Monitoring a Range-Wide Seed Collection of Pacific Madrone (*Arbutus menziesii*) for Disease Resistance and Adaptive Characteristics in a Common-Garden Planting

Gary Chastagner[1], Marianne Elliott[1], Gil Dermott[1], Alan Kanaskie[2], Richard Sniezko[3], Jim Hamlin[4] , and John Russell[5]

[1] Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA, 98731, [2] Oregon Department of Forestry, 2600 State Street, Salem, OR 97310, [3] USDA Forest Service - Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR, 97424, [4]USDA Forest Service - Umpqua National Forest, 2900 NW Stewart Parkway, Roseburg, OR, 97471, and [5]British Columbia Ministry of Forests and Range, Cowichan Lake Research Station, 7060 Forestry Rd., Box 335, Mesachie Lake, BC, V0R 2N0, Canada. Project #WCFHM-11-02.

Abstract

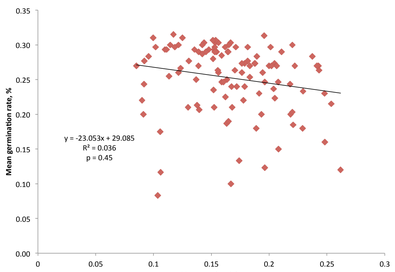
Pacific madrone is a wide ranging species, covering a large latitudinal gradient from southern California to British Columbia. It is an important hardwood species that provides food and habitat for wildlife as well as erosion control in disturbed areas. The species has been in decline for several decades due to a combination of factors such as climate change, forest management, and several endemic fungal pathogens. It is also a host for the invasive pathogen Phytophthora ramorum. Very little is known about the genetics of this species, such as its variation in resistance to pathogens, and adaptation potential in the face of changing climate.

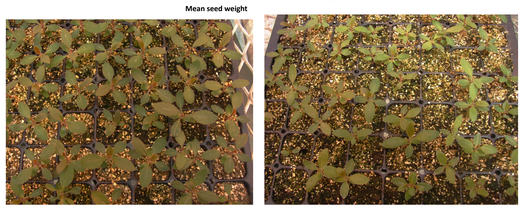
Using material from the Washington State University Pacific madrone seed collection, common garden plantings consisting of 105 families collected from 7 ecoregions were planted in five locations in California, Oregon, and Washington in 2011, with a sixth site planned for British Columbia in 2012. Smaller demonstration plantings with a few trees taken from a range of widely different geographic seed sources, and including other *Arbutus* species from the western states are planned at other locations to be determined.

This project will use the range-wide collection of Pacific madrone to examine genetic variability. Specific objectives are the following: 1) Screen for resistance to multiple pathogens such as *Phytophthora ramorum*, *P. cinnamomi*, and endemic canker and foliar pathogens. 2) Examine variation in growth and adaptive traits. 3) Identify seed sources or populations that may contain individuals that are best adapted to climate change and for urban and restoration plantings. Some preliminary results show genetic variability in a number of traits within the species.

Seedling production

Seed from 125 families of Pacific madrone representing 7 ecoregions were cleaned and weighed in lots of 100 seed each prior to sowing in plug trays. Plug trays were watered and placed into 34 ˚F cooler for stratification in Feb. 2011. Trays were removed from cooler in April 2011 and placed in greenhouse. Fertilizer was applied twice a week at the rate of 200 ppm N with Technigro 20-9-20 soluble. Seedling germination data was collected in June 2011. Plugs were transplanted into Treepots with media consisting of 50% Specialty Soils standard greenhouse mix + 50% fine fir bark + 6 lb/yard slow release Plantacote 14-9-15. These seedlings were grown outside until planting in winter 2011-2012.



