

# Drying and Cold Storage Affect Germination of Black Huckleberry Seeds

**Bahman Shafii**

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

**Danny L. Barney<sup>1</sup>**

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

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**Abstract.** Trials were conducted to determine the effects of air drying and cold storage on black huckleberry (*Vaccinium membranaceum* Douglas ex Hooker) seeds. Treatments included fresh seeds, seeds air-dried for 7 days, and those air-dried and stored at 2 to 3 °C for either 1 or 7 years. Germination was measured every 7 days. The time course of germination was modeled using a logistic growth curve from which days to 50% germination (T50), germination rate index, and maximum germination percentages were estimated. Germination curves of dried and of dried and cold-stored seeds were significantly different from that of fresh seeds. Seeds stored for 1 or 7 years had germination percentages similar to those for the fresh, nondried seeds. Air drying for 7 days reduced the maximum germination percentage from 73% to 59% (fresh seeds). This induced dormancy was gradually lost during cold storage of dry seeds. Cold storage of air-dried seeds was an effective method for preserving *V. membranaceum* germplasm for at least 7 years.

Black or thin-leaved huckleberry grows in forested areas throughout the northwestern United States and western Canada, with small, disjunct populations in Michigan and Arizona (Vander Kloet, 1988). This species was an important food source for certain Native American nations in the northwest and remains popular with recreational and commercial pickers. In recent years, commercial demand for the fruit has increased markedly while the size and quality of natural stands have declined (Minore, 1972) and access to public forests has become more restricted.

Although interest in its cultivation is increasing, the species has not yet been domesticated and relatively little research has been done to determine how various seed and storage treatments affect germination. Seeds of the related species *V. angustifolium* Aiton and *V. corymbosum* Linnaeus may be stored at 1 to

4 °C for up to 12 years and retain reasonable germination percentages (Dirr and Heuser, 1987). Aalders and Hall (1975) noted that storing *V. angustifolium* seeds dry at room temperature (ca. 21 °C) for 7 months reduced germination percentages, whereas germination was not affected by storing the dry seed at 1, –2, or –23 °C. Stark and Baker (1992) found that storing *V. globulare* Rydberg [synonymous for *V. membranaceum* (Vander Kloet, 1988)] at –20 °C for as little as 1 year decreased germination percentage; after 7 years at –20 °C, only 2% of the seeds germinated. The following study was conducted to document the effects of drying and refrigerated storage on germination of *V. membranaceum*.

## Materials and Methods

**Plant materials.** Seeds were collected from a 1-ha site at an elevation of 1600 m in the Kaniksu National Forest in Bonner County, Idaho, during the summers of 1989, 1995, and 1996. Seeds were extracted from ripe fruit by macerating the berries using a sieve and decanting the pulp and hollow seeds during repeated water washes. The seeds were either sown fresh or air-dried at room temperature for 7 d. After drying, the seed was either sown immediately or placed inside sealed plastic bags and stored in the dark at 2 to 3 °C for 1 or 7 years. Seed moisture content was determined gravimetrically after 1 h at 130 °C, and expressed on a fresh weight basis. Moisture contents were 9.6%, 9.9%, and 8.9% for seed collected in 1996 and sown after 7 d drying, seed collected in 1995 and stored for 1 year,

and seed collected in 1989 and stored 7 years, respectively. Before sowing, seeds with diameters smaller than 600 µm were removed using a sieve, and broken and malformed seeds were removed under a magnifying lens. Seeds of *V. membranaceum* average 0.3 mg per seed (Vander Kloet, 1988). Lots of 100 seeds were sealed inside fine mesh bags and washed in running tap water for 24 h, surface sterilized for 20 min in 0.5% (w:v) sodium hypochlorite in water (10% household bleach solution), and rinsed three times for 5 min each in sterile water. Germination tests with nonsterilized *V. membranaceum* seeds are complicated by seed-borne fungi. Previous trials in our laboratory established that surface sterilization for 20 min with sodium hypochlorite did not reduce maximum germination percentages for *V. membranaceum* seeds, although T50 and rate parameters were affected.

