GEOGRAPHIC AND LOCAL GENETIC VARIATION IN PACIFIC MADRONE LEAF BLIGHT

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Pacific madrone (*Arbutus menziesii*) is an evergreen hardwood species in western North America that occurs from British Columbia to southern California. The species has high cultural and specialty wood values, and is also ecologically important for wildlife habitat, wildfire revegetation, and creating midstory canopy biodiversity in mixed conifer forests. Pacific madrone is experiencing unsustainable mortality (Lintz et al. 2016), but reasons for the decline are unknown. Foliar pathogens such as described by Zeller (1934) have been implicated because of their high visibility and association with defoliation. However, very little is known about the genetics of this species, including variation in resistance to pathogens and other adaptive traits.

Range-wide common garden tests were established to examine patterns of genetic variation in traits such as height growth and survival, to identify relative contributions of abiotic, biotic and genetic causes of health declines, and for identifying resistant seed sources. The Washington State University Pacific madrone seed collection contains seed from 320 families in seven ecoregions. Using this seed collection, common gardens were planted at seven locations in 2011 in California (1 site), Oregon (2), Washington (2), and in 2013 in British Columbia (2). These common garden sites are located in four of the seven ecoregions where seed was collected (fig. 1) and consist of 105 half-sib families representing 42 seed sources. Survival and performance data were collected annually and leaf blight, caused by a complex of fungal pathogens, was also rated annually for the first 3 to 5 years, depending on site. In addition to leaf blight, assessments have been made of growth, other diseases, cold damage, flowering, and phenology. Here we summarize variation in leaf blight severity in the OR and WA common gardens.

Across all years and common gardens, average blight severity was slight to moderate (< 25 to 25–50 percent of current leaves affected) with an average incidence of 25–50 percent. Blight symptoms were more severe in years where a common garden test was particularly stressed by drought and/or cold winter temperatures and tended to be more severe at the WA sites (PH, PV) compared to the OR sites (SF, SO). Patterns of blight severity among sites might be related to site conditions such as moisture and temperature. Typically precipitation decreases and temperatures increase from north to south latitude, thus the WA sites would be cooler and wetter than the SF site, which would be cooler and wetter than the SO site. However, the size of the differences in blight symptoms may be magnified since the OR sites were generally assessed in late fall/early winter while the WA sites were assessed in late winter. Subsequent observations in another year on a subset of trees confirm an increasing degree of blight over this time period.

Fungi associated with severe leaf blight, such as *Phacidiopycnis washingtonensis* (Elliott et al. 2014) and *Phomopsis* sp., were not found at the southernmost site (SO) in 2018 samples (fig. 2).

Blight severity within sites varied significantly ($p < 0.01$) among seed sources for all years at all sites except for SO where sources only differed in 2012 (data not shown). Blight severity among half-sib families within sources also varied significantly ($p < 0.01$) for all years and at all sites except at SF where families only

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Citation for proceedings: Nelson, C. Dana; Koch, Jennifer L.; Sniezko, Richard A., eds. 2020. Proceedings of the Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry—Tree Resistance to Insects and Diseases: Putting Promise into Practice. e-Gen. Tech. Rep. SRS–252. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station. 170 p.

