Cold Stratification of PACIFIC

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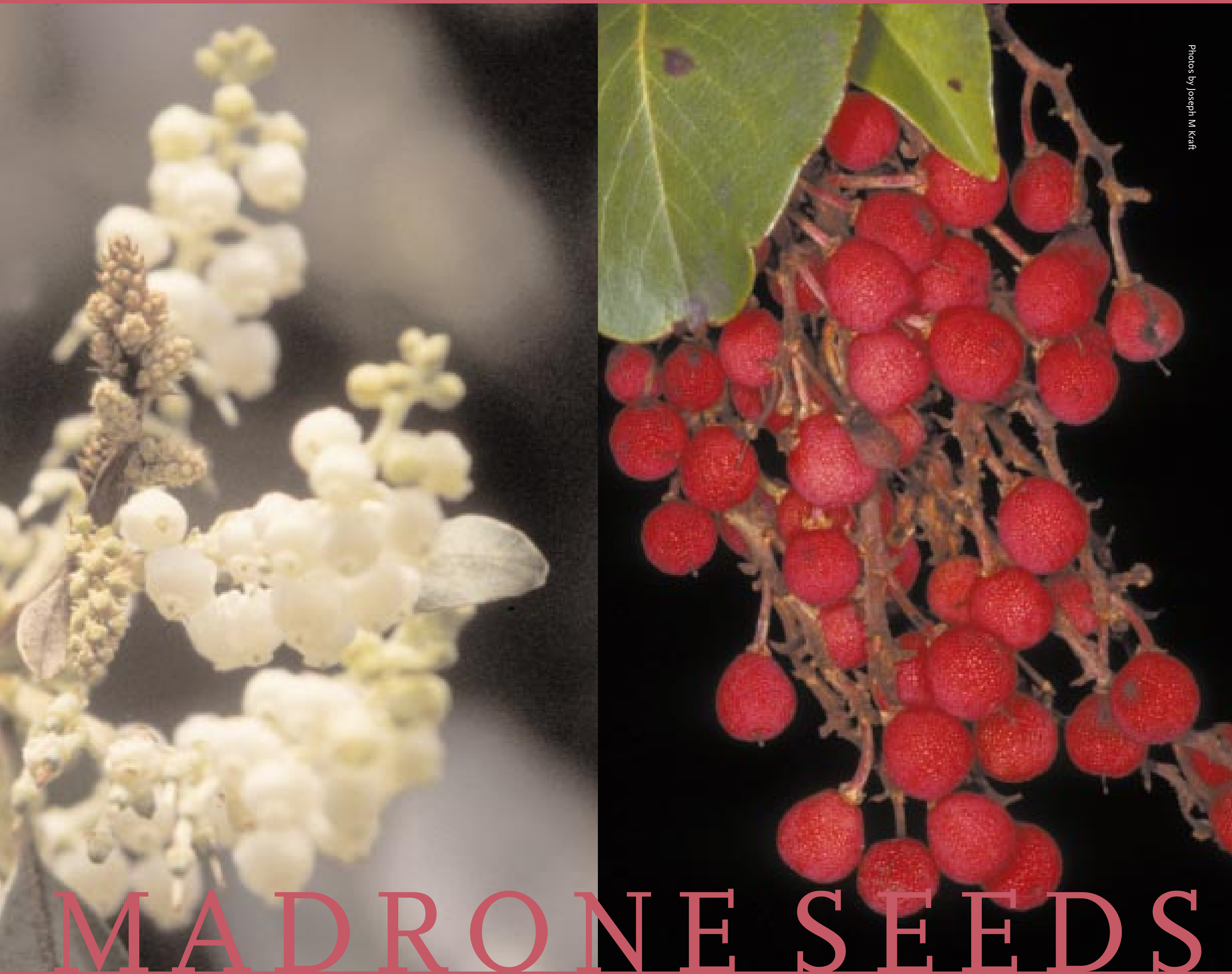
ABSTRACT

