Cold Stratification Delays Germination of Black Huckleberry Seeds

Danny L. Barney¹

Department of Plant, Soil, and Entomological Sciences, University of Idaho, 2105 N. Boyer Avenue, Sandpoint, ID 83864

Bahman Shafii and William J. Price

Statistical Programs, College of Agriculture, University of Idaho, Moscow, ID 83844-2337

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Most *Vaccinium* species germinate without cold stratification (Dirr and Heuser, 1987), but little research has been published on the physiology of seed germination for *V. membranaceum*. Dirr and Heuser (1987) referred to findings by the Arnold Arboretum that *V. membranaceum* seeds germinate without pretreatment, but details on that work were absent. Minore et al. (1979) stated that stratification was unnecessary for *V. membranaceum* seed germination, but provided no details of supporting research. McLean (1967) conducted stratification trials on *V. membranaceum* and found that stratification at 1 °C for 30, 60, 90, 120, or 150 d failed to improve germination. McLean reported only on maximum germination percentages without describing other parameters of germination. Furthermore, statistical treatments supporting the conclusions were absent. The following experiment was conducted to document the effects of cold stratification on germination dynamics of *V. membranaceum*.

Ripe *V. membranaceum* berries were collected in August 1996 from the Kaniksu National Forest in Bonner County, Idaho at an elevation of 1600 m. Seeds were extracted, cleaned, air-dried at room temperature for 7 d to a moisture content of 8.0% on a fresh weight basis, and stored at 0 to 4 °C for ≈180 d. Dry seeds were sieved and those 500–600 µm in diameter were retained. *V. membranaceum*

averages 30 mg per 100 seeds (Vander Kloet, 1988). Lots of 100 seeds were surface sterilized for 20 min in 0.5% sodium hypochlorite solution and rinsed in sterile water. Previous tests (unpublished) in our laboratory established that surface sterilization for 20 min had little, if any, effect on maximum cumulative germination, as compared to nonsterilized controls. Seeds were placed on moist, sterile filter paper inside 10 × 1.5-cm plastic petri dishes, with eight dishes (replications) containing 100 seeds per treatment.

Dishes sealed with plastic film were placed inside a refrigerator at 1 to 3 °C for 0, 14, 28, 42, or 56 d. After stratification, the dishes were moved into an environmental chamber set at 28 °C day/23 °C night. Light was provided 12 h/d by 20 W wide-spectrum plant and aquarium fluorescent tubes. Seeds were examined under a magnifying lens and those exhibiting any radicle emergence were counted and removed from the dishes every 7 d. Sterile water was added to the dishes as necessary to keep the filter paper moist.

Cumulative germination was modeled using a logistic growth curve model, as described by Shafii and Barney (2001):

$$y = M \{1 + \exp[-K \cdot (t - L)]\}^{-1} \quad (1)$$

where y is the cumulative percentage of germination at time t , M the asymptote (theoretical maximum for y), L the time to 50% germination (T50), and K being proportional to the rate of increase in germination. Separate logistic models were estimated for each of the five stratification treatments. Model estimations, assessments, and comparisons were carried out using the methods outlined in Shafii (1995) and Shafii and Barney (2001).

Stratification at 1 to 3 °C delayed germination (Table 1). The T50 estimate for nonstratified seeds was 27 d, while T50 values for stratification treatments varied from 32 to 39 d, averaging 9 d longer than for nonstratified seeds. Statistical contrasts between treatment and control T50 estimates were all highly significant. The germination rate parameter, K , also increased with stratification, but the differences were statistically significant for

only the 28- and 56-d treatments. Stratification increased germination uniformity for seeds germinating within 28 d of sowing.

Maximum cumulative germination ranged from 63% (56 d stratification) to 70% (28 d stratification). Differences between stratified and nonstratified seeds were significant ($P < 0.003$) only for the 14- and 28-d treatments. While statistically significant, the importance of these differences in practical propagation is limited. Stratification for 14 d increased the germination percentage from 65% to 69%, whereas stratification for 28 d increased germination to 70%. In associated trials (Shafii and Barney, 2001), fresh, nondried, nonstratified *V. membranaceum* seeds germinated at percentages about equal to those observed in this trial. The mechanism by which stratification at 1 to 3 °C delayed germination remains to be determined.

Stratification at 1 to 3 °C for 14 or 28 d slightly increased maximum germination percentages for *V. membranaceum*. Any stratification from 14 to 56 d, however, delayed the onset of germination. For propagation purposes, stratification is unnecessary and could delay seedling emergence.

Table 1. Parameter estimates for stratification treatments (moist chilling at 1 to 3 °C) to *Vaccinium membranaceum* seeds.

Treatment duration (d)	L (d) ²	K ³	M (%) ⁴
0	27	0.8	65
14	32*	0.9	69*
28	39*	1.1*	70*
42	37*	1.0	65
56	36*	1.1*	63

²L = T50; days from sowing to 50% germination.

³K = germination rate index.

⁴M = maximum cumulative percentage of germination.

*Parameter estimates that are significantly different from those of the control ($P \leq 0.05$).

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¹To whom requests for reprints should be addressed. E-mail address: dbarney@uidaho.edu