Germination Rates of *Bursera simaruba* Seeds Subjected to Various Scarification Treatments

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Abstract

Bursera simaruba (L.) Sarg. seed were subjected to five scarification treatments to determine their efficacy on subsequent germination. Seeds that were scarified with sandpaper had the highest mean germination, although it was not statistically different than the untreated control. Those treated with hot water had significantly lower germination than the control, suggesting that temperatures may have been too hot. These results indicate that mechanical scarification may improve germination of this species but that further research is needed to refine treatments.

Introduction

Bursera simaruba (L.) Sarg., known as turpentine tree or gumbo-limbo, is easily recognized by its reddish, papery bark and the smell of turpentine when its leaves are crushed or branches are cut (figure 1). The species epithet *simaruba* references the Taino name for the tree (Nellis 1994). The Tainos, also called Arawaks, were the people Columbus encountered on the Caribbean islands when he claimed the Americas for Spain in 1492.

Figure 1. *Bursera simaruba* tree at Buck Island National Monument off the coast of St. Croix, VI. Note the thin, coppery bark and the large crooked branches. (Photo by Michael Morgan, 2014)

Distribution and Characteristics

Bursera simaruba is native to northern South America and the Caribbean Basin (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964). The species is abundant in the U.S. Virgin Islands and Puerto Rico. It has also become naturalized in south Florida, but some discussion remains regarding whether *B. simaruba* is an introduced species to Florida (Navarrete-Tindall and Orellana-Nuñez 2002, Nelson 1994). *B. simaruba* is very tolerant of salt, wind, and drought, making it well adapted to the semiarid Virgin Islands environment. It is found close to the sea and on hilltops, and it is native to limestone-derived soils (Kirk 2009). Although found in primary forests (such as xeric ridgetops), it is more common in secondary forests (Navarrete-Tindall and Orellana-Nuñez 2002).

On a good site, *Bursera simaruba* trees can grow up to 100 ft (30 m) in height and 36 in (1 m) in diameter; however, heights of 30 to 45 ft (10 to 15 m) are more typical. Leaves are alternately pinnate and compound. The length of the whole leaf is 6 to 8 in (15 to 20 cm); the whole leaf consists of leaflets, 1.2 to 1.6 in (3 to 4 cm) long by 0.4 to 0.8 in (1 to 2 cm) wide, occurring in one to three pairs and a single one at the tip. The leaflets are dark green on the upper side and lighter below. Leaves tend to be bunched at the end of the branches, making for an open crown made conspicuous by large, crooked branches (Jones 1995). The bark is thin, reddish or coppery, and peels away, showing green bark below. The small, white flowers are borne in spikes on the branch ends. The species is dioecious; male and female flowers are borne on separate trees. Some flowers, however, are bisexual. The fruit are capsules resembling small, three-sided olives and are green when young but reddish when mature (figure 2). The fruit opens when ripe, revealing a three-sided seed

Figure 2. Closeup view of *Bursera simaruba* leaves, unopened purple seed capsules, and naked seed covered in red pulp in center of photograph. (Photo by Michael Morgan, 2014)

covered by a thin, red pulp (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Nelson 1994).

Phenology

The physiography of Maryland combines features from both The phenology of *Bursera simaruba* is interesting. It is briefly deciduous, losing its leaves during the dry season months of January and February. Then, the tree flowers before or at the same time as new leaves appear around March (Jones 1995). Pollination of *Bursera* species is ambophilous; that is, it is performed by both insects and wind (Bullock 1994). Wind pollination is favored in the absence of rain, low relative humidity, and good air movement—common conditions in tropical dry forests (Bullock 1994). These weather conditions are the exact conditions on the island of St. Croix, VI, when *B. simaruba* flowers (M. Morgan, personal observation). Regarding insect pollination of this species, Nellis (1994) stated, “While flowers only last one day, they begin nectar production before dawn leading to intense honey bee activity at day break.”

After flowering is complete, new fruits form and grow to almost full size within days of pollination, although the seeds take nearly a full year to ripen (Kirk 2009). Fruit become ripe in ones and twos, and they do not ripen off the tree. This uneven ripening makes collecting enough seeds for experimental purposes or for nursery production a challenge. It can take several weeks or months of repeat visits to fruiting trees to collect sufficient seed to conduct an experiment or to produce several dozen seedlings in a tree nursery. Although production from seed can be a challenge because of difficulties in obtaining sufficient seed and variable germination rates, *B. simaruba* is easily propagated by both large and small cuttings.

Products and Uses

Many products are derived from *Bursera simaruba*, especially from its resin. The resin, or the “turpentine,” from the wood is collected by slashing the tree’s trunk and allowing the resin to drip out. The resin is known by various names: cachibou, chibou, copal, gomart, or gum elemi (Nellis 1994). This last name is likely the source from which the common English name gumbo-limbo is derived. The resin is used for incense, insect repellent, varnish, various home remedies, and glue. The Mayans have been using the resin for ceremonies of the tree for centuries, both before and after the arrival of the Spanish to the Americas. Another closely related species, *B. graveolens* (Kunth) Triana and Planch, from the dry coasts of Ecuador and Peru, is used in a similar manner, but, instead of burning blocks of pure resin, pieces of resin-impregnated heartwood are burned. Its Spanish name is Palo Santo, which translates to "holy tree" or "holy stick" (Morgan and Jose 2013).

