COMPARISON OF STRATIFICATION AND SCARIFICATION METHODS OF *SORBUS DECORA, VITIS RIPARIA,* AND *RHUS TYPHINA*

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ABSTRACT

Biderman, M. 2022. Comparison of stratification and scarification methods of *Sorbus decora, Vitis riparia,* and *Rhus typhina.* Natural Resources Management. Lakehead University. 29 pp.

Keywords: Thunder Bay, germination, scarification, stratification, hydrochloric acid, *Sorbus decora, Vitis riparia, Rhus typhina*

This thesis investigates the effectiveness of three methods in breaking seed dormancy of *Sorbus decora, Vitis riparia, and Rhus typhina.* The treatment methods studied were mechanical scarification with sandpaper, hydrochloric acid (HCl), and cold moist stratification. A seed viability float test was performed to establish a control. Nine *Vitis riparia* seeds grew seedlings while no growth was observed in any other treatment method across all species. The results show that to effectively break seed dormancy and stimulate seed germination, a proper dormancy breaking technique must be used.

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INTRODUCTION

Scarification and stratification are naturally occurring processes in nature that allow for a seed to germinate. Almost all seeds have a seed coat that protects the embryo from mechanical damage and the growth of microorganisms. The structure of seeds varies between species, which required different mechanisms of scarification or stratification to break the seed dormancy (Zeng et al 2004; Assogbadjo et al 2011). When planting a seed, the planter has full control over the scarification and stratification techniques employed. Therefore, it is in the planters' best interest to use an effective technique to break the seed dormancy for the given species.

The thesis investigates the effectiveness of three different methods to break seed dormancy of three important commercial mast species: *Sorbus decora* (showy mountain ash), *Vitis riparia* (Manitoba grape), and *Rhus typhina* (staghorn sumac). These three different methods were mechanical scarification with sandpaper, hydrochloric acid (HCl), and cold moist stratification. All three of the species studied are not only consumed by animals but also by humans. *Rhus typhina* is used in spice mixes, teas, sauces, and syrups (Podgorski 2021). Although the leaves of *Sorbus decora* are poisonous, the berries are high in iron and vitamin C and are made into jellies (Olczyk and Geszprych 2017). People consume *Vitis riparia* fruit through jellies and pies but the most common use of the plant in agriculture is using it for rootstock and scion breeding (Lowe and Walker 2006). Due to the importance of the species studied to humans and wildlife the results of the thesis could be beneficial to forest, greenhouse, and agricultural managers interested in planting the species studied in the thesis.

(H_0) Mechanical scarification, chemical scarification, and cold moist stratification have no effect on the germination rates of *Sorbus decora, Vitis riparia,* and *Rhus typhina.*

(H_1) Mechanical scarification, chemical scarification, and cold moist stratification will increase germination rates of *Sorbus decora, Vitis riparia,* and *Rhus typhina.*

LITERATURE REVIEW

SEED DORMANCY

Seed dormancy is a period of growth inactivity even when temperature, water, and day length would usually cause growth (Bidlack and Jansky 2017). The primary function of seed dormancy is to prevent seed germination when conditions are suitable for germination, but the likelihood of the seedling surviving is low (Fenner and Thompson 2005). Species that have evolved to have seed dormancy have done so to improve their survival rates (Foley and Fennimore 1998, Oregon State University 2021). Dormancy distributes germination over time (Foley and Fennimore 1998; Fenner and Thompson 2005; Oregon State University 2021). This benefits plant species because in the event that seeds germinate during unfavourable conditions, other seeds will be left over to germinate at a later date (Foley and Fennimore 1998; Fenner and Thompson 2005; Oregon State University 2021). Delayed germination also protects species from all their seeds and seedlings being eaten by herbivores and can encourage germination when competition for light and water is low (Fenner and Thompson 2005).