Cold moist stratification for 40 d resulted in excellent ($\geq$ 87%) germination for 11 seed lots of Pacific madrone (*Arbutus menziesii* Pursh. [Ericaceae]) from California, Oregon, and Washington. Without stratification, germination was $<$ 2%. Stratification periods longer than 40 d yielded little or no gain for most lots and resulted in premature germination and development of seed coat molds. Germination after 20 d of stratification varied markedly with seed lot (from 10% to 100% of the maximum); 20-d germination was negatively cor-



Photos by Joseph M Kraft

MADRONE SEEDS

related with latitude but not with elevation or mean winter temperature. Growers would need to test individual seed lots to determine if stratification times < 40 d would be adequate; such testing is most likely to be warranted with lots from California or southern Oregon.

KEY WORDS
Arbutus menziesii, germination, seed lots, latitude, elevation

NOMENCLATURE
USDA NRCS (2002)

Pacific madrone (*Arbutus menziesii* Pursh. [Ericaceae]) is native to areas of California, Oregon, Washington, and British Columbia (McDonald and Tappeiner 1990). Interest in growing this species for restoration or reforestation projects, both in forested (McDonald and Tappeiner 1990) and urban (Adams and Hamilton 1999) areas, has increased recently. Madrone berries are an important winter food source for many species of wildlife (Martin and others 1951) as they are available for several months when other food sources are restricted. Limited information is available, however, on how to regenerate Pacific madrone.

Seeds from most North American tree species will not germinate if exposed to moisture but require either special germination conditions or preconditioning to release seed dormancy (Dirr and Heuser 1987; Farmer 1997). The occurrence, nature, or degree of dormancy vary widely and species in the same genus or different species growing in the same locale may have very different types and degrees of dormancy (Krugman and others 1974). Even within a species there are genetic differences in response of seeds to dormancy release treatments; some of these differences appear to be related to elevational or latitudinal gradients (Farmer 1997).

In general, we know that madrone seeds require a cold, moist stratification treatment for germination (Roy 1974; McDonald 1978; McDonald and Tappeiner 1990; McDonald 2003). We lack, however, specific information on how individual seed lots vary in their stratification requirement or if site factors where seeds were collected influence the stratification requirement. Roy (1974) suggested 60 d of stratification was adequate for most lots (based apparently on experiences with California seed lots). McDonald (1978) reported 30 to 40 d were adequate for a mid-elevation California seed source, and initial germination trials in our laboratory for a low-elevation seed source near Olympia, Washington, also indicated 40 d of cold stratification was adequate (Harrington and others 1999). The objective of this study was to test if the results from the low-elevation Olympia lot and the mid-elevation California source would be applicable to seeds from a wider range of elevation and latitude.

MATERIALS AND METHODS

Pacific madrone berries were collected in October and November 1997 from 11 sites in Washington, Oregon, and California (Table 1) that represented a wide range in latitude (> 8°) and elevation (> 1200 m [3937 ft]). The berries were spread out to dry for several days after collection to prevent growth of molds; after drying, the berries were shipped to the Olympia Forestry Sciences Laboratory. Upon arrival, the lots were spread out for additional drying and removal of debris (leaves, twigs). Seeds were extracted from the fruits using the blender method (Harrington and others 1999), thoroughly rinsed, and spread out to dry at room temperature for several days. Dry seeds were stored in small resealable polyethylene bags (~3 mil thick), and then placed in storage at 1 to 4 °C (34 to 39 °F) for about 1 y.

At the start of this study, approximately 300 seeds per lot were assigned to each of 5 stratification treatments: 0, 20, 40, 60, and 80 d. The 80-d treatment was later reduced to 73 d because of high germination during stratification. Starting and ending times for stratification and germination were staggered to reduce the number of seeds to be handled at a time. For each treatment, seeds were soaked overnight in sterilized distilled water at room temperature, rinsed thoroughly with sterilized distilled water, blotted to remove excess water, put into resealable, polyethylene bags (thickness ~3 mil), and stored in a refrigerator for the specified time. Temperature conditions in the refrigerator were monitored with a Hobo™ temperature recorder. Mean temperature was 3 °C (37 °F) with most temperatures between 1 and 4 °C (34 and 39 °F).