Perhaps the most interesting use of *Bursera simaruba* resin is that of birdlime. The use of birdlime is an ancient, worldwide (and usually illegal) practice of smearing a sticky substance on a tree branch to capture birds. Many sticky substances besides *B. simaruba* resin are used as birdlime. Birds land on the branch and are trapped. The hunter then collects the birds.

Bursera simaruba wood is soft and not durable, unless it is treated with preservatives. It has been used for interior carpentry, crates, match sticks, fire wood, and charcoal (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Navarette-Tindall and Orellana Nuñez 2002). As plywood and veneer, it is sold under the commercial name of Mexican White Birch (Longwood 1961). More unusual uses for the wood are the construction of coffins, canoes, carousel horses, and voodoo drums (Nellis 1994).

Because the species is easily propagated via cuttings, both large and small, the tree is also used as living fence posts. One can plant large branches upright in the ground as fence posts and string wire between the posts. After a short wait and some rains, the posts resprout, creating a living fence (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Navarette-Tindall and Orellana Nuñez 2002). Often times on the island of St. Croix, where the authors live, a straight row of evenly spaced trees grows in the woods, suggesting a former field or property boundary created from cuttings. These fence line trees provided seed for the new forest that developed after the field was abandoned.

A more recent use of *Bursera simaruba* is that of landscape plantings because of its attractive coppery bark.

Seed Dispersal and Scarification

Throughout the wide geographical range of *Bursera simaruba*, various bird species disperse the seeds. The birds like to eat the pink, lipid-rich flesh that surrounds the seeds. They swallow the fruit, and seed within it, whole. As the aril's flesh is digested, the seeds pass through the bird's stomach and intestines. The seed ideally is deposited far from the parent tree to generate new seedlings (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964). The number of bird species inhabiting the island of St. Croix is rather impoverished compared with other Caribbean islands and the North and South American mainlands. The primary author of this article, who is an avid birdwatcher, suspects that *B. simaruba* seeds are dispersed by pearly-eyed thrasher (*Margarops fuscatus*) and the gray kingbird (*Tyrannus dominicensis*). Both species feed primarily on insects but supplement their diets with fruit (Bond 1992, Evans 1990). These two bird species are so common on St Croix that one would not even bother to remark on their presence. They are seen repeatedly throughout the day, around houses, in open areas, and in the woods.

Seeds with physical dormancy, such as those of *Bursera simaruba*, have hard seedcoats that need to be cracked to allow for the entrance of water and air so the seed embryo can imbibe water and start metabolizing. Many of these seeds are animal dispersed. The seedcoat protects the seed embryo while the overlying fruit is being consumed, allowing for later dispersal. Passage through an animal's digestive tract often increases seed germination via exposure to stomach acid and the grinding actions of teeth or, in the case of birds, gizzards (Smith et al. 2002). This process serves to scarify the seedcoat, permitting the entrance of air and water to the seed embryo so that germination can begin.

Various scarification techniques can be used for seed propagation. One technique is to use sandpaper on a seed until it loses its shine, a result of abrading away the oily lipids that seal the seed to water. Another is to crack the seed with a hammer (Smith et al. 2002) or nick the seedcoat with a knife. Acid baths and hot-water soaks have been used to imitate stomach acids (Smith et al. 2002) and allow for scarification of many seeds at once. The problem with this method is that the seed embryo can be damaged or killed by soaking too long (i.e., be cooked). It is also potentially dangerous because hot water and acid can spill and burn nursery staff. A safer method is to soak seeds in cool water for several hours or even days to leach out chemicals that inhibit germination. With this method, the water must be changed daily to get rid of leachate or pathogens (Smith et al. 2002). If seeds soak too long, however, they may rot. For example, Cascol (*Caesalpinia paipai* Ruiz Lopez and Pavon), is a tropical dry forest tree with a hard-coated seed. Because the cascol seed is eaten and dispersed by ruminants, one would think that soaking overnight would be an appropriate pretreatment. Soaking for more than 4 hours, however, leads to rotting seeds (Morgan, n.d.). Less conventional pretreatments include feeding seeds to livestock, or even birds, and collecting the defecated or regurgitated seeds, setting fire to the seeds to burn off a thick pericarp, enabling ants to eat the pericarp, and even treating the seeds with fungal spores (CATIE 2000).

Scarification treatments may improve seed germination. In germination trials with the closely related *Bursera graveolens*, which also has bird-dispersed seeds, Morgan and Jose (2013) found that different scarification treatments (mechanical, chemical, and heat) increased seed germination compared with the untreated control. In that study, germination averaged 21 percent for untreated seeds and increased to 53 percent when seeds were immersed in water heated to 158 ºF (70 ºC) and allowed to soak for 24 hours as the water cooled. Using sandpaper to weaken the seedcoat before planting increased germination to 34 percent. The objective of this study was to determine if seed germination of *Bursera simaruba* could also be improved by scarification treatments before planting.

