Seed Scarification Techniques in Silver Leaf Lupine

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Abstract A major problem affecting the populations of the endangered mission blue butterfly of the northern coast of California is the declining numbers of its host plant, the silver leaf lupine (*Lupinus albifrons*), due to competition with non-native species. An attempt to ensure the restoration of populations of *L. albifrons* through germination in greenhouses and subsequent transplantation was initiated in 1984 and has continued to date within the Golden Gate National Recreation Area (GGNRA). This work analyzes the germination rates, necessary expertise, and time efficiency of three different scarification techniques applied to rupture the seed coat and induce germination, in a greenhouse setting. The three scarification techniques applied were: 1) manual nicking with a razor blade near the attachment point, 2) an acid bath in concentrated sulfuric acid for 30 minutes, and 3) scarification by agitation between two pieces of sandpaper for 5 minutes. Sample sizes of 98 seeds were studied at three different greenhouses in GGNRA, California. The experiment was controlled for soil composition and sowing procedure. The data provided no conclusive evidence for determining the most successful treatment for increasing *L. albifrons* germination rates. There was no significant difference between the germination rates of the three scarification treatments including the control but there was a strong difference in the rates of imbibition. The success of the control provides an impetus for further studies regarding the need for scarification on lupine seeds. Among the three scarification treatments, manual nicking had the highest imbibition rate, had the lowest cost and was the most time efficient. The significance of these results will aid in the restoration of *L. albifrons* to its native habitat and encourage increased populations of the mission blue butterfly.

Introduction

The threat of extinction for native species of North America has been a pressing issue since the time of European colonization, with the unchecked dominance of invasive species from across Europe. It is only in the past few decades that the environmental advantage of restoring native ecosystems has been recognized and the large task of restoration been undertaken (Mackay 1995, 2001). One such threatened species, the mission blue butterfly (*Plebejus icarioides missionensis*), native to the coast of northern California, has been federally listed as endangered since 1976 (Elliott 2003, pers. comm.). The population decline of this tiny blue butterfly is principally attributed to the loss of its host plant, the shrub-like silver leaf lupine (*Lupinus albifrons ssp. collinus*) (La Pierre 1998).

Figure 1. Mission blue butterfly with *L. albifrons*

The mission blue butterfly (Figure 1) relies on one of three species of lupine to lay its eggs and for nourishment of larvae (Arnold, 1994). Due to a decline in numbers resulting from a loss of habitat, restoration of lupine to its native ecosystem is necessary in order to increase butterfly populations. In 1984 a recovery plan drawn up by the U.S. Fish and Wildlife Service (USFWS), outlined the need to protect mission blue habitat and to repair habitat damaged by urbanization, off highway vehicle traffic, and invasion by exotic, non-native plants (Arnold 1987). This recovery plan advocates the restoration of the native habitat of the mission blue butterfly by planting high numbers of native species of lupine, including *L. albifrons*.

Due to development and construction, what is left of the native habitat of the mission blue butterfly is mainly the area that comprises the Golden Gate National Recreation Area (GGNRA).

Within this protected area, the survival of the silver leaf lupine is impeded by non-native species and seed predation. This competitive environment has provided an impetus for the development of new methods to reestablish the lupine habitat. To decrease seed predation, biologists at GGNRA in the past, have collected lupine seeds in the field, germinated them in greenhouses and later transplanted them back to the field once they have grown to a substantial height (Setty 2003, pers. comm.). But low greenhouse germination yields have called for an inquiry into better methods for seed germination of the silver leaf lupine.

Past studies have been done with seeds of other classes of *Lupinus* to increase germination rates (Kaye 2001), but there is no information on studies of *L. albifrons*. Of the techniques applied to bush lupine (*L. sulphureus*), which included a variety of scarification and temperature treatments, the method which produced the highest percentage of germination was nicking the seed coat with a razor blade before sowing (Kaye 2001). Scarification techniques, such as nicking or the use of sandpaper, are meant to simulate the scarification of the seed coat that would occur in the natural habitat of the coastal sand dunes (Setty 2002, pers. comm.). A comprehensive experiment that specifically studies different scarification methods and the correlating germination percentages for silver leaf lupine seeds will facilitate lupine restoration, and in turn, the restoration of mission blue butterfly populations.

