

# Optimizing acid scarification and stratification combinations for RUSSET BUFFALOBERRY SEEDS

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## ABSTRACT

Seed propagation literature for russet buffaloberry (*Shepherdia canadensis* (L.) Nutt. [Elaeagnaceae]) is varied, recommending acid scarification, cold moist stratification, or both treatments. When combinations of sulfuric acid scarification (0 or 5 min) and stratification (0, 9, or 14 wk) treatments were tested on a Montana seedlot, the optimal treatment combination was a 5-min acid soak and 14-wk (98-d) stratification. Although stratification was more effective than scarification when treatments were applied alone, germination improved from 27% to 38% when seeds were scarified prior to the 14-wk stratification treatment, and, for seeds germinating after stratification, germination occurred almost 4 d earlier on average. A 1-wk imbibition test, where increase in seed weight is measured following scarification and 1-wk cold-stratification, is proposed as a convenient method to determine the most effective acid scarification duration for individual seedlots. The optimal stratification duration was achieved 4 wk after 10% of viable seeds had germinated during stratification.

## KEY WORDS

imbibition, dormancy, mean germination time, *Shepherdia canadensis*, Elaeagnaceae

## NOMENCLATURE

(plants) ITIS (2001);  
(bacteria) Euzéby (1999)

Russet buffaloberry (*Shepherdia canadensis* (L.) Nutt. [Elaeagnaceae]). Photo by Tara Luna

Russet buffaloberry (*Shepherdia canadensis* (L.) Nutt. [Elaeagnaceae]) is a deciduous shrub 0.9 to 3.9 m (3 to 13 ft) in height found on well-drained soils in the US and Canada from Maine and Nova Scotia west to South Dakota, from Newfoundland west through northern Canada to Alaska, throughout the Pacific coast ranges and in the Rocky Mountains as far south as Arizona and New Mexico (Walkup 1991). Russet buffaloberry occurs as an understory species in fir (*Abies* P. Mill. [Pinaceae]), pine (*Pinus* L. [Pinaceae]), spruce (*Picea* A. Dietr. [Pinaceae]), Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco [Pinaceae]), and quaking aspen (*Populus tremuloides* Michx. [Salicaceae]) forests throughout its range (Walkup 1991). Russet buffaloberry forms a nitrogen-fixing symbiosis with *Frankia* (*Frankia brunchorst* [Frankiaceae]) (Euzéby 1999) bacteria on low-nitrogen soils (Walkup 1991). Russet buffaloberry provides generally poor forage for livestock but is used as forage by deer and elk; birds eat its red berries in late summer and fall (Walkup 1991).

Recommendations for seed propagation of russet buffaloberry include acid scarification, cold moist stratification, or combinations of both treatments. Heit (1971) attributed buffaloberry seed dormancy primarily to hardseededness (physical dormancy). A 30-min acid soak resulted in 78% germination, whereas 1 mo of moist stratification at 2 to 4 °C (36 to 40 °F) resulted in only 12% germination. Combinations of scarification and stratification treatments were not reported. Luna and Wick (2002) classified seed dormancy as both physical and physiological but recommended only stratification for 60 to 90 d. Baskin and Baskin (2002) recommended a 60-d stratification without scarification. McTavish (1986) found a 5- to 7-min sulfuric acid soak followed by a 30-d stratification to be the most consistently effective treatment combination.

Limited literature documents the relative contributions of both physical and physiological dormancy to overall seed dormancy using different scarification and stratification treatment combinations on the same seed lot. Both physical and physiological dormancy likely exist species-wide in russet buffaloberry, but in certain seed lots one of these dormancy components may be weak, and good germination can be obtained by only treating the other component. Faced with an untested seed lot, however, a grower cannot assume either component is insignificant at the risk of poor germination due to inadequate treatment. Likewise, the assumption that both physical and physiological dormancy are significant incurs the risk of over-treatment—acid damage or induction of secondary dormancy during an excessive stratification treatment. This article describes the effects of acid scarification and stratification treatment combinations on a single russet buffaloberry seed lot. A single seed lot was used to demonstrate techniques growers can use to quickly ascertain effective treatment combinations for any given seed lot, rather than to assess species-wide variability in dormancy characteristics, which would require multiple seed lots encompassing the range of the species.

We used commercial seeds (Western Native Seeds, Coaldale, Colorado) collected in Montana. Seeds were purchased about 1 y after collection and then stored at 3 °C (37 °F) for about 6 m until the start of the study. There were approximately 144 000 seeds per kg (65 450 seeds/lb). Seeds were mostly 3 to 5 mm (0.1 to 0.2 in) in length.