**Germination tests.** Tests were conducted using 10 × 1.5-cm plastic petri dishes lined with one sheet of Whatman No. 2 filter paper (Whatman Intl., Maidstone, England) moistened with sterile, distilled, deionized water. Under sterile conditions, the seeds were removed from the mesh bags and placed inside petri dishes, which were then sealed with Parafilm® (National Can Co., Neenah, Wis.). Each dish contained 100 seeds, with seven dishes (replications) per treatment. After sowing, the dishes were kept at 28°C day/23 °C night with a 12-h photoperiod inside an environmental chamber. Light was provided by two 20-W wide-spectrum plant and aquarium fluorescent tubes (General Electric F20T12-PL/AQ, Cleveland). Seeds were examined under a magnifying lens and those exhibiting any visible radicle emergence were counted and removed from the dishes every 7 d. Sterile, distilled, deionized water was added to the dishes as necessary to keep the filter paper moist.

**Statistical procedures.** Typically, cumulative germination of a population of seeds appears as an S-shaped curve, in which a lag phase representing delay in onset is followed by an approximately linear phase of radicle emergence, and ending with an upper asymptote at the maximum germination percentage. The ideal description of germination should include each of these three phases.

Many mathematical growth models have been proposed to describe the time course of germination (e.g., Brown and Mayer, 1988; Shafii et al., 1991). Most of these models are nonlinear, and given proper specification, can provide parameter estimates with relevant biological interpretations. In this study, an excellent fit was obtained using the logistic function:

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

with *y* being the cumulative percentage of germination at time *t*, *M* the asymptote (theoretical maximum for *y*), and *L* the T50 time scale constant, with *K* being proportional to the rate of increase in germination. This function is commonly used in modeling germination (Hsu et al., 1984; Janssen, 1973;

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

Roche et al., 1997; Thompson et al., 1994; Torres and Frutos, 1990), is sigmoidal and symmetrical, and is similar to the cumulative normal distribution. Parameter estimation is accomplished with an iterative nonlinear least square technique, e.g., the Gauss–Newton algorithm.

Parameter L in Eq. [1] measures the days required to reach 50% of maximum estimated germination (T50). Parameter K measures the rate of increase, and hence the spread of germination over time, providing an index of germination speed. Finally, parameter M measures the maximum germination percentage. Germination rate at T50 was calculated as  $MK/4$ .

Separate logistic models were estimated for each of the four specified treatments. Lack of fit tests were completed to measure the adequacy of fit for each logistic model. Residual analyses were also completed to examine the validity of the nonlinear regression assumptions for each model. A full model dummy variable regression procedure (Shafii, 1995) was used to contrast specific parameters among estimated models or test for coincidence (equality) of regression lines. Statistical computations and graphics were performed using SAS/STAT (SAS Institute, 1991).

## Results and Discussion

Estimated maximum germination percentages ranged from 59% (air-dried 7 d) to 74% (fresh), and T50 from 16 to 21 d (Table 1). The treatments appeared to have varying spread of germination over time with the rate parameter, K, estimated in the range of 1.1–1.6. The germination rates at T50 for seeds that were: 1) fresh; 2) dried 7 d; 3) stored 1 year; and 4) stored 7 years were 20, 24.2, 24.9, and 18.6, respectively. Parameter estimates for each specified treatment were significant based on the 95% asymptotic confidence limits (Table 1), suggesting that the model was reasonable and all parameters were required. In addition, the lack of fit tests were nonsignificant ( $P > 0.05$ ) indicating an adequate model fit for each treatment. Furthermore, the correlation coefficients for the parameter estimates associated with each model were  $<0.57$ , indicating that the models were not overparameterized.

For each of the four treatments, the logistic model followed data trends (Fig. 1). The expected S-shaped pattern was evident; however, seeds stored 1 or 7 years appeared to vary more in germination over time than did those sown fresh or air-dried for 7 d, as is evident from comparing the observed data points with the predicted germination curves. This finding relates to the importance of uniformity of seed germination for horticultural crops, as discussed by Hacısalihoglu et al. (1999). The results suggest that aging, even in refrigerated storage, can potentially reduce germination uniformity of *V. membranaceum*. Studentized residuals plotted against time and against predicted values of cumulative germination for each treatment were also examined (data not shown). In each case, the residuals were evenly

Table 1. Parameter estimates for the logistic model for germination of *V. membranaceum* seeds, along with asymptotic standard errors and lower and upper 95% confidence limits.