The objective of this research was to determine a scarification technique that would produce the highest yield of germination for the silver leaf lupine in the GGNRA. Three different scarification methods were tested: manual nicking, acid bath washing (Simms 2002) and sandpapering (Natoli 2003). While recording imbibition and germination rates, other factors were noted, including time efficiency of each treatment and necessary expertise. Based upon past studies of different classes of lupine (Sholars 2002), my hypothesis was that nicking seeds near the attachment point would produce the highest percentage of seed germination, but would also be the most labor intensive.

To determine the scarification technique that is best for the entire GGNRA, treated seeds were sown in three different greenhouses throughout the Recreation Area. This study thus yields results for germination that can be compared across greenhouses, to determine the method that is best overall, or that is particularly best for a specific greenhouse. This technique can then be the basis for more efficient restoration projects of the silver leaf lupine at all greenhouses in GGNRA.

Methods

All *L. albifrons* (LUAL) seeds used in this experiment were purchased by the GGNRA from *S & S Seeds*, collected from Los Alamos, California. The effects of scarification were examined with three different scarification techniques:

Manual nicking The nicking of seeds was performed using a one sided razor blade. The seeds were nicked on the side of the radicle, near the attachment point, and a small portion of the seed coat was removed. The dried weight of the seeds was taken, after which the seeds were soaked in tap water for one hour, dried and then weighed to confirm that they had imbibed water, signified by a 50% increase in weight (also signified by a transparent seed coat). After imbibition was confirmed, seeds were sown (Setty and Heimbinder 2002, pers. comm.).

Acid bath Seeds in batches of 25 were counted into small stopped glass flasks of 25 ml. In a chemical hood, concentrated sulfuric acid was poured onto seeds in sufficient volume to cover seeds with some excess. The flasks were swirled occasionally over a period of 25-35 minutes. The acid was then poured off into a glass beaker and water was immediately added to the seeds in the flask, which was then capped and shaken. Water was then poured off into a second beaker, more water was added, and the flask was again capped and shaken; repeating the rinsing in this manner five more times with each batch. Seeds were then soaked in water and refrigerated overnight at 4˚ C and then sown the next day (Simms 2002, pers. comm.).

Sandpaper Seeds in groups of 25 were placed on the bottom of a metal sieve which was lined with sandpaper (60 coarse grit). Using a wooden block covered in sandpaper of the same grade, the seeds were rubbed in a circular motion for 5 minutes between the two pieces of sandpaper. The seeds were then soaked for one hour, dried, and sown.

The experiments were performed at three greenhouses in GGNRA: (1) Fort Funston, San Francisco, (2) Presidio, San Francisco, and (3) Headlands, Marin. The soil standard at all greenhouses was a 3:1 mixture of GGNRA compost mix to perlite for all treatments; in addition, one set of nicked seeds was potted in 100% Sunshine Mix #4 at the Headlands nursery. This variable in soil composition was tested upon the request of the GGNRA native plant nursery staff since the mix is easily available and its success with lupine seeds is unknown. Due to space and monetary limitations at the Fort Funston and Presidio greenhouses, only the sandpaper and

nicked treatment were tested. At the Marin Headlands greenhouse, which is the newest and largest facility, all treatments were tested including a control with no scarification applied.

In total, three different treatments plus a control, with no treatment applied, were tested, and one soil variation. At each location, a sample size of 98 seeds was used for each treatment. All plants were sown in *Stuewe & Sons* brand Ray Leach "Stubby" Cells (1.5"x5.5"), one seed per pot, in racks with a capacity of 98 (14 x 7). All pots were sterilized before use by being dipped in Clorox water (10% solutions for 30 seconds) before sowing and then rinsed with water. Seeds were sown at a depth of 0.5cm. At Fort Funston and the Presidio, the pots were checker boarded. At Headlands the pots were staggered by placing them in the rack in an ordered sequence (treatments 1-5) up and down each column. Plants at Fort Funston and Headlands greenhouses were protected from predation by being placed inside of wire mesh enclosure cages. At the Presidio, where predation is not a problem, a plastic mesh sheet was placed over the racks. Germination was determined by the appearance of true leaves (Young 2002).

All seeds at the Fort Funston and Presidio greenhouses were placed under an automatic irrigation system which was set according to the discretion of the particular nursery manager, keeping soil barely moist, but never dry. Seeds at the Headlands greenhouse were watered with a misting system which keeps soil constantly damp at a setting of 25 VPDs (*vapor pressure deficit*).