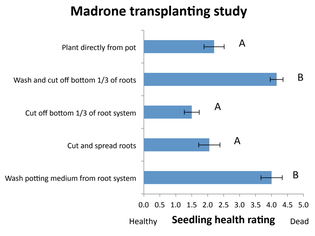


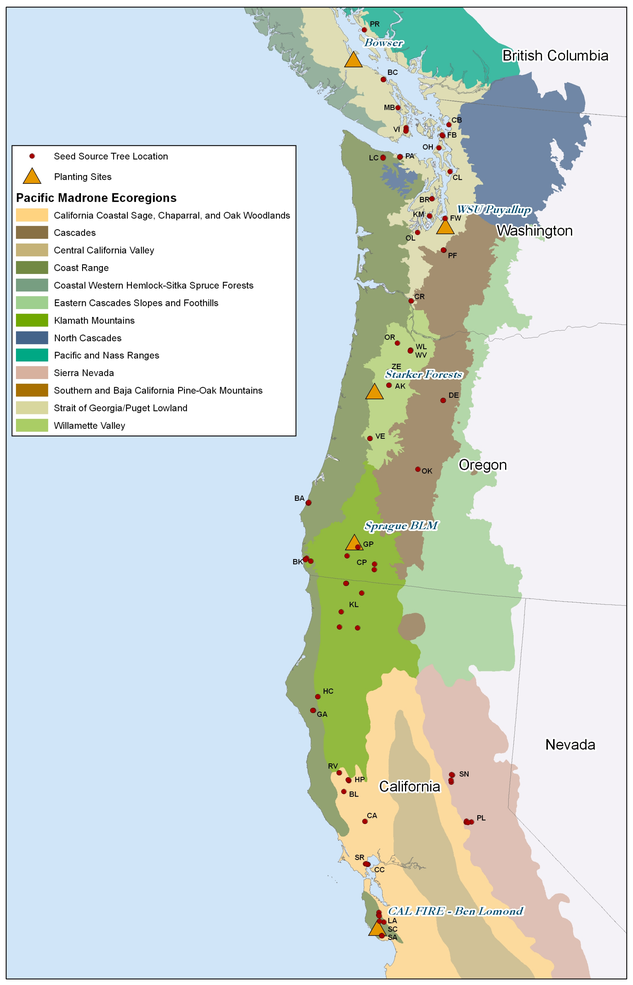
Relationship of seed weight to germination rates and differences in germination rate and seedling size seen among families.

Transplanting madrone seedlings

Pacific madrone has a reputation for being difficult to transplant since its roots react poorly to disturbance. Before transplanting into the common garden sites, studies were undertaken to determine the best methods for planting madrone seedlings.

Two-year-old seedlings from three families were subjected to five treatments and planted in December 2010. Seedlings were examined in May 2011. Treatments that involved washing the roots to remove soil prior to planting resulted in the most seedling mortality.



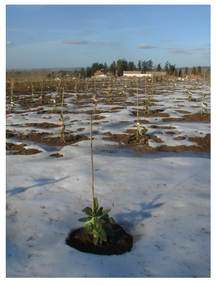


Fruit of madrone ripens in October-November and the seed was extracted by macerating the berries in a blender for four minutes, after which the bare seed was allowed to dry before storage at 1 ˚C.

Differences in fruit production may be due to genetics on these madrones at China Camp State Park, CA.

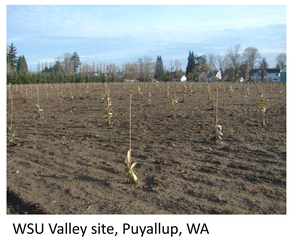
Common garden sites

Bowser seed orchard, Vancouver Island, BC, to be planted in 2013



WSU South Hill site, Puyallup, WA

CalFire Ben Lomond Camp, Felton, CA



WSU Valley site, Puyallup, WA

Starker Forests, Corvallis, OR

BLM Sprague seed orchard, Merlin, OR

Visit the WSU Pacific Madrone Research Website at http://www.puyallup.wsu.edu/ppo/madrone/

Get the free mobile app for your phone http://gettag.mobi

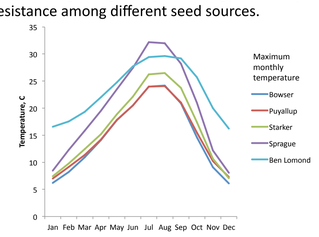
Climate change and disease

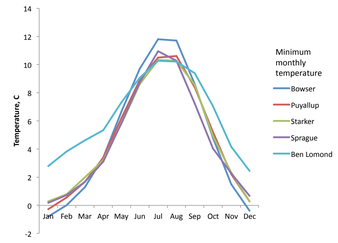
Climate change has the potential to drastically alter the health of our native forests and urban landscapes. Knowledge of genetic variation in resistance and adaptive traits will help provide the knowledge to ascertain future viability of species such as Pacific madrone. This baseline data will allow us to consider proactive measures before native stands are severely affected. For example, tanoak, another west coast native hardwood species, was not a priority until it was shown to be an important host for *P. ramorum*, which could eliminate this tree from certain ecosystems.

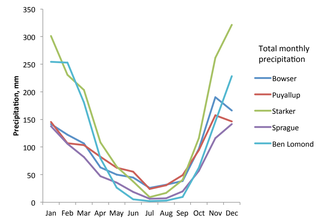
Several diseases affect the health of Pacific madrone throughout its range. These include the endemic canker disease caused by species of *Fusicoccum* (*F. arbuti* and *F. aesculi*), foliar pathogens, and introduced diseases such as *Phytophthora ramorum* and *P. cinnamomi*. The incidence and severity of these diseases will be affected by changes in temperature and precipitation and may cause certain populations of Pacific madrone to go extinct under predicted climate change scenarios. Little is known regarding the epidemiology of these pathogens on Pacific madrone and the level of resistance among different seed sources.