At the end of the stratification periods, any seeds that had germinated were counted and then discarded. Ungerminated seeds were thoroughly rinsed, then placed in a small beaker filled with dilute bleach (0.095% sodium hypochlorite) solution for ~20 to 30 min to retard growth of seed coat molds; the beaker's contents were stirred occasionally using glass rods. After further rinsing with sterile distilled water, 4 or 5 replications of 50 seeds each were spread on damp germination blotters in plastic Petri dishes. Very moldy seeds, and those that appeared to be shriveled or empty were not transferred to the petri dishes. Seeds in the 0-d stratification treatment were treated in the same manner as those assigned to the longer stratification treatments (soaked overnight, treated with the bleach solution, spread on blotters in Petri dishes).

The dishes were placed, in a random distribution by lot and stratification time, inside an Enconair® germinator (90% relative humidity [RH], 10-h photoperiod, 30 °C [86 °F]; 95% RH, 14-h dark, 20 °C [68 °F]). Conditions were very consistent throughout the trials; temperature was maintained within 0.2 °C (0.4 °F) and RH within 5% of set values.

Germination was recorded daily for the first 3 to 5 d, and 2 or 3 times per week thereafter until germination was complete or up to a maximum of 20 d. Germinated seeds (primary roots > 1 cm [0.5 in] long) were removed from the dishes as they were recorded. Sterile distilled water was added to the blotters as necessary to keep the pads and seeds moist. Following the final count, any remaining seeds were squeezed and/or cut to determine if they were empty, had been destroyed by fungi inside the seed coat, or had seed coat molds growing on the outside of the seeds.

Mean germination for the 4 or 5 dishes for each seed lot and stratification period was used in all statistical analyses. We judged results to be significant at $P \leq 0.05$. Regression analysis (Proc Reg; SAS Institute 1999) was used to examine the effects of elevation, latitude, and winter temperature. Because seed lots differed most in their response to 20 or 40 d of stratifica-

TABLE 1

Location and general site description for Pacific madrone seed lots, and notation of which lots were used in separate analyses of elevation or latitude.

Seed lot	Nearby city or town	Number of trees sampled	North Latitude	West Longitude	Elevation (m) [a]	Mean Daily Temperature (°C) [a] November–March	Analysis
Bellevue High School	Bellevue, Washington	1	47° 36'	122° 20'	<100	6.3	Latitude
Priest Point Park	Olympia, Washington	1	47° 04'	122° 53'	<100	4.9	Latitude
Grants Pass	Grants Pass, Oregon	5	42° 27'	123° 36'	300	6.4	Latitude
Greyback	Happy Camp, California	7	41° 56'	123° 29'	910	3.1 [b]	Elevation
Walker Creek	Happy Camp, California	1	41° 50'	123° 10'	600	5.0 [b]	Elevation
Wingate	Happy Camp, California	4	41° 43'	123° 26'	300	7.0 [b]	Elevation
Elk Creek	Happy Camp, California	6	41° 38'	123° 19'	730	4.2 [b]	Elevation
Persido	Happy Camp, California	1	41° 32'	123° 31'	275	7.1 [b]	Latitude and Elevation
Specimen Creek	Orleans, California	1	41° 20'	123° 10'	1270	0.3 [b]	Elevation
Ishi – Pishi	Orleans, California	1	41° 19'	123° 31'	140	7.7 [b]	Elevation
Hopland	Hopland, California	8	39° 00'	123° 04'	300	9.3 [b]	Latitude

[a] Conversions: 1 m = 3.3 ft; (°C • 1.79)+32 = °F.
[b] Estimated by applying the average environmental lapse rate (0.65 °C per 100 m [1.17 °F per 328 ft] in elevation) to the mean daily temperature of a nearby NOAA weather station.