There are two categories of organic seed dormancy: exogenous and endogenous (Oregon State University 2021). Seeds that display exogenous dormancy use their external structures, such as seedcoat, pericarp, or fruit walls to protect the seeds' embryo

(Baskin and Baskin 2001, Oregon State University 2021). Exogenous dormancy is controlled by factors outside of the seed (Baskin and Baskin 2001, Oregon State University 2021). Endogenous dormancy is controlled by factors within the seed (Baskin and Baskin 2001). Chemical reactions in the seed embryo and endosperm control the dormancy of endogenous seeds (Wareing 1965; Baskin and Baskin 2001).

There are three classes of endogenous dormancy: physiological, morphological, and morphophysiological (Nikolaeva 1977). Physiological dormancy is the most common form of dormancy (Finch-Savage and Leubner-Metzger 2006). Physiologically dormant seeds have an embryo that produces physiological inhibiting mechanisms that prevent the radicle from emerging (Baskin and Baskin 2001). Physiological dormancy exists at various levels and can be defined as deep, intermediate, or nondeep (Baskin and Baskin 2004). Seeds with deep physiological dormancy cannot be grown through excising the embryo (Baskin and Baskin 2004; Baskin et al 2005). The seeds will not grow or will produce irregular seedlings (Nikolaeva 1977; Baskin and Baskin 2004; Baskin et al 2005). Additionally, gibberellic acid treatments are unable to break deep physiological dormancy (Baskin and Baskin 2004; Baskin et al 2005). To germinate seeds with a deep physiological dormancy, several months of warm or cold stratification are required (Baskin and Baskin 2004; Baskin et al 2005). Most physiologically dormant seeds are classified as nondeep (Baskin and Baskin 2004). Nondeep physiological dormancy can be broken by excising the embryo or through gibberellic acid treatment (Finch-Savage and Leubner-Metzger 2006). Depending on the species, the dormancy of these seeds can be broken by scarification, after-ripening in dry storage, and cold or warm stratification (Finch-Savage and Leubner-Metzger 2006). Intermediate physiological dormancy is similar to nondeep dormancy in that the dormancy can be

broken by excising the embryo or through gibberellic acid scarification (Baskin and Baskin 2004).

Morphological dormancy is a class of endogenous dormancy characterized by an embryo that is underdeveloped in size (Baskin and Baskin 2001; Finch-Savage and Leubner-Metzger 2006). Before germination can occur, there must be time given for the embryo to differentiate and grow inside the diaspores (Baskin and Baskin 2001; Finch-Savage and Leubner-Metzger 2006; Jaganathan 2020). Morphological dormancy is broken when suitable conditions for embryo growth and germination are established (Baskin and Baskin 2001). Morphophysiological dormancy combines the underdeveloped embryo found in morphological dormancy with the dormancy-breaking requirements characteristic of physiological dormancy (Finch-Savage and Leubner-Metzger 2006). Consequently, warm and/or cold stratification is required to break dormancy and gibberellic acid scarification can sometimes be substituted for the stratification (Baskin and Baskin 2001; Finch-Savage and Leubner-Metzger 2006).

Exogenous dormancy is classified as physical, chemical, or mechanical (Baskin and Baskin 2001). Physical dormancy is caused by a seedcoat or fruit coat that is impermeable to water (Baskin and Baskin 2001). Seedcoat impermeability is achieved through at least one impermeable palisade layer made up of sclereid cells with thick and lignified secondary walls (Esau 1964). Seeds with chemical dormancy delay germination by producing or translocating growth inhibitors in the pericarp, seed, or surrounding fruit (Nikolaeva 1969; Nikoleave 1977). There is evidence that abscisic acid could be the growth inhibitor controlling chemical dormancy (Nikolaeva 1969; Nikoleave 1977). To break chemical dormancy the pericarp must be removed, or the surrounding fruit must be leached (Baskin and Baskin 2001). Mechanical dormancy is caused by a hard,

woody fruit wall (Nikolaeva 1969). This structure is usually the endocarp but the mesocarp can be woody as well (Hill 1933).

