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# *Sulfuric Acid Scarification of* Wax Currant Seeds from New Mexico

**Lee S Rosner, John T Harrington, David R Dreesen, and Leigh Murray |**

ABSTRACT

The germination response of 6 New Mexico sources of wax currant (*Ribes cereum* Dougl. [Grossulariaceae]) to combinations of 0 to 120 d cold stratification and 0 to 8 min of acid scarification varied widely among seedlots. For most seedlots, cold stratification was more effective than scarification in improving germination, and scarification improved germination only at low, ineffective levels of cold stratification. For 3 of 6 seedlots, maximal germination was achieved without scarification. For the remaining 3 seedlots, optimal scarification duration varied. Variability in sensitivity to acid scarification is discussed in terms of environmentally induced effects on seed coat structure and physiology.

KEY WORDS
cold stratification, variability, dormancy

NOMENCLATURE
ITIS (2001)

Wax currant (*Ribes cereum* Dougl. [Grossulariaceae]) is a shrub species found throughout western North America including New Mexico. Wax currant occurs on dry slopes, ridges, and plains within numerous habitat types and plant communities (Vines 1960; Marshall and Winkler 1995). This species grows on diverse soil types and occurs across a range of temperatures and precipitation levels (Marshall and Winkler 1995). Wax currant provides browse for deer when better browse is not available, and fruits are eaten by numerous bird species (Marshall and Winkler 1995). Wax currant is valued for use in the reclamation of disturbed lands.

The primary seed dormancy mechanism in *Ribes* species is embryo dormancy, but seed coat dormancy controlled by growth inhibitors and/or an impermeable seed coat is suspected to occur as well (Pfister 1974; Goodwin and Hummer 1993). Cold stratification for 60 to 300 d has been used to overcome embryo dormancy for most *Ribes* species (Fivaz 1931; Quick 1936; Heit 1971, Pfister 1974; Young and Young 1992; Goodwin and Hummer 1993). Among New Mexico

TABLE 1

*Seedlots used in wax currant germination studies.*

| Seedlot | Latitude | Location in New Mexico | Elevation m (ft) | Collection date (1997) |
|---|---|---|---|---|
| Capulin | 36° 42' N | Questa | 2987 (9800) | 10 Aug |
| Raspberry Ridge | 36° 42' N | Questa | 2987 (9800) | 12 Aug |
| Pinon Knob | 36° 42' N | Questa | 2896 (9500) | 21 Aug |
| Mahogany Hill | 36° 42' N | Questa | 2774 (9100) | 13 Aug |
| Rociada | 35° 50' N | Rociada | 2377 (7800) | 17 Aug |
| Gila | 34° 06' N | Gila National Forest | 2499 (8200) | 21 Aug |

seedlots of wax currant, the cold stratification requirement ranges from 120 d or more for northern seedlots, to none for at least 1 seedlot in the southern third of the state (Rosner and others 2001). Others have also observed variability in seed dormancy level between and within seedlots in *Ribes* (Pfister 1974; Young and Young 1992).

Acid scarification using various concentrations of sulfuric acid has broken seed coat dormancy in some *Ribes* species. A 5-min soak in 50% sulfuric acid improved germination of European black currant (*Ribes nigrum* L.) (Adam and Wilson 1967). Germination of Appalachian gooseberry (*Ribes rotundifolium* Michx.) was improved by a 35-min soak in "commercial" sulfuric acid (Fivaz 1931). Five-min soaks in 2% to 10% sulfuric acid solution improved germination of prickly currant (*Ribes lacustre* [Pers.] Poir.) and sticky currant (*Ribes viscosissimum* Pursh) (Pfister 1974). There is a lack of published literature documenting the use of acid scarification as a seed treatment for wax currant.

For many native shrub species, propagation techniques are not well researched, resulting in increased production costs (Dreesen and Harrington 1997). In reclamation, locally adapted seedlots are usually preferred, but seed crops are often limited, and the need to maximize germination is paramount. For wax currant, the combination of acid scarification and cold stratification may result in higher germination than cold stratification alone. The purpose of this research was to evaluate the efficacy of sulfuric acid scarification and variability in treatment response among wax currant seedlots encompassing both a range of latitudes throughout New Mexico and a range of elevations at 1 location in northern New Mexico.