The temperature at each greenhouse was monitored with a digital temperature recorder which was placed inside the cages for a three week period. Each recorder was hung inside an upside-down plastic cup to protect if from water damage due to constant watering. These recorders produced temperature graphs which facilitate the comparison of the temperature differences in the three greenhouses.

Germination data was collected and compiled into tabular form, documenting the germination progress for all treatments.

Results

Treatment Requirements and Imbibition Rates The manual nicking treatment for 98 seeds requires approximately 40 minutes; the average person can nick approximately 150 seeds per hour with little prior training. The only cost required for this treatment is the initial purchase of the razor blades, which is relatively minimal. Nicking yielded an 80% imbibition rate after

soaking for one hour. For the acid scarification of a total number of 98 seeds, three hours of labor were required. The acid treatment must be performed under a fume hood using concentrated sulfuric acid, an extremely dangerous substance and necessitates training in laboratory safety. Sulfuric acid must be bought in a large quantity and is relatively expensive. This treatment yielded a 63% imbibition rate. The sandpaper treatment required 5 minutes to scarify 98 seeds and can be performed with no prior experience. A large quantity of sandpaper can be purchased at a very low cost. This technique resulted in a high percentage of destroyed seeds, yielding a 32% imbibition rate.

Imbibition indicates that the seed coat has been successfully scarified which is necessary for germination of seedlings (Young 2002). Only seeds that displayed visible signs of imbibition, determined through the display of a transparent seed coat and increased size, were sown. Figure 2 demonstrates that there was a significant difference ($p < 0.000$) in the imbibition rates between the three treatments. The control had a 1% imbibition rate, but was not included in the figure.

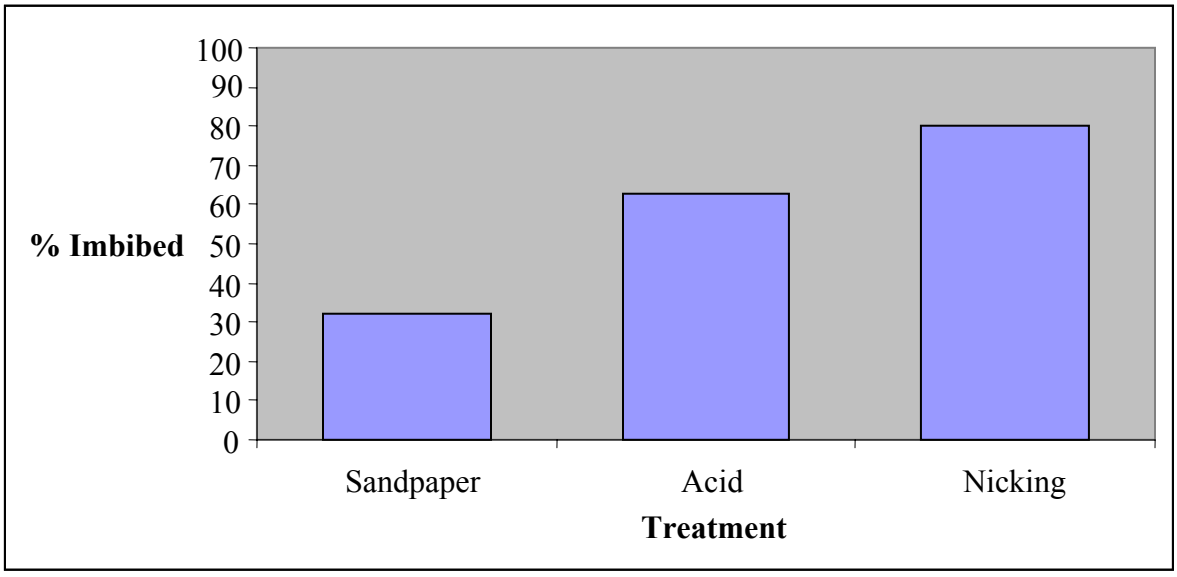

Figure 2. Imbibition rates for all three treatments ($p < 0.0001$, $\chi^2 = 48.6$, $df = 5$)

Germination Rates and Statistical Analysis Germination data collection was initiated approximately a week after the first seeds were sown, February 21, 2003, and was continuously collected on a weekly basis until April 7, 2003. Germination was determined by appearance of true leaves. At each greenhouse a sample size of 98 seeds was sown for each treatment.