A single seed can have a combination of physical and physiological dormancy (Fenner and Thompson 2005). These seeds display a class of dormancy called combinational dormancy and are rare (Fenner and Thompson 2005; Finch-Savage and Leubner-Metzger 2006). Seeds with combinational dormancy have water-impermeable coats and physiological dormancy in the embryo (Baskin and Baskin 2004). Only non-deepp physiological dormancy has been observed in studied seeds (Baskin and Baskin 1998; Cho et al 2020). To break combinational dormancy, a treatment to break physical dormancy is used in combination with a treatment to break physiological dormancy (Baskin and Baskin 2014; Cho et al 2020). In some species, the seeds should be after ripened at warm temperatures to break physiological dormancy before scarifying to break physical dormancy (Baskin and Baskin 2014; Cho et al 2020). Other species require that physical dormancy is broken first through scarification (Cho et al 2020). Then the scarified seeds can be cold stratified to break physiological dormancy (Cho et al 2020).

GERMINATION REQUIREMENTS OF RHUS TYHPINA

Non-stratified seeds of *Rhus typhina* have been found to have germination percentages of less than 20% (Norton et al 1985). The findings of Norton et al (1985) are not in line with Li et al (1999) who reported a 99% germination rate in *Rhus typhina* seeds without stratification. The 99% germination rate was unchanged when the seeds underwent a 21-day cold stratification (Li et al 1999). *Rhus typhina* exhibits physical dormancy because of a water-impermeable seed coat (Norton et al 1985; Li et al 2007). Scarification by immersing the seeds in boiling water yields better germination results

than sulphuric acid scarification (Li et al 2007). Li et al (2007) found that 100% of *Rhus typhina* seeds absorb water and germinate when they are immersed in boiling water for one minute. In Li et al's (2007) study, only about 15% of *Rhus typhina* seeds absorbed water and germinated when soaked in sulphuric acid for one hour. There is no procedure published for the scarification of *Rhus typhina* with HCl and literature on *Rhus typhina* acid scarification is limited. However, acid scarification procedures for other species of the *Rhus* genus are available (Norton et al 1985; Li et al 1999; Pullman et al 2021). In 2021, Pullman et al. developed a scarification protocol for *Rhus glabra* and *Rhus michauxii.* The researchers found that soaking the seeds in concentrated sulfuric acid for 5 hours resulted in germination percentages of 98% for *Rhus glabra* (Pullman et al. 2021). Their findings were in line with other literature on scarification of *Rhus glabra* (Brinkman 1974; Boyd 1943). *Seeds* germinated within one month of scarification (Pullman et al. 2021). Studies on *Rhus* scarification have also used gibberellic acid for scarification and similar germination rates were found (Li et al. 1999; Norton 1985; Pipinis et al. 2017; Tang et al. 2019). For example, Li et al. (1999) found that *Rhus glabra* seeds that underwent gibberellic acid scarification had a germination success rate of 93%. However, seeds must remain soaked in gibberellic acid for 24 hours to obtain the high germination rate seen in Pullman et al's (2021) study.

GERMINATION REQUIREMENTS OF *VITIS RIPARIA*

The regeneration of all *Vitis* cultivars was poorly studied until 2017. A study by Orsenigo et al. (2017) examined the effects of cold and cold/warm stratification on *Vitis labrusca, Vitis vinifera, and Vitis riparia*. This study was the first demonstration of how *Vitis* hybrids are capable of regeneration by seed, even without animal dispersal (Orsenigo et al 2017). The researchers found that all 3 species had reduced germination

