from the coast at the northernmost latitude of the species and at 245 m elevation. At DTIC the experimental design was a randomized, complete block with six blocks and 300 seed lots (includes two bulk lots) arranged in 3-tree family row plots. At DTIC all six blocks were artificial raised beds with organic rooting medium 40 cm deep over a gravel base. At DTIC, three of the blocks were shaded with 47% shade-cloth during the growing season and three were unshaded. At HN, the design was similar, except three of the blocks were raised beds (HN-RB) and about 20 m away were three conventional nursery beds (HN-NB). All HN blocks had native mineral soil and were transplanted one month later than DTIC. In addition, HN-RB were sheltered from wind and partially shaded by a mature spruce canopy, while the HN-NB were fully exposed to sun and wind. Thus, HN treatments represented two contrasting microenvironments confounding differences in exposure to wind, sun, and temperature extremes. Spacing of trees was 210, 204, and 160 cm^2 per seedling, respectively, for DTIC, HN-NB, and HN-RB. All trees were irrigated and fertilized at maintenance levels.

Traits Measured and Statistical Analysis

Treatment design inconsistencies (spacing, type of bed, and shading) at HN resulted from lack of funding and materials for constructing additional raised beds. Confounding effects from other factors with treatment and treatment interaction effects at HN necessitated analyzing the treatments first as separate experiments (by location-treatment), then by location combined across treatment, and finally, combined across both treatment and location. This approach aided interpretation of results.

Seed weight and percent filled seed were available for 223 families. Total height (longest leader), mortality (summarized as arcsin $[\text{proportion dead}]^{1/2}$), and shoot condition (dieback, foliage discoloration) were taken at the end of the first and second year after transplanting. Simple correlations among traits were made using 223 families with both seed and seedling data. Regressions for height on location variables were based on all 298 families sown.

For height traits, dead and unhealthy trees were removed prior to

analysis. Means for missing plots (15 at DTIC, 12 from shaded blocks, 2 at HN) were estimated by adjusting location means for block and family means. All analyzes involving height were based on plot means and were balanced at the family level. SAS (ver. 6.12) was employed for all analyses. Where necessary, F-tests were synthesized from expected mean squares, and degrees of freedom were calculated (Satterthwaite, 1946).

Expected mean squares for a "combined experiment" analysis across locations and treatments are shown in Table 2. Locations and treatments were considered as fixed effects, blocks and families as random. For the purposes of examining genotype × environment interactions (G-×-E) and estimating variance components, the family effect was partitioned into the nested genetic sources: watersheds, stands within watersheds, families within stands, along with their associated interactions with locations.

RESULTS

Locations and Bed Treatments

Trees at Dorena grew rapidly, averaging 117 cm for total height (2-yrs after transplanting) which was 21% taller than trees at Hum-

TABLE 2. ANOVA and expected mean squares for combined experiments across sites and treatments

	Source	Deg Freedom	MS	Expected Mean Squares
F	Location	$p-1=1$	1	$\sigma^2 + 298\,\sigma^2_B + 3\,\sigma^2_{PF} + 1788\,\Sigma P^2/1$
F	Trt	$t-1=1$	2	$\sigma^2 + 298\,\sigma^2_B + 6\,\sigma^2_{TF} + 1788\,\Sigma T^2/1$
	Loc × Trt	$(p-1)(t-1)=1$	3	$\sigma^2 + 298\,\sigma^2_B + 3\,\sigma^2_{PTF} + 894\,\Sigma PT^2/1$
	Blocks (Loc × Trt)	$pt(b-1)=8$	4	$\sigma^2 + 298\,\sigma^2_B$
	Families	$f-1=297$	5	$\sigma^2 + 12\,\sigma^2_F$
	Loc × Fam	$(p-1)(f-1)=297$	6	$\sigma^2 + 6\,\sigma^2_{PF}$
	Trt × Fam	$(t-1)(f-1)=297$	7	$\sigma^2 + 6\,\sigma^2_{TF}$
	Loc × Trt × Fam	$(p-1)(t-1)(f-1)=297$	8	$\sigma^2 + 3\,\sigma^2_{PTF}$
	Error	$pt(b-1)(f-1)=2376$	9	σ^2
	Corr. Total	$ptbf-1=3575$		

$p=2$, $t=2$, $b=3$, $f=298$, MS = sample mean square, F-test for Locations = MS1/(MS4 + MS6 − MS9), F-test for Treatments = MS2/(MS4 + MS7 − MS9), F-test for Loc × Trt = MS3/(MS4 + MS8 − MS9)

boldt. At DTIC, shaded trees grew only slightly taller than trees in open sun the first year, but the second year, trees in open sun grew 26% taller ($p < 0.01$) than shaded trees. At HN, the response pattern was different. Sheltered trees were 13% taller the first year ($p = 0.06$), but subsequently trees grew at the same rate in both treatments (Table 3). This location-×-treatment interaction was significant ($p < 0.01$) for second year height growth and for total two-year height ($p = 0.04$). A delay of 1-month in planting date in HN-NB may account for its slower first-year growth. Overall mortality was very low (1.2%) at HN, but was 6.7% at DTIC, where most mortality occurred in shade. At HN, the reverse was true. Gophers caused most mortality in open, barrier-free nursery beds (HN-NB).