Differences in germination rates between treatments in each greenhouse, and for specific treatments throughout all three greenhouses, were analyzed using a chi squared test.

Germination rates from all three greenhouses were put into tabular form to compare the success of the different treatments at each location (Figures 3, 4, and 5). At each greenhouse the germination rates among the different treatments were not significant.

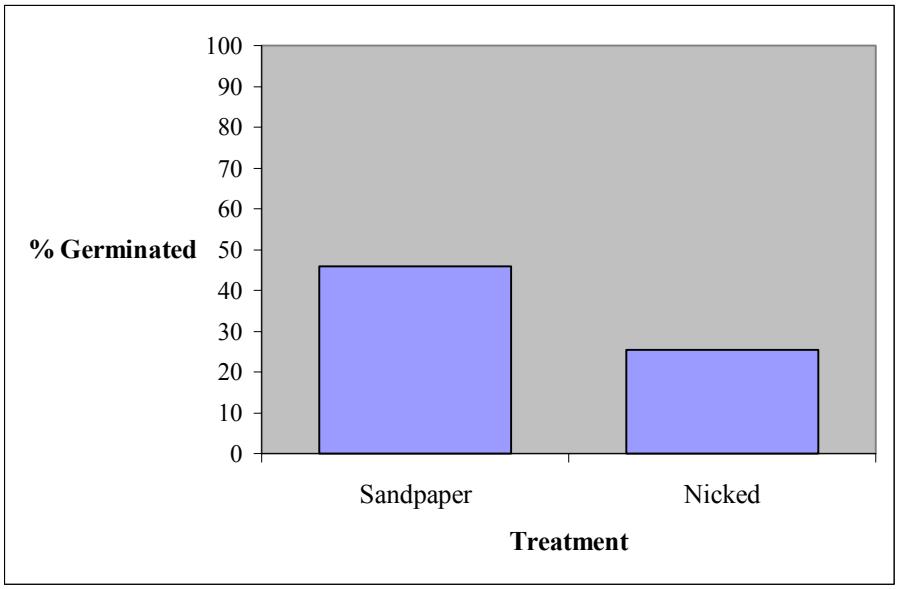

Figure 3. The Fort Funston germination results showed a significant difference between treatments ($p = 0.003$, $\chi^2 = 8.9$, $df = 1$).

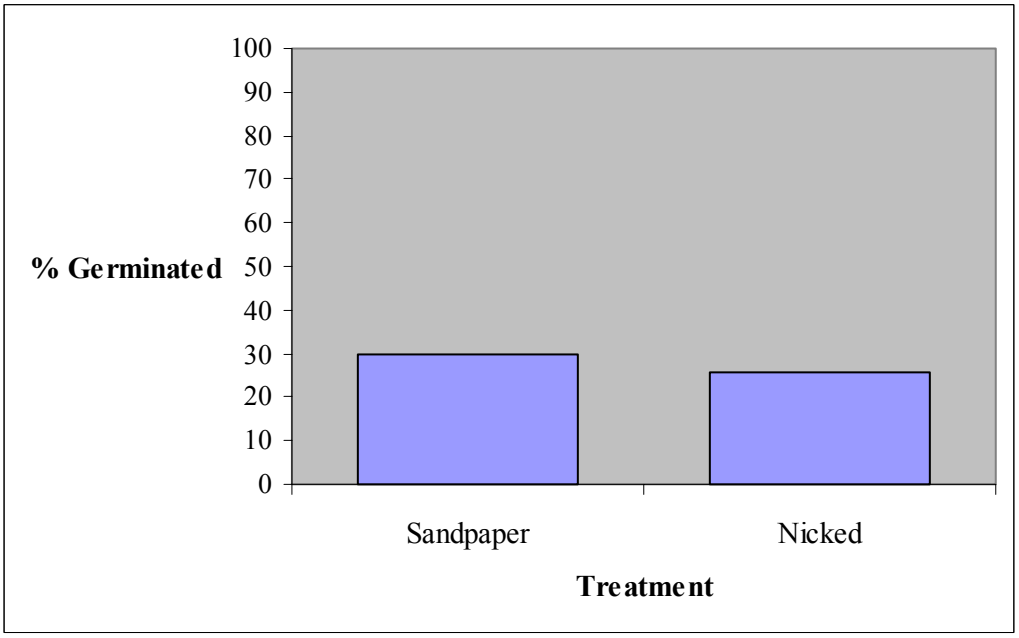

Figure 4. Presidio germination results showed no significant difference ($p = 0.4$, $\chi^2 = 0.4$, $df = 1$)