tion, percent germination after 20 d and after 40 d of stratification were used as the dependent variables in the regression analyses. First, the data were analyzed for the effects of latitude using just the 5 low-elevation lots (< 300 m [984 ft]) that represented the geographic range sampled (from 39° N to 47° 36' N); the independent variables in this set of regression analyses were latitude and mean winter temperature. Next, 7 Klamath National Forest sites that covered range in elevation (from 140 to 1270 m [459 to 3937 ft]) at 1 latitude (near 41° N) were assessed for the effects of elevation; the independent variables in the regression analyses were elevation and mean winter temperature. One seed lot, Persido, was used in both groups. Mean temperature during the winter (November 1 to March 30) was calculated from the nearest weather station and adjusted for the actual elevation of the seed source using an average moist adiabatic lapse rate of 0.65 °C per 100 m (1.17 °F/ 328 ft). We used mean winter temperature as an index of the amount of chilling the sources would have received naturally. Finally, all sites were combined for a third set of regression analyses to examine the effects of elevation, latitude, and mean winter temperature (in 1- or 2-variable models).

RESULTS

All lots had ≥ 99% filled seeds, no or minimal germination without stratification, and responded to cold stratification with increased germination (Figure 1). Regardless of lot, stratification of at least 20 d resulted in the first germinants appearing within 1 to 3 d after being placed in the warm-moist

MOLD AND MADRONE SEEDS

Development of seed molds can be a problem with many species but is a particular problem with madrone. Many people have tried planting madrone berries (rather than extracting and then planting the seeds), but the berries quickly were covered with mold and few seedlings emerged. In our early trials with madrone seeds, we had trouble with seed coat molds that developed during both stratification and germination. We modified our procedures and now have very few problems. Based on our experience we would recommend using:

- a seed separation procedure that results in very clean seeds (no pulp should be visible on the seeds);
- products made from non-porous materials such as a stainless steel sieve (kitchen strainer or tea infuser) for rinsing and transferring seeds (Do not use cheesecloth!);
- sterilized distilled water for rinsing seeds (and blotting excess water from seeds);
- a dilute bleach solution (0.095% sodium hypochlorite) to treat seeds prior to placing them in the germinator.

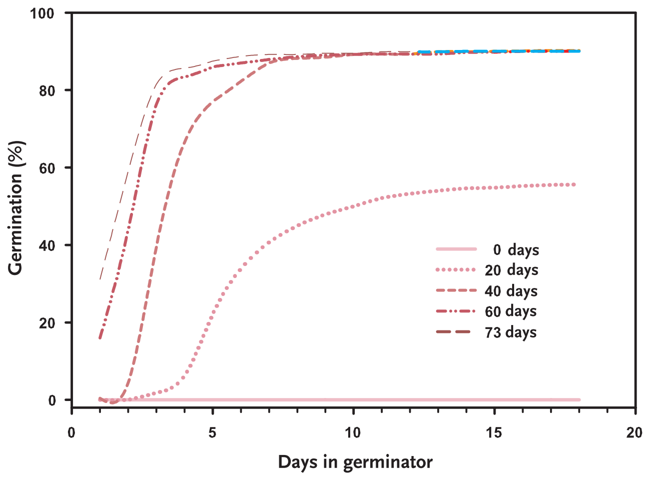


Figure 1. Germination by time in germinator for all lots combined.

TABLE 2

Germination (%) during stratification by seed lot and length of cold stratification treatment. No germination occurred during stratification for the 20- or 40-d periods.

	Stratification period (d)	
Seed lot	**60**	**73**
Bellevue High School	3	11
Priest Point Park	7	15
Grants Pass	7	30
Greyback	11	28
Walker Creek	31	38
Wingate	13	25
Elk Creek	12	35
Persido	15	51
Specimen Creek	11	31
Ishi – Pishi	14	35
Hopland	16	40

germinator environment. All lots except one had greater germination with 40 d than with 20 d of stratification, and germination after 60 or 73 d of stratification was generally equal or within 5% of the germination after 40 d of stratification. Averaged over all lots, less than 2% of the unstratified seeds germinated, 9% to 93% of the 20-d, and 77% to 100% of those stratified for 40 d or more germinated (Figure 1). At the end of the stratification periods, no obvious molds were growing on the seeds from the 20- or 40-d treatments, and only a small amount of mold was growing on the 60- and 73-d seeds. No premature germination (germination while in stratification) was observed during the 20- or 40-d stratification treatments but premature germination occurred in all lots during the 60- and 73-d stratification treatments (Table 2).