percentages when stratification took place in dark conditions (Orsenigo et al 2017). When alternating temperatures were used during seed stratification, all 3 *Vitis* species responded with higher germination percentages (Orsenigo et al 2017). The most important temperature for *Vitis riparia* was a simulation of winter conditions at 0°C (Orsenigo et al 2017). Orsenigo et al. (2017) found that at least 3 months of winter conditions were required for *Vitis riparia* to germinate (Orsenigo et al 2017). Depending on the population, germination was increased by 58%-98% (Orsenigo et al 2017). *Vitis riparia* seeds display deep morphophysiological dormancy (Baskin and Baskin 2014; Orsenigo et al 2017). *Vitis riparia* seeds should be stratified with a combination of cold and warm stratification that simulates changing seasons (Orsenigo et al 2017). The temperature of stratification should be changed daily on a 12-hour cycle (Orsenigo et al 2017). The following regimes should be used for stratification and incubation: 4 weeks at 25/15°C (early autumn), followed by 8 weeks at 15/5°C (late autumn), 12 weeks at 0°C (winter), 4 weeks at 15/5°C (early spring), 8 weeks at 25/15°C (late spring) (Orsenigo et al 2017). Orsenigo et al (2017) achieved a germination rate of 96% in *Vitis riparia* by developing this method. The winter period was the most important period for breaking dormancy and the seeds germinated in the late spring simulation (Orsenigo et al 2017). Other literature recommends 6 weeks of cold stratification (Plants for a Future na). *Vitis riparia* did not respond to gibberellic acid scarification in the 2017 study by Orsenigo et al.

GERMINATION REQUIREMENTS OF *SORBUS DECORA*

Species that belong to the genus *Sorbus*, exhibit physiological dormancy (Finch-Savage and Leubner-Metzger 2006; Afroze and O'Reilly 2013; Tang et al 2019).

Mechanical seed coat abrasion is harmful to *Sorbus decora* seeds and results in low rates of germination (Hilton et al. 1965). *Sorbus decora* responds well to sulfuric acid scarification when used in combination with cold stratification (Hilton et al 1965). However, the soft and thin seed coat only requires 10 minutes of soaking for the best germination rate (Hilton et al 1965). A standard germination test for *Sorbus* has not yet been developed. Additionally, Barclay and Crawford (1984) found that the after-ripening requirements of *Sorbus* can vary, depending on the time and place the fruit is collected. Chilling to break dormancy can take 9 – 26 weeks depending on the species (Gordon and Rowe 1982; Harris and Stein 1974; ISTA 1993). A study by Hilton et al (1964) found that high *Sorbus decora* seed germination rate is directly related to low stratification temperatures and long stratification periods. *Sorbus decora* exhibited the highest germination rates when stratified for 120 days at 2 degrees Celsius (Hilton et al 1964). *Sorbus alnifolia* seeds germinate at a rate of 91% when stratified at 5 degrees Celsius for 150 days then incubated at alternating temperatures of 5 and 15 degrees Celsius on a 12-hour cycle (Tang et al 2019). Afroze and O'Reilly (2013) studied *Sorbus aucuparia* germination and developed a method that does not require a temperature cycle for incubation and remains a constant 15 degrees Celsius. In the study, *Sorbus aucuparia* underwent 24 – 28 weeks of cold stratification for a germination rate of about 90% (Afroze and O'Reilly 2013). *Sorbus* seeds are commonly stratified through a cold stratification method (Gordon and Rowe 1982; Lenartowicz 1988; Taylor and Gerrie 1987). Multiple studies have found it beneficial to store *Sorbus decora* seeds in a warm environment for at least 2 weeks and then switch to a cold stratification for 14-16 weeks (Gordon and Rowe 1982; Lenartowicz 1988; Taylor and Gerrie 1987). Although, it has also been found that a warm period before cold stratification does not affect germination

if cold stratification of the *Sorbus decora* population was relatively high initially (Lenartowicz 1988). When cold stratification results in low germination rates of the *Sorbus decora* population then preceding with a warm period of at least 6 weeks significantly improves germination (Lenartowicz 1988). Variations in germination rate between *Sorbus* populations are common (Lenartowicz 1988; Var 2010). A study by Var et al. (2010) found that one population of *Sorbus torminalis* had a 96.6% germination rate while another had a germination rate of 13.3%. Both populations underwent the same lab conditions and were stratified in a mixture of 80% peat and 20% stream sand (Var et al 2010).