### Preliminary Study—1-wk Imbibition Test

First we studied the efficacy and optimal duration of acid scarification for overcoming physical dormancy in this seed lot. Of particular interest was the effect of scarification on the seeds' ability to rapidly (1 wk) imbibe water. For the study, 50-seed samples were scarified in concentrated sulfuric acid (Reagent ACS, 95% to 98%, VWR International, West Chester, Pennsylvania) for 5, 10, or 15 min with 3 replications per treatment. (Note: Controls [0-min acid treatment] were omitted because they were going to be included in the main study, regardless of the outcome of this preliminary study. When using this test to assess the optimal scarification treatment for a seed lot, controls must be included to determine if physical dormancy is even a factor.)

Scarification was accomplished by placing each seed sample in 5 ml of acid (the ratio of acid to seeds was about 14:1 (v:v) during scarification), stirring vigorously for 30 s to disperse the seeds, allowing the seeds to remain undisturbed for the appropriate duration of treatment, and thoroughly rinsing seeds under running tap water following treatment. Samples were allowed to air-dry for 12 h after scarification before weighing (initial weight). Samples were then cold stratified 1 wk on filter papers (VWR Qualitative 413) within 100 mm (3.9 in) petri dishes wetted with 4 ml (0.14 fl oz) of deionized water. Petri dishes were placed in a low-temperature incubator (Fisher Scientific, Hampton, New Hampshire) set to 3 °C (37 °F) for stratification. After completion of stratification, seeds were surface dried on paper towels and reweighed (final weight). The percentage increase in seed weight during stratification was calculated as follows:

#### Equation 1.

$$\text{Percentage increase} = ((\text{final weight} - \text{initial weight}) / \text{initial weight}) * 100\%$$

Seeds scarified 5, 10, and 15 min increased in weight 97.5% (standard error [ $s_{\bar{x}}$ ] = 1.12], 97.5% [ $s_{\bar{x}}$ ] = 1.10], and 100.2% [ $s_{\bar{x}}$ ] = 1.28], respectively) following 1 wk of stratification. For all 3 treatments, near doubling of seed weight following treatment indicated seeds imbibed water readily, and physical dormancy, if present, was overcome. The shortest duration treatment (5 min) was selected for the main study because it was least likely to result in damaged seeds, although no seed damage was evident for any treatment in the study.

## Main Study

The combined effects of acid scarification and stratification on germination were tested using a completely randomized design with a factorial treatment structure. Factors were duration of scarification in concentrated sulfuric acid (0 or 5 min) and duration of stratification (0, 9, or 14 wk). This resulted in 6 treatment combinations, each having 4 replications of 100 seeds.

All seeds were surface sterilized with a 2-h soak in 3% hydrogen peroxide followed by a thorough rinse in running tap water and brief soaks in several changes of fresh water prior to other treatments. Seeds were then allowed to dry overnight. The following day, seeds were randomly assigned into the appropriate number of 100-seed samples and weighed. A representative sample of the remaining surface-sterilized seeds was sent to the National Tree and Native Plant Seed Laboratory (USDA Forest Service, Dry Branch, Georgia) for viability testing via tetrazolium staining of 400 seeds using methods prescribed by the Association of Official Seed Analysts (AOSA 1993).

Scarification using concentrated sulfuric acid was accomplished as described for the preliminary study, except each 100-seed sample was scarified in 10 ml (0.34 fl oz) of acid. Stratification was carried out by the same methods as described for the preliminary study, except stratification start dates were staggered so that all samples completed stratification concurrently. In addition, seeds were checked weekly during stratification to monitor germination and to add water, if necessary. Seeds were considered germinated when the radical had emerged through the seed coat. After 1, 2, 3, and 4 wk of stratification, each sample was weighed to assess increase in water content. Seeds were removed from the petri dish, surface dried on paper towels, weighed, and returned to the same petri dish for further stratification. Water was added to the petri dish as needed.

Following stratification, each sample was rinsed 1 min under tap water and germinated on sterilized sand (ISTA 1999) in petri dishes. Petri dishes were arranged on a single greenhouse bench using a completely randomized design. Germination was monitored daily for 2 wk and weekly for an additional 2 wk. Daytime high temperatures were set at 30 °C (86 °F) and nighttime low temperatures at 15 °C (59 °F) during the test period. Day length during that period (March 28 through April 25) ranged from 12.4 to 13.3 h. Daily high greenhouse temperatures averaged 29 °C (84 °F) +/- 0.3 °C (0.6 °F) with a maximum of 33 °C (91 °F). Daily low temperatures averaged 17 °C (62 °F) +/- 0.2 °C (0.3 °F) with a minimum of 15 °C (59 °F).