Lots varied in their response to stratification (Figure 2). Only the 2 northernmost lots (Bellevue High School and Priest Point Park) showed any significant benefit from longer stratification; for these lots the maximum germination was at 60 or 73 d and the maximum value was 9% or 10% greater than the value after 40 d. Premature germination during the 73-d stratification period varied substantially across lots (Table 2) and was least (≤ 15%) for the 2 northernmost seed lots. One southerly, mid-elevation lot (Wingate) reached maximum germination (93%) after only 20 d of stratification, and 2 other California lots (Walker Creek and Specimen Creek) had germination ≥ 79% with 20 d of stratification. One seed lot (Specimen Creek) was remarkable for the absence of mold associated with its seeds during all phases of the trial.

In the analysis of the 5 low-elevation lots, germination after 20 d of stratification was negatively related with latitude, but the relationship was nonsignificant (Table 3); the relationship with mean temperature was even weaker. The results from the analysis of the 5 lots after 40 d of stratification were similar to the 20-d results for latitude (negatively related but nonsignificant), however, winter temperature was significant in this analysis. In the analysis of the 7 lots at 1 latitude, neither elevation nor winter temperature were significant in explaining germination after 20 or 40 d of stratification. When all 11 lots were combined for analysis, latitude was the only variable in a 1-variable model with a significant R^2 for germination; latitude was significant both in the 20-d and 40-d models. Adding a second variable to the model predicting germination after 20 d of stratification did not result in any significant results; however, the 2-variable models of elevation plus latitude, and elevation plus mean winter temperature were both significant in predicting germination after 40 d of stratification.

DISCUSSION

Early growers of Pacific madrone tried stratification periods of 31 to 93 d resulting in a recommendation that 60 d was probably adequate for most lots (Roy 1974). A later trial (McDonald 1978) with a California seed source (39° 30' N, ~780 m [2558 ft] elevation) indicated excellent germination (94%) with 30 to 40 d of stratification at 2 °C (36 °F), premature germination after 50 d, and seed mortality from mold increased over time. Our previous trials (Harrington and others 1999) with a low-elevation lot from western Washington had similar results. In this trial, we found that 40 d of stratification was adequate for all 11 Pacific madrone seed lots. Because these 11 lots represent a range of more than 8° in latitude and 1200 m (3937 ft) in elevation, we feel confident that a 40-d stratification period would be adequate for most, if not all, madrone seed lots.