Seed treatment	Parameter	Estimate	Asymptotic SE	95% Asymptotic confidence limits	
				Lower	Upper
Fresh	L <sup>z</sup>	19.1	0.77	17.6	20.7
	K <sup>y</sup>	1.08	0.11	0.86	1.30
	M <sup>x</sup>	74.0	1.4	70.6	76.3
	F <sup>w</sup> = 1.265	P > F <sup>w</sup> = 0.2806			
Dried 7 d	L	16.2	0.55	15.1	17.3
	K	1.64	0.18	1.27	2.00
	M	59.0	1.0	56.9	60.6
	F = 1.435	P > F = 0.1985			
Stored 1 year	L	19.1	0.67	17.8	20.5
	K	1.42	0.17	1.09	1.75
	M	70.0	1.3	67.3	72.5
	F = 0.954	P > F = 0.4792			
Stored 7 years	L	21.3	0.97	19.4	23.3
	K	1.05	0.13	0.79	1.31
	M	71.0	1.8	67.6	74.7
	F = 1.3967	P > F = 0.2144			

<sup>z</sup>L = T50; days from sowing to 50% germination

<sup>y</sup>K = Germination rate index

<sup>x</sup>M = Maximum cumulative germination percentage

<sup>w</sup>F represents the calculated F ratio for the lack of fit, and P > F is the probability of larger F.

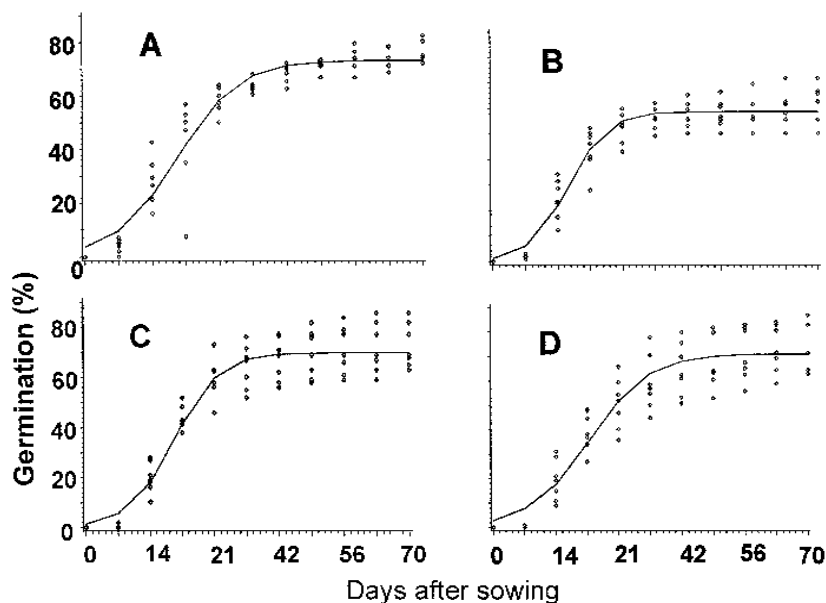


Fig. 1. Logistic model fit to cumulative germination of *V. membranaceum* for seeds (A) sown fresh, (B) air-dried for 7 d, (C) stored for 1 year, or (D) stored for 7 years. Points are values for seven replicates for each time of observation.

and randomly distributed about zero with acceptable magnitudes.

Contrasts performed on the estimated regression parameters revealed that drying plus cold storage significantly altered germination behavior in comparison with that of fresh seeds (Table 2). Drying and storage for 1 year increased the germination rate, K, from 1.1 to 1.4 and reduced maximum predicted germination by  $\approx 4\%$  (Table 1). Both of these effects were highly significant ( $P < 0.003$ ), although T50 for the freshly-sown and 1-year-old seeds were statistically similar ( $P = 0.9696$ ) (Table 2). Drying and storage for 7 years increased T50

from 19.1 to 21 d ( $P = 0.0013$ ), whereas rate and extent parameters were statistically similar ( $P = 0.7476$ , and  $P = 0.0633$ , respectively) to those of fresh seeds (Table 2).

For both storage treatments, the differences observed, while statistically significant, were minimal and partially a function of the large number of samples analyzed. From a practical perspective, cold storage of dried seeds did not adversely affect propagation. For example, seeds stored for 7 years attained 50% germination only 2.2 d after fresh seeds. Likewise, storage for 1 year reduced predicted germination percentages by  $<5\%$  (Table

Table 2. Contrasts for parameters and joint hypothesis of L, K, and M (lines) for the estimated regression model of *V. membranaceum* seeds sown fresh vs. after other drying and storage treatments.