## MATERIALS AND METHODS

Seeds were collected 10 August 1997 through 21 August 1997 at 6 locations throughout New Mexico (Table 1). Seeds were collected at varying heights within the canopy of each plant and from a minimum of 5 plants at each location. Seedlot locations were selected to encompass both a range of latitudes within New Mexico and a range of elevations at Molycorp mine in Questa, New Mexico. Before cleaning, seeds were soaked overnight in tap water (seeds that floated were discarded), allowed to ferment for 48 h, mashed, and dried. Dried fruits were processed in a rubbing box, and a Dakota blower was used to separate seeds from pulp. Cleaned seeds were stored at 5 °C (41 °F) until use.

This study utilized a completely randomized design with a factorial treatment structure. Factors were seedlot, scarification treatment, and cold stratification duration. Seeds from 6 seedlots underwent acid scarification in concentrated sulfuric acid for durations of 0, 2, 4, or 8 min followed by cold stratification for 0, 60, 90, or 120 d. Germination data were analyzed as a 6 (seedlot) by 4 (cold stratification) by 4 (scarification) factorial and then separately by seedlot. Two replications of 100 seeds were used to test each treatment combination.

Scarification treatments were conducted using concentrated sulfuric acid (Reagent ACS, 95.0-98.0%, VWR Scientific Products, West Chester, Pennsylvania). Each seed sample (100 seeds) undergoing scarification was placed in 10 ml acid, stirred vigorously for 30 s to disperse the seeds, and then allowed to soak in the acid for the remainder of the treatment duration (2, 4, or 8 min). Following treatment the seeds were removed from the acid and thoroughly rinsed under running tap water for 1 min.

Seeds were placed between 9.0-cm (3.5-in) filter papers (VWR Qualitative Grade #3) moistened with distilled water for cold stratification treatments. Filter papers were placed in 100-mm petri dishes sealed in 15 x 16 cm self-sealing poly bags within a walk-in cooler. Cooler temperatures fluctuated from an average daily low of –1.2 °C (30 °F) to an average daily high of 5.4 °C (42 °F). Seeds underwent cold stratification immediately following scarification. Scarification/cold stratification start dates were staggered so that all seeds completed cold stratification at the same time.

Seeds were maintained between filter papers in petri dishes within poly bags for germination testing. Petri dishes in poly bags were placed directly on greenhouse benches under natural light (filtered through shade cloth) with fluctuating temperatures. A 30-cm (1-ft) border on all sides of each bench was left

empty in order to minimize temperature differences between samples. The greenhouse thermostat was set to maintain daytime highs near 30 °C (86 °F) and nighttime lows near 15 °C (59 °F). Daytime high temperatures averaged 34 °C (93 °F) +/- 0.5 °C (0.9 °F) and nighttime low temperatures averaged 15 °C (59 °F) +/- 0.3°C (0.5 °F) during the experiment. Germination testing took place from 14 May 1999 through 11 June 1999.

Germinated seeds were counted and removed when samples were taken out of cold stratification and after 7, 14, 21, and 28 d of incubation. Filter papers were remoistened as needed. Seeds were considered germinated if the radicle was visible to the naked eye. Fungal contamination of petri dishes caused some problems. Seeds covered in mycelium, discolored, softened, or oozing were removed from the petri dish and counted as rotten.

Categorical analysis (SAS PROC CATMOD) was used to determine treatment differences in total germination using a factorial treatment structure (SAS Institute 1989; Stokes and others 1995). Traditionally, analysis of variance (ANOVA) has been used to analyze germination data. The ANOVA assumes continuous, normally distributed data with equal variances, but germination percentage data has unequal variances between treatments and is frequently skewed and, therefore, non-normal. Usually percentage data are arcsine transformed to achieve normality and then analyzed by ANOVA. Categorical analysis eliminates the need for data to be transformed. The procedure 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—as the response (Grizzle and others 1969). 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 interactions. Approximate pairwise Z statistics were used to conduct pairwise comparisons of main treatment effects using a conservative alpha value of 0.05 divided by the number of comparisons. Due to a highly significant 3-factor interaction, main effects and all 2-factor interactions except cold stratification by acid scarification are not discussed here. This interaction is presented because growers will not be using the seedlots tested in this study, and it may be useful to understand how cold stratification and acid scarification interact averaged across multiple seedlots.