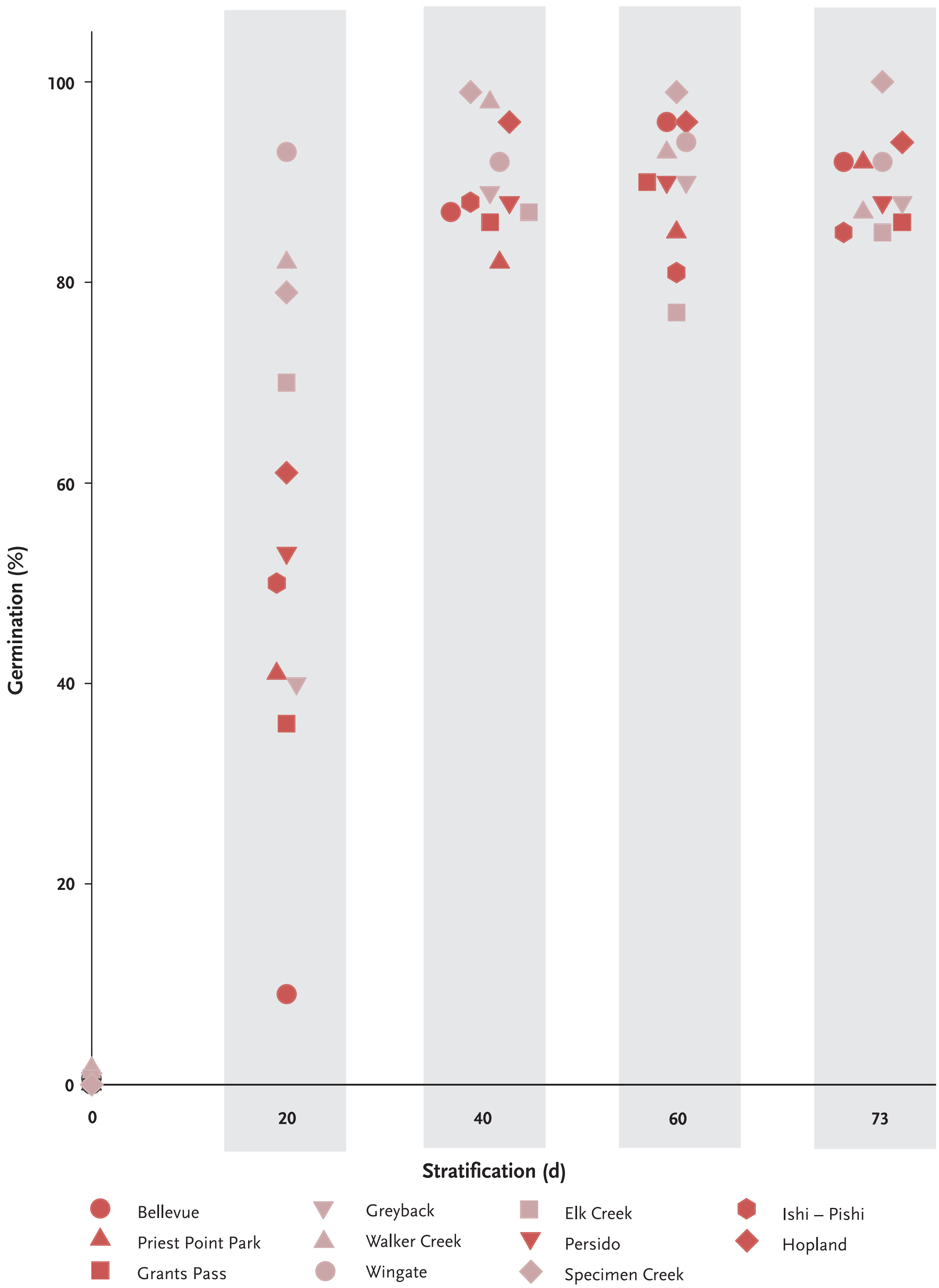


Figure 2. Percent germination for each seed lot after 0, 20, 40, 60 and 73 d of stratification. Data points for each seed lot are shown dispersed around the discrete stratification periods only for clarity.

COLLECTING, TREATING, STORING, AND GERMINATING PACIFIC MADRONE SEEDS

- COLLECT RIPE FRUITS
- EXTRACT SEEDS USING BLENDER OR SIMILAR DEVICE
- REMOVE AS MUCH DEBRIS AS POSSIBLE
- RINSE THOROUGHLY WITH TAP WATER

- STORING SEEDS FOR USE LATER
  – Spread seeds in single layer on absorbent material.
  – Dry for several days at room temperature.
  – Place in sealed container and store at 3 °C (37° F). Seed viability will remain high for several years under these conditions.
  – When needed for planting, remove seeds and soak overnight.

- SEEDS FOR IMMEDIATE USE
  – Blot to remove excess water.
  – Place in sealed container (plastic bags okay). Do not use cheesecloth to hold or transfer seeds.
  – Store seeds at 3 °C (37 °F) for 40 d.
  – Remove from cold, discard any damaged seeds, sow immediately. If sowing into cold soils, a bleach treatment is recommended (see germination tests below).

- GERMINATION TESTS
  – After seed extraction, rinse with sterilized distilled water instead of tap water.
  – After stratification, discard seeds that germinated during storage.
    - Rinse with sterile distilled water, then soak in 1 part household bleach to 50 parts sterile distilled water for about 25 min. Rinse with sterile distilled water.
    - Spread on pads or blotters in Petri dishes or similar containers.
    - Place in germinator (10-h day, 30 °C [86 °F]; 14-h night, 20 °C [68 °F]) with high relative humidity.
    - Check daily. Keep pads moist. Classify seeds as germinated when emerging roots are > 1 cm (0.5 in) long.