Contrast	df	F <sup>z</sup>	P > F <sup>z</sup>
<i>Fresh vs. dried 7 d</i>			
L <sup>y</sup>	1	20.053	0.0001
K <sup>x</sup>	1	12.610	0.0004
M <sup>w</sup>	1	164.442	0.0001
Lines	3	55.594	0.0001
<i>Fresh vs. stored 1 year</i>			
L	1	0.001	0.9696
K	1	7.942	0.0052
M	1	9.309	0.0025
Lines	3	4.984	0.0022
<i>Fresh vs. stored 7 years</i>			
L	1	10.596	0.0013
K	1	0.1037	0.7476
M	1	3.475	0.0633
Lines	3	9.575	0.0001

<sup>z</sup>F represents the calculated F ratio for the specified contrasts, and P > F is the probability of larger F.

<sup>y</sup>L = T50; days from sowing to 50% germination

<sup>x</sup>K = Germination rate index

<sup>w</sup>M = Maximum cumulative germination percentage

1). Indeed, predicted cumulative percentage germination responses for the aforementioned treatments appeared to have very similar patterns over time (Fig. 1).

Some differences seen in the fresh and stored treatments may be unrelated to drying and storage. Plots of predicted germination curves vs. observed values showed that seeds stored for 1 or 7 years displayed greater variability in germination than did fresh seed, particularly 28 d or more after sowing (Fig. 1 A, C, and D). Although seeds for fresh and stored treatments were collected from the same site each year, test samples came from pooled collections made throughout a 1-ha colony. The required sampling protocol probably resulted in genetic differences among samples. In addition, variable environmental conditions at the sites during the three collection years may have affected germination responses.

More interesting than the minor differences in germination parameters among fresh and stored seeds were the differences between fresh seeds and those dried for 7 d. The samples for these two treatments were taken from the same pooled collection of seeds made on 26 Aug. 1996. Despite the lack of genetic and environmental variability, dried seeds exhibited significantly different germination parameters (Tables 1 and 2) and greater variability (Fig. 1 A and B) than did fresh seeds. Dried seeds germinated more rapidly than did fresh seeds, as evidenced by a reduction in T50 from 19.1 to 16.2 d and an increase in rate of germination from 1.1 to 1.6. Also, the predicted maximum germination percentage decreased significantly from 74% for

fresh seeds to 59% for dried seeds (Tables 1 and 2). This inhibition in germination may be attributed to induced or secondary dormancy that results from drying (Berrie, 1984), and is similar to the induced dormancy seen in hazel (*Corylus avellana* L.) (Jarvis, 1975). Other factors may also be involved.

The induced dormancy declined during storage. In a separate trial, *V. membranaceum* seeds collected from the same site on 1 Aug. 1996, were air-dried and stored at 2 to 3 °C for 6 months. The estimated maximum germination percentage was 64%. Germination percentages of seeds treated similarly and stored for 1 or 7 years were 70% and 71%, respectively; the latter was not statistically different from the value for fresh seeds. While genetic and environmental factors for the various seed lots may have contributed to the observed differences, drying *V. membranaceum* seeds appears to induce a dormancy that disappears during cold storage. The controlling mechanism remains to be determined.

The dormancy induced by drying provides a potential survival advantage. Most *V. membranaceum* fruits ripen between mid-July and fall frosts, depending on elevation and weather. In the huckleberry's native region, summer weather conditions are typically hot and dry, with occasional thunderstorms. Seedlings of *V. membranaceum* are small, delicate, and survive poorly under natural forest conditions (Minore et al., 1979; Stark and Baker, 1992). Stark and Baker (1992) suggested that the main problem with huckleberry seedling establishment is drought during summer, especially at lower and mid-elevations. Germination during late summer and early fall rains could, however, expose the tender seedlings to killing frosts and freezes. Inducing dormancy in some seeds increases the likelihood of some germination during the following spring, when temperatures are mild and moisture is abundant. Why dormancy was induced in only some seeds is unknown.

The results of this study indicate that *V. membranaceum* seeds, like those of lowbush and highbush blueberries, store well and can be kept dry for at least 7 years at 2 to 3 °C while retaining germination percentages in the 70% range.

### Conclusions

Air-drying *V. membranaceum* seeds for 7 d reduced the maximum percentage of seeds that germinated. The effect was transitory, however, and air-dried seeds stored at 2 to 3 °C for 1 or 7 years germinated at percentages near those for fresh, nondried seeds. Effects of drying and storage on T50 and germination rates were minimal, although stored seed appeared to exhibit greater variability in cumulative germination than did fresh seeds or seed collected fresh and sown after drying for 7 d.

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