Categorical analysis (SAS Proc Catmod; SAS Institute 1989) was used to determine differences in total germination (the sum of germination during and after stratification) using the factorial treatment structure. Categorical analysis is a generalization of the chi-square ( $X^2$ ) test of homogeneity, using the “logit”—the natural log of the ratio of germinated to non-germinated seeds for each treatment—as the response. Generalized least squares were used to calculate  $X^2$  test statistics.

Observed significance levels less than  $\alpha=0.05$  were considered significant. Percentages and standard errors were calculated for main effects and interaction combinations. Approximate pairwise Z-statistics were used to conduct comparisons of main effects using an alpha value of 0.01.

Germination rate, the average number of days required for seeds to germinate, was expressed as mean germination time (MGT). MGT was calculated by the equation:

$$\text{Equation 2.} \quad \text{MGT} = \frac{\sum g_i n_i}{G}$$

where  $g_i$  is the number of seeds germinating on the  $n_i$ th day of germination testing and  $G$  is the total number of seeds germinating during the 28-d test (Ellis and Roberts 1981). Because germination counts were conducted weekly after day 14,  $n_{21}$  was considered to be the midpoint between days 15 and 21 or  $(15 + 21)/2$  and  $n_{28}$  was considered to be the midpoint between days 22 and 28 or  $(22 + 28)/2$  (Jones and Gosling 1994).

Mean germination time was calculated for 2 different data sets. First, MGT was calculated for seeds germinating after stratification only, as a practical measure of the rate of germination that might be expected in greenhouse production following sowing of non-germinated seeds. The second data set included all germinating seeds (counting seeds germinating during stratification as germinating on day 0) in order to better measure the effectiveness of treatments in breaking dormancy and improving germination energy. Differences among MGT means were determined using ANOVA (SAS Proc GLM; SAS Institute 1989) for the data set including seeds germinating after stratification only. Observed significance levels less than  $\alpha=0.05$  were considered significant. Pairwise comparisons of means were conducted using an alpha value of 0.01. The second data set was not formally analyzed due to problems with normality associated with counting seeds germinating during stratification as germinating on day 0.

Differences in percentage increase in seed weight during stratification due to acid scarification and duration of incubation were determined using ANOVA (SAS Proc GLM; SAS Institute 1989). Observed significance levels less than  $\alpha=0.05$  were considered significant. Pairwise comparisons of means were conducted using an alpha value of 0.01.

## RESULTS

Tetrazolium staining indicated 44% seed lot viability. Germination percentage was analyzed on the basis of 100 seeds per sample; germination as a percentage of viable seeds (estimated for the seed lot, not measured for individual samples) is reported in parentheses in the following discussion. Both acid scarification ( $P=0.019$ ) and stratification ( $P<0.001$ ) main effects were

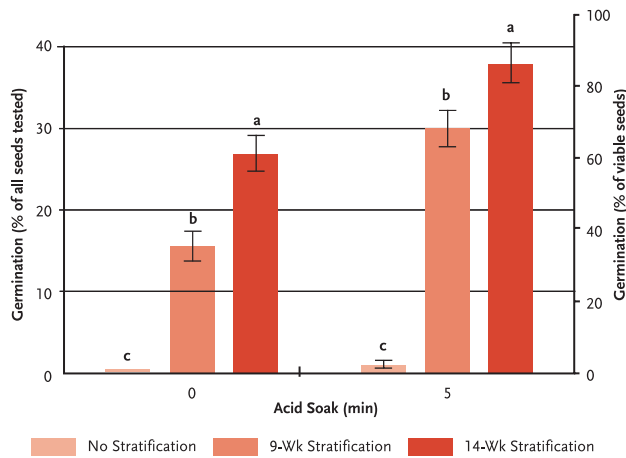


Figure 1. Russet buffaloberry germination following combinations of acid scarification and stratification treatments. For seeds undergoing neither stratification nor scarification, no seeds germinated. Within acid soak durations, means labeled with the same letter are not different at the 0.01 level of significance. Bars indicate  $\pm 1$  standard error.

significant, but the interaction between factors was not ( $P < 0.221$ ). On average, 14.3% (32.4%) of non-scarified seeds germinated, while 23.0% (52.3%) of seeds scarified for 5 min germinated. Increasing stratification durations improved germination with each increase, from only 0.5% (1.1%) for non-stratified seeds to 22.9% (52.0%) for seeds stratified 9 wk, up to 32.5% (73.9%) for seeds stratified 14 wk. Maximum germination was achieved with a combination of scarification and stratification treatments (Figure 1).