Magnified image of madrone seeds, showing the ridges on the seed surface that make them difficult to clean. Photo by Joseph M Kraft

Effectiveness of the 20-d stratification period varied widely compared to longer stratification times (10% to 100% of maximum). Also, 40-d stratification showed no negative effect on the lots with high (≥79%) germination at 20 d; however, growers may wish to determine which lots will have their stratification requirement completed in less than 40 d to allow for additional flexibility in scheduling stratification and sowing (see sidebar this page). Testing of individual lots is most likely to be warranted for sources from southern Oregon and California.

Some northern seed lots might have more germinative energy or slightly greater germination if stratified for longer than 40 d; even for most northern seed lots, however, we suspect the slight benefits associated with longer stratification periods would not outweigh the potential negative impacts of premature germination and growth of seed coat molds. Both seeds that germinated prematurely and those that were moldy would usually be discarded and thus would represent a loss in seed yield. For northern seed lots, stratification times of 45 or 50 d could be tried. It may be possible to reduce premature germination during stratification periods longer than 40 d by using colder temperatures than those used in this trial (for example, a mean temperature of 1 or 2 °C [34 or 36 °F] rather than 3 °C [37 °F]).

The relationship between seed germination and the elevation of the seed source has been studied in several eastern hardwood species. In general, low-elevation sources of several hardwood species in the southern Appalachian mountains had lower chilling requirements for seed germination than high elevation sources (Farmer and Barnett 1972; Farmer 1974;

TABLE 3

Regression coefficients (R^2) for 1- and 2-variable models predicting percent germination of madrone seed after 20 or 40 d of stratification. See Table 1 for identification of the latitude and elevation seed lots. Coefficients significant at $P \leq 0.05$ are in bold.

Lots used in analysis	Model variables	Germination after 20 d	Germination after 40 d
5 Latitide	Latitude (L)	0.67	0.62
5 Latitude	Winter Temperature (WT)	0.32	**0.98**
7 Elevation	Elevation (E)	0.01	0.27
7 Elevation	WT	0.21	0.30
All	E	0.18	0.34
All	L	**0.40**	**0.36**
All	WT	0.03	0.05
All	E + L	0.43	**0.56**
All	E + WT	0.30	**0.56**
All	L + WT	0.46	0.45

Barnett and Farmer 1978; Farmer and Cunningham 1981). In our study, elevation alone had only a fairly weak relationship with germination after either 20 d or 40 d of stratification (in the analyses of the 7 elevation lots or all 11 lots). Elevation added as a second variable did significantly improve the models of germination after 40 d of stratification but the range in germination was not great (82% to 99%).

Latitude of the seed lot was the best predictor of germination after 20 d and 40 d for all lots and had the highest R^2 for germination for the 5 latitude lots. In other species, the effects of latitude of seed lot on the need for stratification have been quite mixed. Greater chilling was required for more northerly lots of sweetgum (*Liquidambar styraciflua* L. [Hamamelidaceae]) (Wilcox 1967; Winstead 1971), red oak (*Quercus rubra* L. [Fagaceae]) (Farmer 1974), and eastern hemlock (*Tsuga canadensis* L. Carr. [Pinaceae]) (Stearns and Olson 1958) but decreased chilling was required for northerly sources of eastern white pine (*Pinus strobus* L. [Pinaceae]) (Mergen 1963; Fowler and Dwight 1964). For paper birch (*Betula papyrifera* Marsh. [Betulaceae]), southern sources had a chilling requirement but more northern sources did not (Bevington 1986), while for Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco. [Pinaceae]), the reverse is true and northern sources generally benefit from stratification while southern sources do not (Stein and Owston 2003). It has been suggested that dormancy is an adaptation to fluctuating, uncertain environments (Levins 1969); thus, some species may have adapted to variable winter conditions with the development of a stratification requirement to prevent germination during a mid-winter thaw.

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