THE USE OF HYDROCHLORIC ACID IN SEED SCARIFICATION

Hydrochloric acid (HCl) is used in a variety of concentrations to scarify seeds (Lautenschlager 1997; Abubakar and Maimuna 2013; Chac 2014). Seeds are soaked for varying durations of time depending on the thickness of the seed coat and the concentration of the acid (Lautenschlager 1997; Abubakar and Maimuna 2013; Chac 2014). Soaking can also be done intermittently or all at once (Lautenschlager 1997; Abubakar and Maimuna 2013; Chac 2014). There has been no research published on HCl scarification of *Sorbus, Vitis, or Rhus* genus'. The recommended concentrations of HCl and durations of soaking vary widely between studies and species. Chac (2014) found that soaking *Solanum lycopersicum* (Roma tomato) and *Citrullus lanatus* (crimson sweet watermelon) seeds for 2 hours in 0.15M HCl increased germination rates to 95% and 85% respectively. This can be compared to the non-scarified control seeds which had germination rates of 80% and 55% respectively (Chac 2014).

MATERIALS AND METHODS

SEEDS

The seeds of showy mountain ash (*Sorbus decora)*, Manitoba grape (*Vitis riparia)*, and staghorn sumac (*Rhus typhina)* were all collected from the Thunder Bay area in September and October of 2021. Manitoba grape seeds were collected from the Braun Building courtyard at Lakehead University. Showy mountain ash seeds were collected from a tree on the Lakehead University Campus. Staghorn sumac seeds were collected from a tree on the lawn of 63 Kenwood Ave. A total of 1200 seeds were collected for all species. To collect seeds, bundles of berries were removed from the trees using secateurs. Only new growth was removed to minimize damage to the tree and allow for fruit production the next year. Seeds were immediately extracted from the fruits manually. The fruit was gently crushed to expose the seed. Any fruit material left on the seed was wiped off and the seeds were stored inside a refrigerator at 3° C until they were planted.

TREATMENTS

There were four treatments in this study: cold stratification, sandpaper scarification, HCl scarification, and a control where no stratification or scarification was applied to the seeds. Each treatment method was preceded by soaking the seeds in water for 24 hours while stored at 3° C inside a refrigerator. The cold moist stratification was carried out on all 3 species. Each species group underwent the same stratification process in separate containers. Each container contained 100 seeds of a single species sandwiched between 2 pieces of moist paper towel. The seeds were stored in a refrigerator at 3° C and examined daily for signs of germination. The paper towel was

misted with a spray bottle when it began to dry out. Before they were stratified the seeds were stored in a refrigerator at 3° C while soaking in water for 24 hours.

The scarification was carried out using 120 grit sandpaper. The seed coat was sanded away until a small portion of the seed was exposed (Figure 1). Next, the seeds were soaked in water for 24 hours at 3° C and then planted. 100 seeds were used for each of the species studied. The acid scarification was carried out using hydrochloric acid. 100 showy mountain ash seeds were soaked in HCl for 30 minutes, washed off with water, soaked in water at 3° C for 24 hours, and then planted. Manitoba grape and staghorn sumac followed the same process except that they were soaked in HCl for 60 minutes. Acid scarification was carried out with 0.15M hydrochloric acid. All three species were soaked in separate containers for 2 hours while being kept at 3° C. The seeds were then moved into a sieve where they were washed thoroughly with water. 100 seeds of each species were used as controls to the other treatments. A seed viability float test was used to determine the viability of each seed. Seeds were placed in a beaker of water and examined after 15 minutes. The seeds that were floating were categorized as nonviable and the seeds that sank were categorized as viable.

Seeds were planted in groups of 5 per pot in a mixture of 30% vermiculite and 70% sphagnum peat moss. 20 planting pots per species per scarification method were used (120 pots in total). All the pots were placed in a greenhouse on the same bench and watered when the soil was dry (every 2-3 days). The experiment was carried out in the main greenhouse of the Lakehead University Forest Ecology complex in Thunder Bay. The experiment lasted for 107 days and germination was observed daily.