Both acid scarification and the longest stratification duration (14 wk) increased the number of seeds germinating during stratification treatment (Figure 2). MGT analysis, for seeds germinating after stratification only, revealed that seeds scarified 5 min in sulfuric acid germinated more rapidly than non-scarified seeds ( $P < 0.0001$ ), but increasing stratification length from 9 to 14 wk did not affect MGT ( $P = 0.373$ ) (Figure 3). When seeds germinating during stratification were counted as germinating on day 0 in MGT calculations, seeds scarified 5 min in sulfuric acid still germinated more rapidly than non-scarified seeds. MGT fell from  $8.1 \pm 0.6$  and  $5.9 \pm 0.6$  for non-scarified seeds stratified 9 and 14 wk, respectively, to  $2.5 \pm 0.6$  and  $1.7 \pm 0.6$  for seeds scarified 5 min and stratified 9 and 14 wk, respectively.

Seeds that underwent acid scarification for 5 min achieved near complete imbibition (increase in seed weight of 95% to 100% on a dry weight basis) after 1 wk of stratification (Figure 4). In comparison, non-scarified seeds increased seed weight by 63% after 1 wk and approached complete imbibition only after 4 wk of stratification, achieving a 90% seed weight increase.

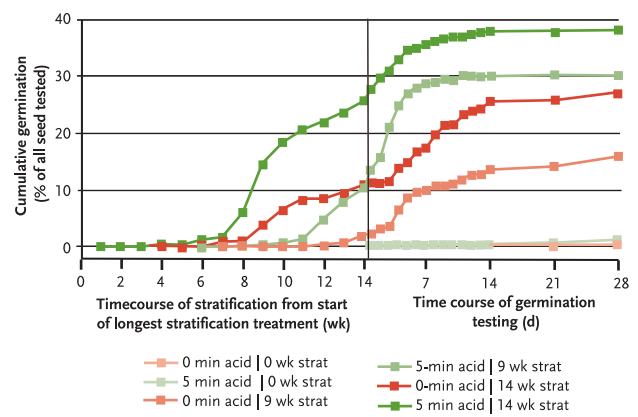


Figure 2. Timecourse of germination during stratification and germination testing for each acid scarification/stratification treatment combination. The 14-wk stratification treatment began on week 0 and the 9-wk stratification treatment began on week 5. acid = acid scarification, strat = stratification.

## DISCUSSION

Various authors recommend scarification only (Heit 1971), stratification only for 60 to 90 d (Baskin and Baskin 2002; Luna and Wick 2002), or the combination of both treatments (McTavish 1986) to propagate russet buffaloberry. Our study found a 5-min acid soak followed by a 14-wk (98-d) stratification to be optimal for this seed lot. The stratification requirement for this seed lot was on the upper end of the 60- to 90-d range recommended in the literature. Although the stratification requirement was stronger than the scarification requirement (only 2.3% of viable scarified non-stratified seeds germinated, whereas up to 61% of viable stratified non-scarified seeds germinated), scarification prior to the 14-wk stratification treatment improved germination percentage by 11% (25%) and scarified seeds germinated nearly 4 d earlier on average (using either MGT calculation).

In this study, full imbibition was associated with an increase in seed weight of 95% to 100%. The maximum increase in seed weight (full imbibition) may vary among seed lots. If the increase in seed weight following a 15-min acid soak is much less than 95%, longer treatment durations should be tested. The shortest treatment duration resulting in nearly full imbibition after 1 wk successfully overcomes physical dormancy and is the least likely to damage embryonic tissues.

This procedure can be adapted for other species in which acid scarification is known to facilitate imbibition in physically dormant seeds. However, species susceptibility to damage from

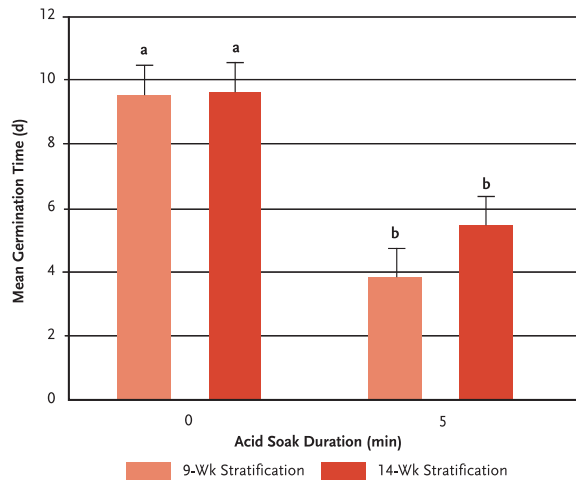


Figure 3. Russet buffaloberry mean germination time (MGT) following combinations of acid scarification and stratification treatments. Analysis was conducted for seeds germinating after stratification (pink bars). MGT was recalculated for all germinating seeds, using  $d = 0$  for seeds germinating during stratification (red bars). Means labeled with the same letter are not different at the 0.01 level of significance. Bars indicate  $\pm 1$  standard error.