## RESULTS

Cold stratification, seedlot, acid scarification, and all interactions of these factors impacted germination (Table 2). Germination response to combinations of cold stratification and acid scarification treatments varied by seedlot (Figure 1). For all seedlots except the southernmost (Gila) and the highest elevation Questa seedlot (Capulin), cold stratification was a more robust treatment than acid scarification—the simple effect of cold stratification improved germination more than the simple effect of acid scarification for those seedlots. For the Gila seedlot, however, scarification improved germination more consistently than cold stratification, whereas for the Capulin seedlot, which appeared to have the lowest overall quality, the combination of cold stratification and acid scarification treatments resulted in threefold improvement in germination over the best level of either treatment alone. Interestingly, the seedlot with the best cold stratification-only germination (Raspberry Ridge) was most negatively affected by acid scarification

TABLE 2

*Categorical analysis of variance table for wax currant germination response to stratification, seedlot, and acid scarification.*

| Component | DF | Chi-square | Observed significance level |
|---|---|---|---|
| Stratification (S) | 3 | 774 | < 0.001 |
| Lot (L) | 5 | 1010 | < 0.001 |
| Acid scarification (A) | 3 | 24 | < 0.001 |
| S x L | 15 | 570 | < 0.001 |
| S x A | 9 | 138 | < 0.001 |
| L x A | 15 | 251 | < 0.001 |
| S x L x A | 45 | 257 | < 0.001 |