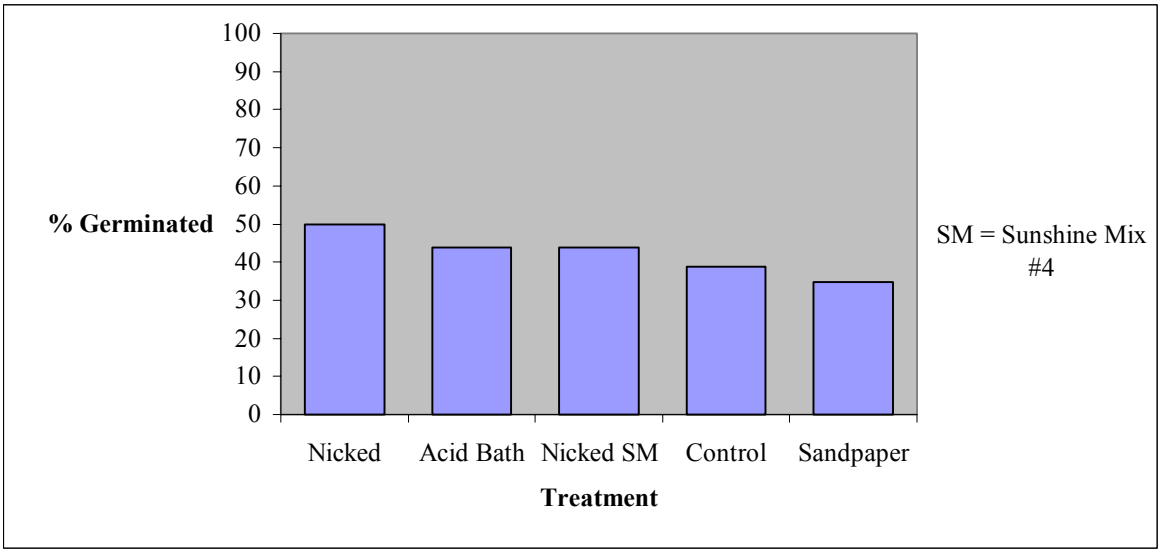

Figure 5. The Headlands germination results showed no significant difference between treatments ($p = 0.25$, $\chi^2 = 5.4$, $df = 4$).

The data collected was then compared for the different treatments among the three greenhouses. The two treatments that were tested at all greenhouses were the nicked and sandpaper methods. The results presented in Tables 1 and 2 represent the difference in germination rates among greenhouses using either the nicked or sandpaper technique.

Greenhouse	Germinated	Not germ
Fort Funston	25	73
Presidio	25	73
Headlands	49	49

Table 1. Results for nicked treatments at all greenhouses yielded a significant difference ($p < 0.0001$, $\chi^2 = 17.5$, $df = 2$).

Greenhouse	Germinated	Not germ
Fort Funston	45	53
Presidio	29	69
Headlands	34	64

Table 2. Results for sandpaper treatment at all greenhouses yielded no significant difference ($p = 0.053$, $\chi^2 = 5.9$, $df = 2$).

Discussion

The results of the experiment do not support my hypothesis that manual nicking would produce the highest germination rates. At Fort Funston, the sandpaper treatment produced significantly higher germination rates than the nicking treatment (Figure 3), while at the Presidio nursery the seeds sown showed no significant difference between the two treatments (Figure 4). The data for all five treatments performed at Headlands showed no significant difference between the treatments (Figure 5). These findings are quite unlike what were expected, especially considering that the control, which was not scarified at all, had a germination rate higher than that of the sandpaper treatment. This result challenges the accepted standard practice of seed scarification for lupines. The sample of nicked seeds sown in the Sunshine Mix #4 demonstrated a germination rate that was comparable to that of the other treatments sown in the compost/perlite mixture. This also questions the standard practice at GGNRA of sowing lupines in the compost/perlite mix.