Figure 1. Sandpaper scarified Manitoba grape seed.

RESULTS

Germination rates were mostly 0 across all species and treatment methods. Sandpaper scarification of Manitoba grape was the only treatment and species that showed signs of germination. Table 1 summarizes the germination rates of each species group for all the methods studied. In the seed viability float test, 100 staghorn sumac seeds floated, 8 Manitoba grape seeds floated, and 18 showy mountain ash seeds floated.

Table 1. The number of showy mountain ash, staghorn sumac, and Manitoba grape seeds that sprouted/germinated/sank in stratification, scarification, and float test trials.

	Staghorn sumac	Manitoba grape	Showy mountain ash
Sandpaper scarification	0	9	0
Sulphuric acid scarification	0	0	0
Cold moist stratification	0	0	0
Control (Float Test)	0	92	82

DISCUSSION

No seeds of *Sorbus decora* and *Vitis riparia* HCl and sandpaper scarification germinated. *Sorbus decora* and *Vitis riparia* seeds exhibit physiological and morphophysiological dormancy, respectively (Finch-Savage and Leubner-Metzger 2006; Afroze and O'Reilly 2013; Baskin and Baskin 2014; Orsenigo et al 2017; Tang et al 2019). By only treating the seeds with scarification, the seeds did not experience the correct dormancy breaking temperatures before they were planted (Afroze and O'Reilly 2013; Orsenigo et al 2017; Tang et al 2019). Sandpaper scarification has been shown to damage *Sorbus decora* seeds and lower their overall germination rate (Hilton et al 1965). This likely contributed to the low germination rates in the sandpaper scarification trials. Therefore, it is not surprising that no germination occurred in seeds that were not stratified. The stratification of *Sorbus decora and Vitis riparia* did not yield any germination results because the seeds were never incubated after they were stratified (Orsenigo et al 2017; Tang et al 2019). It is possible that the dormancy of the seeds was

broken, but seeds should have been incubated in a peatmoss and vermiculite mixture and observed for signs of germination. By not doing this, it is unknown if any of the seeds broke dormancy or could have germinated. The results of this thesis suggest that a combination of scarification and cold stratification should be more effective to break the dormancy and promote germination of *Sorbus decora* and *Vitis riparia* seeds.

Rhus typhina germination was 0% for the stratification trial because the seeds' dormancy was not properly broken. Since *Rhus typhina* exhibits physical dormancy, the water-impermeable seedcoat must be compromised to allow water into the seed and break dormancy (Li et al 2007). To test the effect of cold stratification on *Rhus typhina,* the germination rates of seeds that were stratified and scarified should be compared to seeds that were only stratified. Once the stratification period is over, the seeds must be germinated in a combination of peatmoss and vermiculite to observe and calculate the germination rate. Proper dormancy breaking treatments were used in the scarification of *Rhus typhina*, but the germination rate was 0%. This is likely because the seeds were inviable. The float test for *Rhus typhina* showed that 100% of the seeds collected were inviable.

The thesis could be improved by collecting the seeds from more than one individual plant for each species. This would create a greater sample size for the species and represent the population more accurately. Seeds were immediately sealed in an airtight bag for storage after they were extracted from their fruit. Proper seed storage practices state that seeds should be dried to a moisture content of 3 – 7% before they are stored (Peterson and Facelli 1992; De Vitis et al 2020). Seeds should be air-dried by spreading them out in a thin layer in an airconditioned room at least for 72 hours (Peterson and Facelli 1992; De Vitis et al 2020).

CONCLUSION

The results of this thesis show that an improper dormancy breaking technique will not be effective in breaking seed dormancy and stimulating seed germination. The seeds should have been collected from multiple individual plant species to account for genetic variation. The seeds should have been dried before storage and seed dormancy should have been broken with techniques that correspond to the class of dormancy in that species. Therefore, there is plenty of room to improve the treatment design in future studies examining seed treatment to improve seed germination.

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