Site	Bowser, BC	Puyallup, WA	Starker, OR	Sprague, OR	Ben Lomond, CA
Lat	49.42998	47.184742	44.458412	42.540568	37.131939
Long	-124.702007	-122.328794	-123.479068	-123.412465	-122.170134
Elev (m)	108.81	10.05	363.93	312.12	780.59

Closest weather station	Nanaimo A	Puyallup	Corvallis Water Bureau	Grants Pass	Ben Lomond 4
ID	1025370	456803	351877	353445	040673
Lat	49.05444	47.19	44.5	42.43333	37.08333
Long	-123.87	-122.33	-123.45	-123.31667	-122.08333
Elev (m)	28.04	10.36	155.45	283.46	137.16



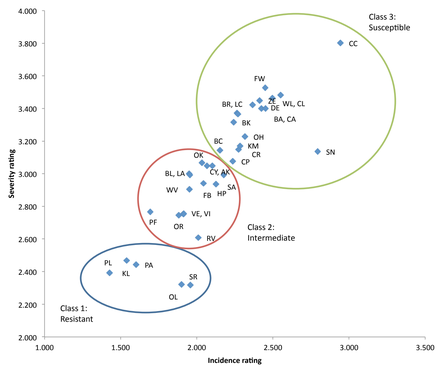




Variation in susceptibility to *P. ramorum* infection in a range-wide collection of Pacific madrone seedlings

Seedlings of Pacific madrone grown from seed collected from the range of the species were inoculated with *Phytophthora ramorum* to examine susceptibility to this pathogen within the species. Seedlings grown from 81 seed sources from 36 sites within 7 ecoregions in the range of Pacific madrone were used. Zoospore inoculum was prepared from one isolate from each of the three clonal lineages of *P. ramorum* and sprayed on six-month-old seedlings in plug trays. Seedlings were incubated under optimum conditions for infection, and disease incidence and severity was rated after 8 days.

Phytophthora ramorum on madrone seedlings. Photo by Kathy Riley, WSU.



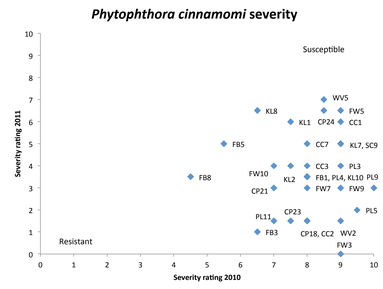
There were significant differences in both incidence and severity of foliar infection caused by each of the three isolates of *P. ramorum*. The NA2 isolate was the most aggressive, EU1 intermediate, and NA1 least aggressive. Cluster analysis of incidence and severity ratings for seed sources of Pacific madrone formed three groups: resistant (24%), intermediate (40%), and susceptible (36%). There was no interaction between *P. ramorum* isolates and susceptibility of madrone seedlings: if plants from a given seed source were resistant to one isolate of *P. ramorum*, they were resistant to the others. There was no relationship between susceptibility and the ecoregions where seed was collected, but there were differences in disease incidence and severity on seedlings among individual sites.

A first look at genetic variation in resistance to the root pathogen *Phytophthora cinnamomi* using a range-wide collection of Pacific madrone

Phytophthora cinnamomi causes root disease and basal canker on a number of hardwood and conifer hosts, including Pacific madrone. In recent years increasing mortality of Pacific madrone and the related shrub species manzanita (*Arctostaphylos* spp.) have been attributed to this disease in California forests.

To obtain information on the genetic variation in resistance to *P. cinnamomi*, greenhouse trials were conducted in 2010 and 2011 on one-year-old seedlings. After inoculation and flooding, seedlings were monitored for six weeks and rated according to the amount of wilting (0-4) and browning of foliage (0-10). Afterwards the condition of the roots was assessed (1-5) and dry weights were taken of roots and aboveground portions of each plant.

Disease severity differed widely between the two trials for some families and less for others (right). Some families were susceptible or resistant in both trials, but most were variable. Cluster analysis results are shown below. There was no relationship of disease resistance with ecoregion or site where the seed was collected.



Seed source	2010	2011
CC1	1	1
KL7	1	1
WV5	1	1
CP24	2	1
KL10	1	2
PL3	1	2
PL9	1	2
CC2	2	2
CC7	2	2
FW5	2	2
FW9	2	2
KL1	2	2
KL2	2	2
PL4	2	2
SC9	2	2
FB5	4	2
FB8	4	2
FW3	1	3
PL5	1	3
CC3	2	3
CP18	2	3
CP21	2	3
CP23	2	3
FB1	2	3
FW7	2	3
PL11	2	3
WV2	2	3
FB3	3	3
FW10	3	3
KL8	3	3

Class
1 susceptible
2 intermediate
3 resistant
4 very resistant

After inoculation all plant pots were placed in 32 oz plastic cups which were then flooded with water. Plants remained submerged in water for two days.

Madrone seedlings two weeks after inoculation with *P. cinnamomi*. Inoculated plants (front) are showing wilt symptoms while check plants (rear) appear healthy.

Acknowledgements

The authors wish to thank everyone who helped to collect seed, identify possible sites for the common garden plantings, and with growing and maintaining the seedlings. We also wish to thank Starker Forests, BLM, CAL FIRE, WSU, and BC Ministry of Forests, who provided space for the plantings. Funding for this project was provided by the US Forest Service FHM Project #WCFHM-11-02.

Washington State University

British Columbia

04/16/2012