The significant p-value (<0.0001) that was obtained when comparing the nicked treatment rates between greenhouses (Table 1) can be attributed to the high germination rates for all treatments yielded from Headlands nursery in comparison to the other two greenhouses. The Headlands nursery, which is the newest facility, has the only sophisticated misting system and has the highest degree of climate control of all the greenhouses. This advantage led to the significant difference between germination rates at Headlands compared to the other two nurseries.

In Table 2 the differences between the germination rates for the sandpaper treatment can be compared from all of the greenhouses. While the chi squared test shows no significant difference (with a p-value of 0.053), the data show a moderate difference between the greenhouses. The difference between the germination rates for nicked and sandpaper treatments in the three greenhouses leads to the conclusion that there may not be one scarification method that is best for all greenhouses. Due to the variation within the different greenhouses, including modernity, watering regime, and climactic factors, it is possible that the best scarification technique has to be determined on a case by case basis.

In response to the results found at the Headlands nursery with high germination of the control, it is possible that this difference from what is known as common practice was actually due to the variety of *Lupinus albifrons* used. The seeds used in the experiment were purchased from a supplier due to the quantity needed and the value of those actually collected from the GGNRA. The seeds from the supplier were collected from a site in Los Alamos, California, located about 15 km from the coast. It is possible that the seed coat of the seeds used was adapted to a less sandy habitat, and because of this, it had a thinner seed coat to compensate for reduced scarification opportunities. This thinner seed coat would make scarification techniques irrelevant because the intact seed coat would not impede germination. Thus, until further studies can be performed with the specific variety of *L. albifrons* growing natively in GGNRA, the success of seeds without scarification can not be fully supported.

The data resulting from the imbibition rates is also very important in analyzing the results of the experiment. The significance of the high 80% imbibition rate (Figure 1) resulting from the nicked treatment should not be ignored. Since imbibing water signifies seed coat scarification, the success of the nicked treatment indicates that, regardless of all other factors, it is the most successful technique in breaking the seed coat. Since there are other factors to consider including greenhouse variation, time, and cost efficiency; imbibition rates were not solely used to determine the best treatment. If this experiment had been performed sowing all seeds post-treatment, not considering whether they had imbibed water or not, then the germination rates may have reflected the imbibition rates that were obtained. For this reason, figure 1 represents the efficiency of each treatment in scarifying the seed coat, which was the initial goal of the experiment.

The purpose of this study was to determine the treatment that produced the highest germination rates, was the most time efficient and that required the least amount of expertise. The latter is important so that seed scarification could easily be performed by volunteers if needed. With this consideration, the utility of implementing the acid bath treatment as a common practice in GGNRA is low. This treatment must be performed in a laboratory with the required safety equipment to prevent injury while working with concentrated sulfuric acid. To this date, GGNRA has no access to a facility that would allow them to integrate this treatment into their normal routine.

The treatment that necessitates the least amount of time and expertise is the sandpaper treatment. This method requires about five minutes to perform and could be done by even the school-age volunteers. But with such a low imbibition rate of 32%, which can be mainly attributed to seed destruction during sandpapering, this should not be performed on seeds that are of scarce supply, which is the case of *L. albifrons*. Further experimentation of this method could lead to a higher imbibition rate; by modifying the pressure used, the grain of sandpaper and the time scarified, seed mortality could be greatly decreased.

The nicked treatment, relative to the other treatments among the nurseries, had the highest germination percentages at the Headlands nursery and proved to have the highest imbibition rate. This technique, which is the common practice at the Headlands and Fort Funston greenhouses, can be performed at a moderate rate of 150 seeds per hour per person. The time required reflects the accuracy with which it scarifies the seed coat, thus not damaging the seed and leading to a lower mortality rate. Older volunteers would be able to perform this treatment without difficulty. Due to the high accuracy, low mortality rate, and moderate performance difficulty, this treatment is the best method for the germination of *L. albifrons* seeds at GGNRA until further studies conclude otherwise.

The conclusion to recommend manual nicking as the preferred treatment over the other scarification treatments and the control is valid. While the proposition of not using a scarification technique at all seems to have had similar germination rates as the other treatments in this experiment, these tests cannot compete with the past experience of all of the experts with whom I had personal communication who have found scarification necessary. It is acknowledged though, that further tests should explore germination without scarification on *L. albifrons* seeds from the field to provide a stronger argument in its favor. In regards to the implementation of a technique in the meantime, manual nicking is advised as the most effective and most efficient scarification method.

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