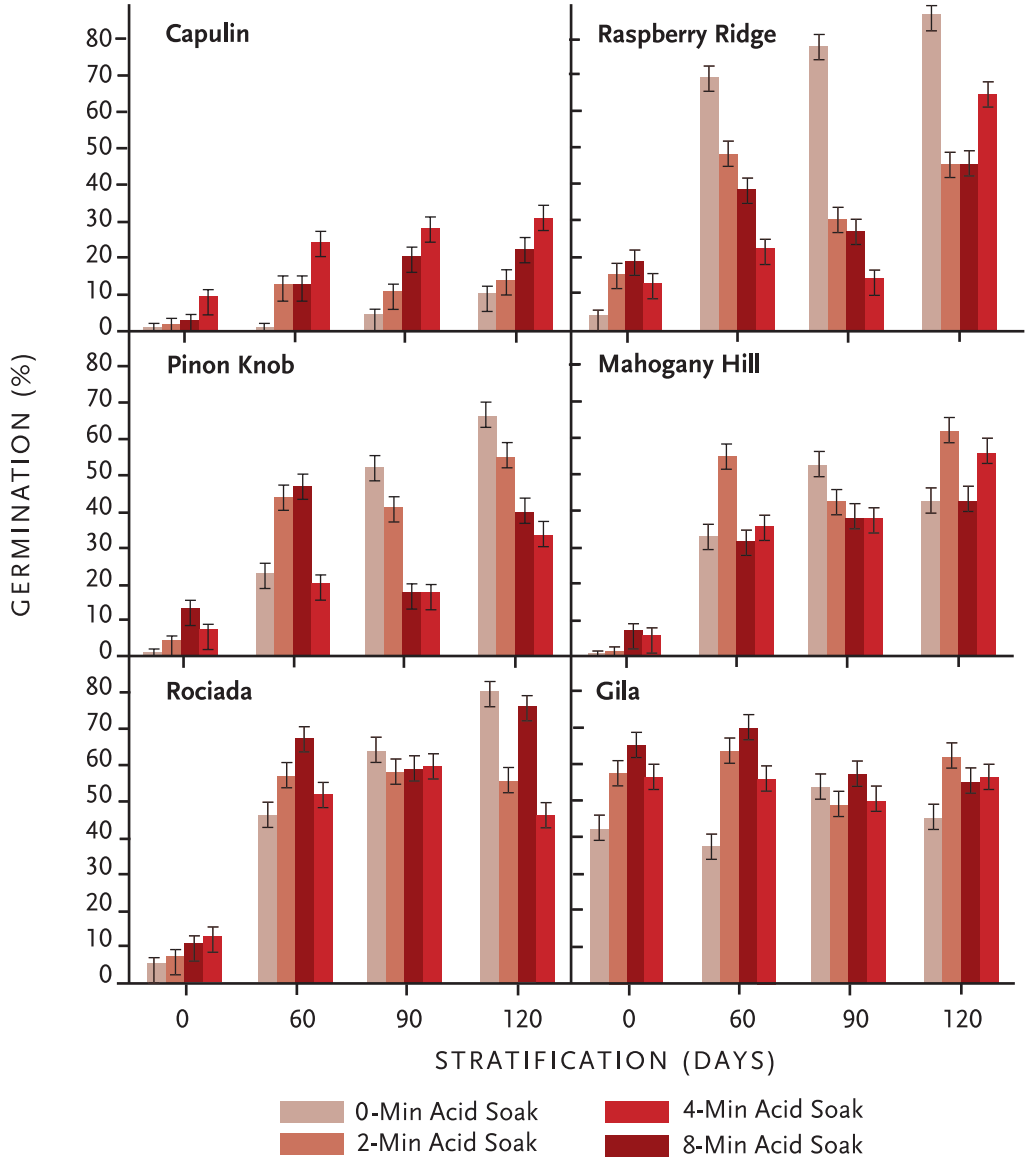


*Figure 1.* Effect of interaction between acid scarification duration, stratification length, and seedlot on wax currant germination.

treatments, while the seedlot with the lowest cold stratification-only germination (Capulin) was most positively affected.

The effect of scarification varied widely among cold stratification durations for all but the Capulin seedlot, for which germination improved with increasing acid soak duration at all cold stratification levels. For the remaining seedlots, acid scarification tended to improve germination only at low, less effective levels of cold stratification. Averaged over all seedlots, 2- or 4-min acid scarification treatments improved germination in combination with 0- or 60-day cold stratification treatments, but all acid scarification durations reduced germination for seeds undergoing 90 or 120 d of cold stratification (Figure 2). As a result of this interaction, for 3 of 6 seedlots (Raspberry Ridge, Pinon Knob, and Rociada), maximal germination could be achieved without the use of acid scarification (Figure 1). For those 3 seedlots, no treatment combination resulted in better germination than cold stratification for 120 d alone. For the remaining 3 seedlots, acid scarification was necessary to achieve maximal germination, but the optimal treatment duration varied.

The percentage of seeds rotting during the course of cold stratification and germination testing (based on the total number of seeds) increased sharply with increasing duration of acid soak, for both pooled seedlots and by seedlot (Figure 3). The differences among seedlots in percentage of rotting seeds corresponded to differences in acid scarification treatment efficacy. The 2 seedlots with the highest percentages of rotting seeds at all treatment levels (Raspberry Ridge and Pinon Knob) were 2 of the 3 seedlots that did not benefit from acid scarification. In contrast, 3 of the 4

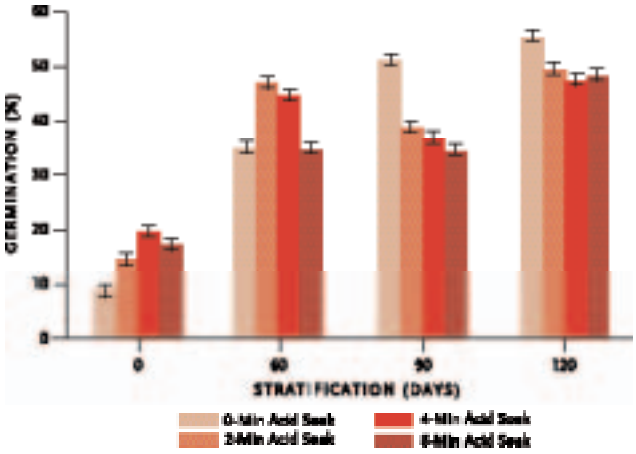


*Figure 2.* Effect of interaction between acid scarification duration and stratification length on wax currant germination.

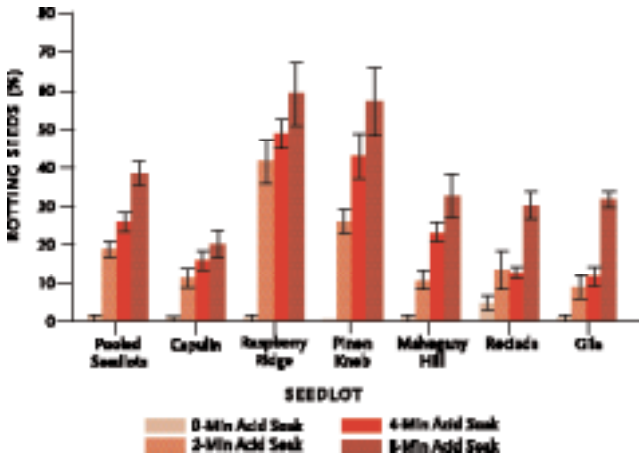


*Figure 3.* Effect of interaction between acid scarification duration on percentage wax currant seeds rotting during the course of stratification and germination testing for pooled seedlots and by seedlot.



