Inbreeding depression in Port-Orford-cedar: early survival and growth of self-fertilized and open-pollinated progeny

Scott E. Kolpak, Richard A. Sniezko, and Christine F. Hayot
USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon 97424

POC containerized resistant orchard

Introduction:

Phytophthora lateralis is a non-native, invasive root pathogen that has caused extensive mortality of *Chamaecyparis lawsoniana* (Port-Orford-cedar) in some areas of its natural range in southwest Oregon and northwest California. An operational breeding program to develop populations of Port-Orford-cedar that are resistant to *P. lateralis* is underway. Several containerized orchard populations have been established and more are being developed as the first cycle of disease resistance testing is completed.

We are investigating inbreeding as a tool to elucidate the patterns of inheritance for root disease resistance. Several well characterized families suggest a single dominant resistant gene is present in high survival families (Sniezko et al, 2004). Disease resistance testing on progeny from self-fertilized parents is a potential tool to determine the prevalence of single gene resistance in the orchard populations, including any recessive genes. However this strategy requires a minimal impact of inbreeding depression on seed yield and seedling survival. In addition, selfing or related matings may be common in natural populations that have been decimated by *P. lateralis*. In these populations, if significant inbreeding depression exists, then Port-Orford-cedar may lose competitiveness with other tree and shrub species causing a further loss of POC in natural populations.

Inbreeding depression has been examined in several conifer species. Generally, inbreeding depression causes a decrease in young seedling height and survival, and a reduction of filled seed due to the expression of embryonic lethals (Sorensen & Miles, 1974; Williams & Savolainen, 1996). We examined the effects of inbreeding in Port-Orford-cedar by examining growth and survival in self-fertilized and open-pollinated progeny.

Distribution of POC and P. Lateralis

Container study:

•Introduction

A container study was undertaken to provide a more detailed examination of inbreeding in Port-Orford-cedar by examining growth and survival in self-fertilized and open-pollinated progeny. 18 parent trees having both first generation self-fertilized (S1) and open-pollinated (W1) seed were sown in March 2005. The self-fertilized and open-pollinated seedlots were from containerized seed orchards at Dorena Genetic Resource Center. Seedlings were grown in 'supercell' containers (164 cm^3) in a greenhouse for several months then moved into raised beds. Seedlings were exposed to several treatments including a shade, drought, and control treatment while planted in containers. Early survivorship and growth are currently being measured. An analysis of percent filled seed of the self and open-pollinated seedlots is summarized here.

Photo 1: Raised bed control treatment

•Material and Methods

Seedling Culture

Seedlings were sown in family blocks March 2005 in 164 cm^3 containers and cultured in a greenhouse for several months. Seedlings were thinned to one seedling per container in mid-June 2005 when seedlings were robust and an approximately 5 cm tall. An average of 1.6 seedlings were rogued from 55% of the containers. 3151 seedlings were randomized 2 weeks later and placed outdoors in raised beds.

Experimental design

Seedlings were randomized into a three treatments: shade, drought and control (see photos 1,2). Each treatment consisted of the same 19 parents with two levels of inbreeding (S1, W1). For each parent a split-plot was formed with adjacent self and open-pollinated row plots. Parent split-plots were randomized into blocks within treatments (see diagram 1). Each treatment consists of four blocks with 2 – 8 seedlings per plot. Filler trees were added to each row plot when necessary and a single row of border trees surrounded each treatment to reduce edge effects.

Measurements

Survival % filled seed Growth increments germination rate Diameter root and shoot biomass

Photo 2: Raised bed shade treatment

•Results

- Initial height measurements have been recently taken (July 5-7, 2005).
- A couple families already have dramatic differences in height between the self and open-pollinated crosses (see photos 3,4).
- There is a 61% decrease in filled seed in the self seedlots compared to open-pollinated seedlots (see figure 2).