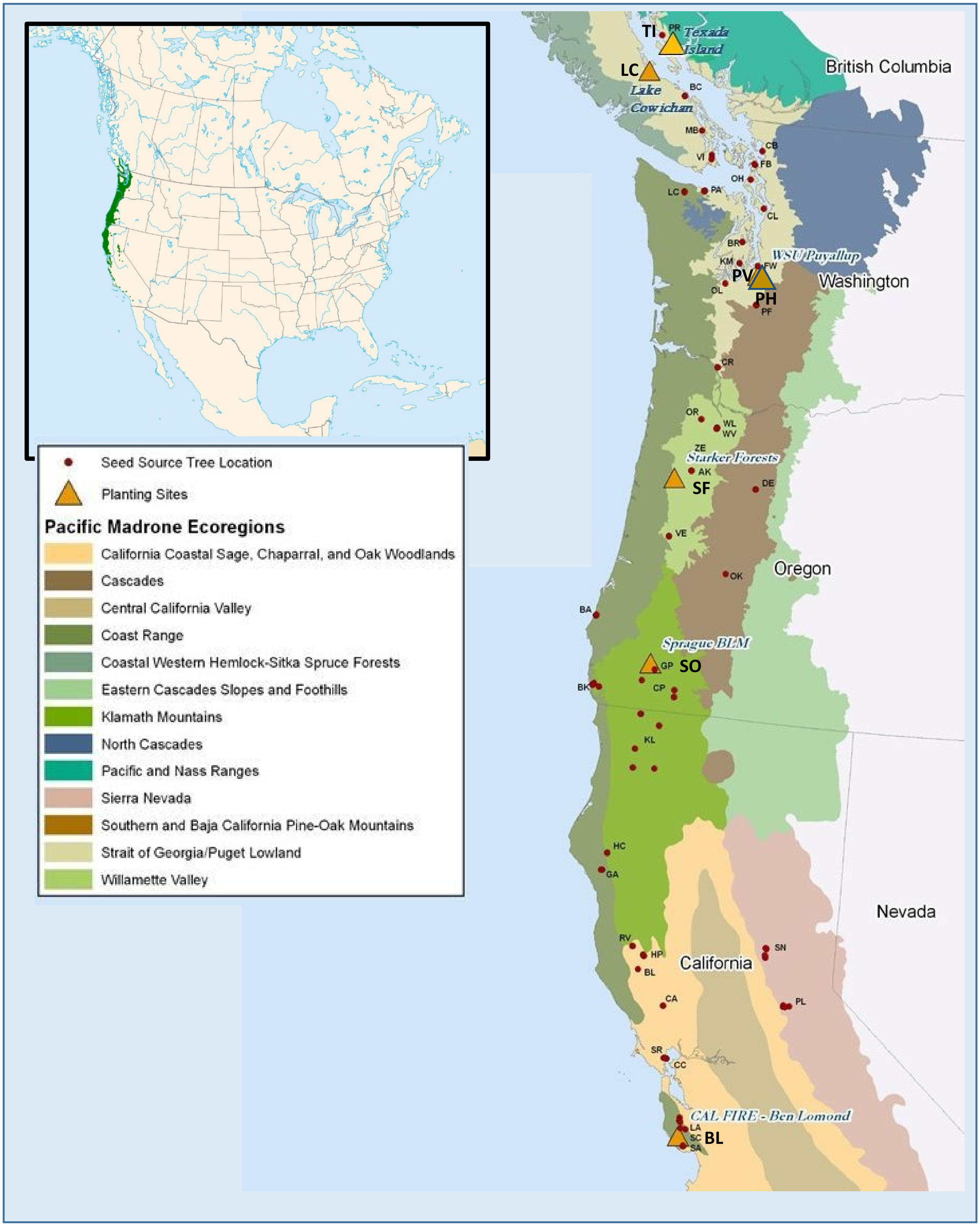

Figure 1—Locations of madrone common garden sites, seed sources, and ecoregions. (Insert: full range of Pacific madrone). Sites used in this study: PV, PH (WSU Puyallup), SF (Starker Forest), and SO (Sprague Orchard).

	Oregon		Washington	
	SO	SF	PH	PV
Large leaf spots				
Cryptostictis arbuti		x		
Didymosporium arbuticola	x	x	x	x
Rhytisma arbuti	x			
Small leaf spots				
Epicoccum nigrum	x	x	x	x
Mycosphaerella sp.			x	
Shoot and leaf blight				
Diaporthe spp. (=Phomopsis spp.)		x		x
Phacidiopycnis washingtonensis		x	x	x
Endophytes				
Allantophomopsis cytisporea		x		
Aureobasidum pullulans	x	x	x	x
Colletotrichum clavatum		x		
Fusarium sp.			x	x
Stemphylium sp.		x		
Sydowia polyspora	x		x	x

Figure 2—Fungi associated with leaf blight. Fungi were identified from madrone foliage samples at the common garden sites using morphological and molecular techniques, from south (SO) to north (PV) in 2018.

differed in 2015. Averaged across all sites, variation in blight severity among sources was not related to longitude of origin but was significantly ($p < 0.01$) related to latitude of origin, ($R^2 = 0.09$- 0.69), and elevation of origin ($R^2 = 0.13$-0.16). When analyzed separately by site, variation in blight severity among sources was not related to longitude at any site, and at SO variation was also not related to latitude or elevation. Relationships with latitude were strongest for PH ($R^2 = 0.48$-0.65) but at PV latitude was only significantly related in 2014.

The strongest relationships between seed source origin and site were at the most northern common gardens (PH & PV). Averaged across sites, trends in table 1 show that in general, southern sources had higher blight severity than northern sources. Sources with the lowest blight severity were from the north: OH, CL (Oak Harbor and Clinton, WA), OR (Cornelius, OR). Sources with the highest blight severity were from the south: e.g., HC, HP, LA (Humboldt County, Hopland, Los Altos, CA). However, some northern sources such as PA, DE (Port Angeles, WA , Detroit, OR) had relatively

Table 1—Blight severity score averaged across common garden sites for each Pacific madrone seed source for each year