seedlots with the lowest percentages of rotting seeds (Capulin, Mahogany Hill, and Gila) had optimal treatment combinations involving some level of acid scarification. It should be noted that seeds must be filled in order to rot, but seed fill was not measured. The percentage of filled seeds may have varied among seedlots, and some of the observed differences in rotting may have been influenced by differences in seed fill.

## DISCUSSION

Variability in acid scarification requirement among seedlots has been found to occur in numerous species including California redbud (*Cercis canadensis* var. *texensis* [S. Wats.] M. Hopkins [Fabaceae]) (Heit 1967), honeylocust (*Gleditsia triacanthos* L. [Fabaceae]) (Bonner and others 1974), and cotoneaster species (*Cotoneaster* Medik. [Rosaceae]) (Slabaugh 1974). This variability occurs to such an extent that Heit (1967) and Bonner (2001) recommend testing each seedlot of species requiring acid scarification to determine the optimum duration of treatment. Seed source has been ascribed as a major source of this variability (Heit 1967; Bonner 2001). A good example of source variability is Kentucky coffeetree (*Gymnocladus dioicus* [L.] K. Koch [Fabaceae]), where acid scarification improved germination of seeds collected in Ohio and Illinois, but not in Minnesota (Frett and Dirr 1979; Ball and Kisor 1985). For wax currant in the present study, the effect of sulfuric acid scarification on germination varied widely among seedlots, but this variability conformed to no latitudinal or elevational pattern. In fact, Capulin and Raspberry Ridge seedlots occur at similar elevations within a mile of each other, yet response to acid scarification was nearly opposite; increasing acid soak duration increased Capulin germination, whereas all levels of acid soak, when combined with cold stratification, reduced Raspberry Ridge germination.

This differential response to acid scarification among proximal seed sources may stem both from genetic and environmental differences between these populations. The extent of the contribution of each factor, however, is generally difficult to determine (Anderson and Milberg 1998). Environmental factors during seed maturation have been shown to affect depth of dormancy (Gutterman 1992), seed coat thickness (Pourrat and Jacques 1975), seed coat permeability (Gutterman and Evanari 1972), seed coat thickness and germination-inhibiting polyphenol content (Dorne 1981), and hardness of the seed coat and endosperm (Juntilla 1973). Microclimatic factors such as aspect, slope, vegetative cover, and edaphic conditions can affect temperature, light quality, moisture, wind exposure, and nutrient availability. Anderson and Milberg (1998) suggest that even seeds collected from plants growing side by side may have been subject to different weather conditions during maturation due to small differences in development time, and that water and nutrient status can vary within small areas. Differential response to acid scarification among seed sources in the present study may have been mediated by microclimatic differences, and variability among proximal sources is not surprising.

Seeds damaged or weakened by excessive treatment lose their ability to resist pathogenic deterioration (Murdock and Ellis 1992). For the Raspberry Ridge seedlot, a high percentage of seeds rotted following even the shortest duration of acid soak, indicating that sulfuric acid damaged seeds at all treatment levels. Although the Capulin seedlot responded positively to acid scarification, 10% to 20% of scarified seeds rotted following treatment, depending on treatment level. This occurrence indicates a high degree of within-seedlot variability in seed coat dormancy and a paradox inherent in all scarification treatments; treatments thorough enough to improve germinability for some seeds may be damaging to other seeds within the lot (Young and

others 1984). In some cases, weaker solutions and longer incubation durations may reduce the risk of damaging seeds without loss of treatment efficacy (Young and others 1984).

## CONCLUSIONS

Our results suggest germination in New Mexico seedlots of wax currant is improved by 120 d of cold stratification, but response to acid scarification is variable. For most seedlots, cold stratification for 120 d results in good germination. Further increases in germination can be realized through the use of treatments targeting seed coat dormancy mechanisms, but sulfuric acid may be too reactive to be an effective agent. Better understanding of the mechanisms imposing seed coat dormancy in this species may lead to the development of more effective seed treatments.

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