Photo 3: Height difference between Self and OP in PO-117490

Photo 4: Height difference between Self and OP in PO-DOR-70262

Diagram 1: Example of treatment layout

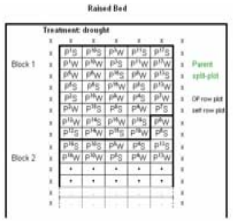
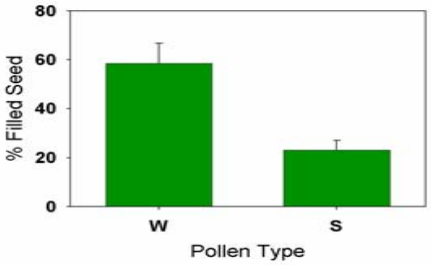


Figure 2: Histogram of % filled seed by pollen type



•In the near future…

- Height increment, diameter, and survival measurements.
- An analysis of root and shoot biomass will occur at the termination of the experiment in Fall 2005.
- A more detailed analysis of % filled seed and germination rate.
- Upon termination of the experiment seedlings may be planted into the field or a raised bed to further monitor inbreeding in Port-Orford cedar.

Field study:

•Introduction

7 parent trees with self (S), open-pollinated (W), and control crosses (C) from the 2000 Camas Valley resistance validation planting were measured for growth to examine the effects of inbreeding in a natural field environment. The seedlings were planted in March 2000 at Camas Valley (Roseburg BLM, Oregon) as 1-year old seedlings in a randomized complete block design. Seedling heights were taken several months later in June 2000.

•Materials and methods

Heights (cm) were taken on approximately 1.5-year old seedlings from 24 seedlots out of the 110 resistant and susceptible seedlots planted in the 2000 Camas Valley resistance validation trial. 7 parent trees are represented in the 24 seedlots as S, W, and C. Each seedlot was planted 2 or 4 blocks with 1- 4 seedlings per block (average = 2.7 sdlg/block). The variation in seedling number per block is caused by poor germination success and any field mortality (diseased and non-diseased sources) occurring before the height measurements were taken.

•Data Analysis

A 3-way nested ANOVA was carried out using Proc GLM with cross type (S = self, W = open-pollinated, C = control cross) family nested within pollen type, and block nested within family and cross type. Duncan's multiple comparison test was used to examine differences in average height amongst inbred and outcrossed seedlots.

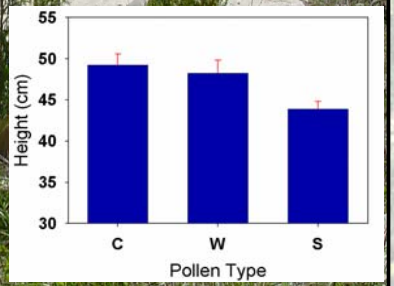
•Results

The main effect pollen type and both nested factors were significant (see table 1). Self families are shorter than outcrossed families (W and C). There is no height differences between open-pollinated and control cross families (see figure 1).

Table 1: Nested ANOVA for Camas Valley inbreeding trial

Source	DF	Type III SS	MS	F	P-Value
Pollen Type	2	811.09	405.55	8.90	0.0004
Family (Pollen Type)	21	2840.73	135.27	2.81	0.0015
Block (Family (Pollen Type))	50	2370.18	47.40	1.72	0.0083

Figure 1: Histogram of tree height by pollen type



References:

•Sniezko, R.A., Hansen, E.M., and Kolpak, S.E. 2003. Simply inherited resistance to *Phytophthora lateralis* in Port-Orford-cedar: greenhouse testing. Western Forest Genetics Association. Whistler B.C., Canada.
•Sorensen, F.C., and R. S. Miles. 1974. Self-pollinated effects on Douglas-fir, and ponderosa pine seeds and seedlings. Silv. Genet. 22: 135-138.
•Williams, C.G., and O. Savolainen. 1996. Inbreeding depression in conifers: implications for breeding strategy. Forest Science 42(1): 102-117

Acknowledgments: Leslie Elliott for measuring heights at the Camas Valley outplanting. Roseburg BLM for Camas Valley site preparation and planting, particularly Leonard Dour and Kirk Casavan.