Source	State or Province	Latitude	2012	2013	2014	2015	Mean, all years
SA	California	37.02010	14.1	37.0	34.5	26.5	28.1
SC	California	37.21705	16.2	28.2	26.9	21.2	23.1
LA	California	37.29174	18.2	31.3	31.1	42.3	30.7
CC	California	38.00579	14.6	26.3	30.4	38.2	27.4
SR	California	38.01276	11.8	29.8	32.9	26.0	25.1
CA	California	38.60876	12.8	24.0	22.7	29.2	22.2
PL	California	38.75026	13.2	22.2	16.5	38.0	22.5
HP	California	39.00000	22.3	31.9	31.5	44.0	32.4
BL	California	39.16684	17.5	33.1	26.1	40.2	29.2
RV	California	39.25356	17.1	29.0	30.7	42.7	29.9
SN	California	39.35246	14.1	27.7	21.0	37.9	25.2
GA	California	40.08990	18.2	31.0	26.7	41.4	29.3
HC	California	40.29190	17.2	37.7	31.9	44.4	32.8
KL	California	41.69240	16.2	27.3	18.6	40.7	25.7
RA	Oregon	42.19585	15.4	25.1	18.7	40.6	24.9
BK	Oregon	42.21448	12.2	23.3	25.4	37.3	24.6
CP	Oregon	42.26213	21.0	33.6	17.1	41.6	28.3
GP	Oregon	42.47669	18.0	28.1	17.0	40.5	25.9
CY	Oregon	42.91553	15.4	35.5	16.6	40.4	27.0
BA	Oregon	43.01794	12.4	20.5	17.7	33.1	20.9
OK	Oregon	43.67947	13.3	27.9	15.0	40.0	24.1
VE	Oregon	44.03646	11.6	21.2	12.3	32.2	19.3
DE	Oregon	44.69452	20.3	30.3	17.4	44.4	28.1
AK	Oregon	44.82509	13.4	22.5	14.0	38.0	22.0
ZE	Oregon	45.01889	10.2	19.0	11.8	31.2	18.0
WL	Oregon	45.35004	7.2	23.8	10.4	32.6	18.5
WV	Oregon	45.36323	15.5	24.8	12.4	33.0	21.4
OR	Oregon	45.44143	10.8	18.6	10.5	28.0	17.0
CR	Washington	46.06361	14.4	20.1	13.2	29.6	19.3
PF	Washington	46.84067	15.7	24.4	13.2	37.7	22.7
OL	Washington	47.05435	10.5	15.7	10.9	33.6	17.7
FW	Washington	47.30014	16.1	23.9	13.6	33.2	21.7
KM	Washington	47.30751	14.0	23.1	16.0	35.7	22.2
BR	Washington	47.55823	14.2	23.3	13.6	33.8	21.2
CL	Washington	47.97990	8.6	17.5	10.7	28.6	16.4
LC	Washington	48.06585	14.9	22.4	15.8	40.5	23.4
PA	Washington	48.10687	20.2	29.2	15.8	37.8	25.8
OH	Washington	48.30342	8.4	16.7	10.8	28.5	16.1
FB	Washington	48.48699	8.7	22.1	14.0	31.1	19.0
VI	British Columbia	48.51078	16.5	26.4	16.7	29.0	22.1
CB	Washington	48.64940	14.7	31.1	13.7	32.4	23.0
BC	British Columbia	49.18983	10.7	23.6	13.4	30.0	19.4

Note: Blight severity was scored as a percentage of blighted leaf area on the most severely impacted current season leaves. Blight incidence was based on percent of the whole tree with leaves having the severity rating described above. Severity and incidence scores were multiplied together for an overall severity rating score ranging from 0 to 100, with 100 being highly susceptible.

higher blight severity while some southern sources such as PL, CA (Placerville & Calistoga, CA) had lower blight severity. These differences may be related to the climate at the site where the seed was collected being similar to that of a southern or northern site, respectively, and will be investigated further (Wilhelmi et al. 2017). The source by common garden site interaction (i.e., genotype X environment) was significant for all years ($p < 0.001$).

These results suggest that wetter, cooler conditions at a site seem to increase blight severity. Sources that moved the farthest north generally had more severe blight. These sources may have been less well adapted, thus more stressed and less able to resist leaf blight. In addition, the results suggest that resistance to leaf blight might exist. Madrone seed sources collected within the ecoregion of a common garden had both high and low blight severity. Blight severity also varied among half-sibling families within seed sources. Relative blight severity of some sources was not consistent across all common garden sites. This indicates that blight symptoms are likely caused by a complex of biotic and abiotic factors. Relatively few details of the blight dynamics were known and assessments of these trials increase it substantially. Detailed observations in 2018 on a small subset of seedlings indicate that blight severity continues to increase from November through May. In addition, some trees double or triple flush and the later flushes tended to have lower blight severity. This information will be useful in planning the timing of future assessments to examine genetic variation in blight. Further study should also include a more refined study of the individual causes of leaf blight in Pacific madrone.

ACKNOWLEDGMENTS

The authors wish to thank CalFire, BC Ministry of Forests, Bureau of Land Management and Starker Forests for providing the sites, and to the people at Dorena Genetic Research Center, WSU, and OSU who helped with annual assessments. In addition, we thank the volunteers who assisted with seed collection, including Marin County Mid Peninsula Open Space, Ellen Goheen, Brenda Callan, Alan Kanaskie, and many others. We also thank Connie Harrington (USFS) for the use of the cameras documenting blight, and Doug Savin (U.S. Forest Service) for statistical help. Funding for establishing common garden sites and growing seedlings is from U.S. Forest Service Forest Health Protection Grant #2010-DG-11062754-015. Funding supporting data analyses and ongoing disease assessment is from U.S. Forest Service Forest Health Monitoring grant #EM-2018-WC-2.

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