Thus, POC benefited slightly from shading and shelter initially before heavy inter-tree shading and root competition began. Then, as inter-tree competition intensified, trees in open sun grew taller and had less mortality at DTIC. However, at HN, mean final height of treatments were similar, perhaps because conditions for growth became less favorable in the sheltered, raised bed where tree spacing was closest and rooting space was restricted. Typical coastal cloudiness reduced the sunlight in both treatments.

TABLE 3. ANOVA for combined experiments across treatments by site and height variable.

		1st Yr Ht		2nd Yr Ht		2nd Yr Ht	Growth
Source	DF	MS	Prob	MS	Prob	MS	Prob
Humboldt:							
Treatments	1	17703.10	.0646	21956.51	.1140	228.74	.6427
Blocks (Trt)	4	2763.39	.0001	5399.85	.0001	911.65	.0001
Families	299	275.73	.0001	1038.35	.0001	343.01	.0001
Trt × Fam	299	36.49	.0158	166.19	.4914	104.74	.2863
Error	1196	30.14		166.26		99.66	
Dorena:							
Treatments	1	1679.14	.8225	52880.56	.1672	73470.43	.0036
Blocks (Trt)	4	29278.45	.0001	18612.34	.0001	1973.22	.0001
Families	299	303.16	.0001	2213.10	.0001	931.66	.0001
Trt × Fam	299	43.65	.0910	255.57	.3207	124.88	.3896
Error	1196	38.75		245.37		121.93	

Genetic Sources and G- ×-E Interactions

Differences among families and the location-×-family interaction were significant ($p < 0.01$) for height both years (Table 4). The family mean phenotypic correlation for total height between locations was 0.81, which reflects high overall stability across locations. The Type B genetic correlation adjusted for heterogeneity of family variances (Surles et al., 1995) was 0.94 for total height, which indicates the interaction was caused by scale effects and not by true rank changes among family genotypes. Significant differences were also evident in 1-yr height for treatment-×-family and location-×-treatment-×-family interactions (Table 4), but these subsequently became non-significant. Also, their underlying cause was examined by conducting a joint regression analysis (see Westfall, 1992) for family height regressed across sequential location-treatment means. Eberhardt and Russell's slope coefficient and residual variance parameters were computed and then correlated with Wricke's ecovalence. This showed a significant positive correlation ($r = 0.81$) for ecovalence with heterogeneity of residual variances, but not with heterogeneity of slopes ($r = 0.07$). Thus, scale effects again caused the G-×-E interactions.

Variance Components for Height Traits

The genetic structure of POC for early height is described in the proportion of total variance residing at various levels and from differ-

TABLE 4. ANOVA for combined experiments across sites and treatments by height variable.

		1st Yr Ht		2nd Yr Ht		2nd Yr Ht	Growth
Source	DF	S	Prob	MS	Prob	MS	Prob
Locations	1	118349.30	.0263	363448.82	.0006	66968.30	.0001
Treatments	1	15143.27	.3594	3344.02	.6120	32750.14	.0014
Loc × Trt	1	4238.27	.6209	71493.04	.0405	40949.03	.0007
Blocks (Loc × Trt)	8	16020.92	.0001	12006.10	.0001	1442.43	.0001
Families	299	532.51		2848.30	.0001	1023.34	.0001
Loc × Fam	299	46.38	.0002	403.15	.0001	251.33	.0001
Trt × Fam	299	40.46	.0275	204.21	.5268	112.71	.4129
Loc × Trt × Fam	299	39.67	.0464	217.56	.2525	116.91	.2590
Error	2392	34.44		205.81		110.79	

ent sources (Table 5). Site location effects were assumed fixed and thus excluded from the SAS Restricted Maximum Likelihood model. Genetic main effects of watersheds, stands within watersheds, and families within stands accounted for 47.5% of the total variability in total height, and most (37.4%) of this resided at the watershed level. Families accounted for twice as much as stands. The difference between the tallest and shortest stand means within watersheds varied by 2-31% (ave. = 12%), while that between family means within stands varied by 2-47% (ave. = 22%).

G-×-E interaction components accounted for only 6.1%. These were largely due to location × family "scale" effects. Environmental block effects, which included shade treatment effects here, accounted for 9.9%. The remaining 36.5% was due to random plot effects.

Compared to the first year height components, a shift occurred in second year growth toward a lower component for family, higher for interaction, lower for block, and higher for plot error (Table 5). Intense competition among trees in different plot neighborhoods apparently resulted in inconsistent performance across blocks and locations, especially at the family (plot) level.