acid scarification varies, and as a result, treatments resulting in near complete imbibition may damage seeds. We have observed that acid scarification durations of 0, 5, 15 and 30 min resulted in 1-wk seed weight increases of 21%, 69%, 82%, and 85%, respectively, for silver buffaloberry (*Shepherdia argentea* [Pursh] Nutt. [Elaeagnaceae]), but the 5-min treatment maximized germination and longer treatment durations damaged seeds (unpublished data).

Few options exist to predetermine the optimal stratification duration for a given seed lot, other than to rely on literature based on different seed sources or to run a lengthy pilot study. An alternative is to determine the optimal stratification duration based on the occurrence of germination during stratification. Suszka and others (1996) suggest adjusting the stratification duration to the dormancy level of the particular seed lot by stratifying seeds for  $(X + 2)$  to  $(X + 4)$  wk, where  $X$  is the time necessary to achieve 10% germination of viable seeds in stratification. In the present study, 10% germination of viable seeds occurred around week 8 of stratification for acid-scarified seeds, so a stratification duration of  $X + 4$  or greater was required to achieve maximal total germination for this seed lot.

The degree of dormancy was highly variable within the seed lot used in this study. Some seeds germinated after 5 wk of treatment whereas others required more than 9 wk of stratification to germinate. A drawback to choosing the optimal (in terms of total germination) stratification duration is that many seeds germinate in stratification and are susceptible to damage before or during sowing. For example, after 12 wk ( $X + 4$ ) of

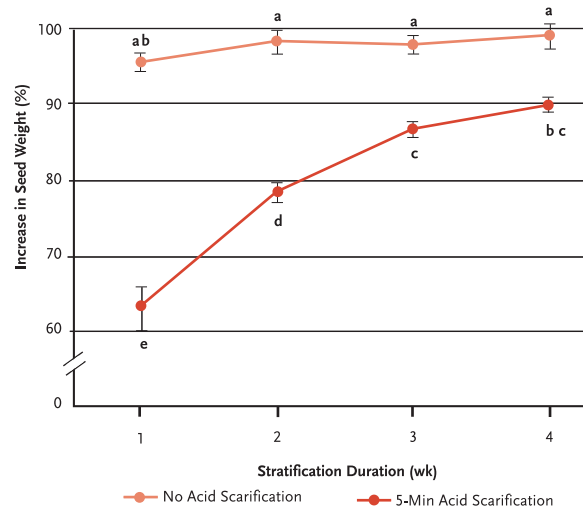


Figure 4. Russet buffaloberry increase in seed weight during the first 4 wk of stratification following no scarification or a 5-min soak in concentrated sulfuric acid. Means labeled with the same letter are not different at the 0.01 level of significance. Bars indicate  $\pm 1$  standard error.

## A QUICK TEST

**I**N LIGHT OF THE DIFFERENT GERMINATION PROTOCOLS noted earlier, simple quick tests to determine optimal scarification and stratification treatment levels for any given seed lot would be useful. A modification of the 1-wk imbibition test used in the preliminary study can be used to determine the optimal acid scarification treatment level. By this method, small seed samples (for example, 50-seed samples or 1-g samples) are scarified in sulfuric acid over a range of durations (0, 3, 5, 10, or 15 min). Following treatments, seeds are dried overnight, weighed, stratified for 1 wk (or imbibed at room temperature for more rapid imbibition), and reweighed. Replicating each treatment with at least 3 samples ensures more reliable results. The percent increase in weight due to imbibition is then calculated (Equation 1).

stratification in this study, 50% of viable seeds had germinated. Rather than reducing the stratification duration to suboptimal, growers can sow germinants periodically during the latter stages of stratification. Reducing the moisture content at which seeds are stratified can prevent this problem of germination in stratification (Jones and Gosling 1994; Poulsen 1996; Suszka and others 1996), but testing is needed to determine if this technique can be adapted to russet buffaloberry.

## CONCLUSIONS

Growers can rapidly assess the degree of physical dormancy and optimal acid scarification duration for any seed lot using a 1-wk imbibition test. The optimal stratification duration need not be guessed at prior to treatment, rather, growers can maximize buffaloberry seedling production by sowing germinants weekly after germination in stratification has begun, and continuing to stratify seeds until at least 4 wk after 10% of viable seeds have germinated.

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