Correlations Among Traits and with Geographic Origin

Using the 134 families and 32 sub-stands (spatially separate groups within stands) having both seed and seedling data from only the 1993 seed collections, stands with lighter seed had lower filled seed, shorter trees, and greater mortality at DTIC (Table 6). Using the 223 families and 57 sub-stands having both seed and seedling traits from all collection years, watersheds and sub-stands from higher elevations, more

TABLE 5. Distribution of variance components for height traits

Varcomp Trait	Water-shed[1]	Stand/W	Family/S	Loc × W	Loc × S	Loc × F	Block	Error
1st Yr Ht	26.5**	2.8**	9.1**	0.1	0.0	1.2**	35.0**	25.4
HG 2nd Yr	28.0**	2.6**	2.8**	3.6**	0.1	6.7**	11.2**	45.0
2nd Yr Ht	37.4**	3.4**	6.7**	1.6**	0.0	4.5**	9.9**	36.5
Total 2nd Ht	<-----	47.5	----->	<-----	6.1	----->	<----- 46.4	----->

[1] ** = significant at $p < 0.01$

southern latitudes, and more eastern longitudes had higher mortality at DTIC and had shorter trees at both locations (Table 7). Also, sub-stands with taller trees after the first year had greater height growth during the second year. Regressions for height on location variables were highly significant for latitude, longitude, and elevation (Table 7), but were strongest for watershed elevation (Figure 1). Height decreased 28.2 cm per 1000 m increase in source elevation. Trees from the low Sixes and Elk watershed averaged 60% taller than those from the high Trinity watershed.

CONCLUSIONS

The environmental components–transplanting location and "shade" treatments–had significant and major effects on height growth. Sur-

TABLE 6. Simple correlations between seed traits and mortality and height traits for the 1993 seed collections based on sub-stand means. Critical values are 0.31 (p = 0.05) and 0.41 (p = 0.01).

Traits[1]	% Filled	Dead_Hn	Dead_DTIC	1st Yr Ht	2nd Yr Ht	2nd Yr Ht Gr
Seed Weight	0.60	−0.18	−0.56	0.49	0.43	0.46
% Filled		−0.18	−0.49	0.20	0.18	0.19

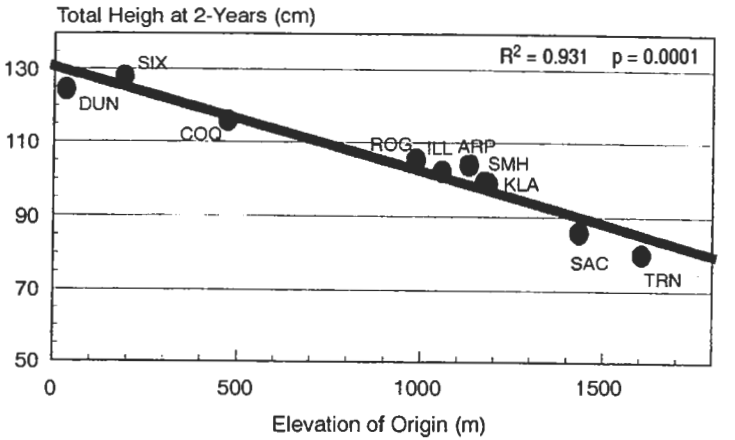
[1] % filled seed, arcsin (proportion dead)$^{1/2}$ at HN and DTIC, total 1-, 2-yr height and height growth 2nd yr

TABLE 7. Simple correlations between height, mortality, and source location variables for all seed collection years based on sub-stand means. Critical values are 0.22 (p = 0.05) and 0.31 (p = 0.01).

Traits[1]	2nd Yr Ht	2nd Yr Ht Gr	Dead_HN	Dead_DTIC	El	Lat	Lon
1st Yr Ht	0.98	0.95	−0.25	−0.57	−0.87	0.77	0.77
2nd Yr Ht		0.99	−0.31	−0.60	−0.89	0.81	0.82
2nd Yr Ht Gr			−0.34	−0.61	−0.88	0.81	0.83
Dead_HN				0.26	0.19	−0.31	−0.29
Dead_DTIC					0.37	−0.37	−0.43
El						−0.78	−0.75
Lat							0.80

[1] arcsin (proportion dead)$^{1/2}$) at HN and DTIC, total 1-, 2-yr height and height growth 2nd yr

FIGURE 1. Watershed mean total height by elevation of origin.



prisingly, the inland location had superior height to the coastal location both years, and "shading" was inferior to open sun the second year. The height growth response of POC families from different geographic regions and stands revealed a strong genetic structure with a well-defined geographic pattern. Height potential was highly related to genetic source at the watershed, stand, and family levels. Strong clinal patterns were found for height potential with source elevation, latitude, and longitude. G-×-E interactions, though statistically significant at watershed and family levels, were minor sources of variability in height, and were due to scale effects rather than rank changes. Southern and high elevation inland sources had low growth potential at both locations, while northern and low elevation coastal sources had high growth potential. Trees from low elevation, northern, and coastal sites had less mortality, higher seed weight and higher filled seed percent.

These tentative results show similar, though much stronger, population structure and geographic patterns as allozymes (Millar et al., 1992, unpublished report). Current results suggest that gene conservation practices should encompass: (a) seed zoning by watershed, subdivided by elevation bands, and (b) protecting the broad gene base for growth, including the adaptive extremes near the northern and southern limits. Long-term field studies and disease screening trials already underway will complement these nursery results and guide restoration planting of resistant stock.

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