Materials and Methods

Seeds were collected from two *Bursera simaruba* trees growing approximately 10 mi (15 km) apart from each other on the island of St. Croix. Because of the uneven seed ripening of this species, seeds were collected weekly for 3 months (October, November, and December, 2014) to accumulate enough seeds for the experiment. Usually 12 to 20 seeds were collected on each visit. Pulp was removed from the seeds by washing the fruits in a 10-percent bleach and water solution. Then the seeds were allowed to air-dry before being stored in a small plastic box placed in a cool, dry room.

The experiment was performed in a glasshouse at the University of the Virgin Islands Agricultural Experiment Station on the island of St. Croix. Christiansted, the nearest town, is located at long. 64°43' W. and lat. 17°45'01" N. (NOAA 2015). The glasshouse is screened on both the east and west sides to allow for air circulation. Winds predominantly blow from east to west in the Caribbean. Air temperatures range from 78 °F (24 °C) to 104 °F (40 °C) (Rhuanito Ferrarrizi, personal communication). Day length varies from 11 hours in the winter month to 14 hours in the summer months. At the time of germination, however, day length was 12 hours.

A total of 180 seeds were divided into five treatment groups of 36 seeds; each group was assigned a scarification treatment. Each treatment had three replicates of 12 seeds each. The five treatments were (1) soaking in concentrated sulfuric acid for 4 minutes, followed by a water rinse; (2) soaking in the growth hormone gibberellic acid for 4 hours; (3) mechanically scarifying the seedcoat with sandpaper until one side of the seed had been roughened to allow for the entrance of air and water to the seed embryo; (4) immersing in hot water heated to 158 °F (70 °C), followed by a 24-hour soak as the water cooled; and (5) maintaining an untreated control.

The seeds were sown in early March 2015 in 6-by-8 in (15-by-20 cm) unibody plastic trays filled with a 50:50 peat moss and perlite mix (figure 3). Each tray was randomly assigned a treatment, and a subset of seeds that received the assigned treatment was planted in that tray. The seeds were watered once daily. The number of germinated seeds in each treatment replication was recorded for the whole month of March, which was until 4 days after germination had ceased.

We had the opportunity to collect more seeds in September 2015 to conduct a second trial. By raking up leaf litter, we were able to uncover 150 seeds on the ground that had fallen from a *Bursera simaruba* tree. The seeds had either simply fallen off the tree and the aril rotted away, or birds had swallowed the fruit, digested the aril, and deposited the seeds in their droppings. An unusual drought in 2015 prevented the seeds from germinating. The seeds were estimated to have an age of 6 or 7 months.

Back in the laboratory, the seeds were washed in a 10-percent bleach solution and allowed to air-dry. Once dried, the seeds were divided into three treatment groups of 50 seeds. These seeds were not subjected to any pretreatment before sowing. On September 8, each group of 50 seeds was planted in a germination tray filled with 50:50 peat and perlite. The seeds were watered daily and monitored for germination as described previously in the March trial.

The number of germinated seeds by treatment was plotted over time. Analysis of variance was performed on the data

Figure 3. Germination of *Bursera simaruba* seeds treated with various scarification treatments was monitored for 30 days after sowing. (Photo by Michael Morgan, 2014)

using JMP® software, a menu-driven version of SAS (SAS Institute Inc., Cary, NC), to determine if a statistically significant difference occurred in germination among treatments and days since sowing. To meet assumptions of the model, the data were normalized before analysis by arcsine transformation. First, the raw data were converted into proportions. Then, the square roots of the proportions were calculated. Finally, arcsines were taken of the square roots (Chen and Maun 1999). A Dunnet's Post Hoc Test was used to compare the control with the different scarification treatments.

Results and Discussion

In the first trial, germination commenced 8 days after sowing and ceased 15 days later. Of the first three seeds to germinate, two had been subjected to the sandpaper scarification treatment and one was from the untreated control treatment. Seeds treated with the sulfuric acid and gibberellic acid treatments began germination 9 and 10 days after planting, respectively. Of the 36 seeds treated with the hot-water treatment, only 2 germinated during the study, 13 and 19 days after sowing. Germination ceased after 18 days for seeds treated with sandpaper scarification, 19 days for seeds soaked in gibberellic acid, 20 days for seeds subjected to the sulfuric acid treatment, and 23 days for untreated control seeds (figure 4). Overall, seeds scarified with sandpaper had the highest percentage of germinated seeds, although this did not differ significantly from those in the control treatment (figure 5). Seeds treated with hot water had significantly lower germination than those in the control treatment (figure 5). Seeds treated with gibberellic acid or sulfuric acid treatments had similar germination rates and did not differ from those in the control treatment. A statistically significant difference between timing of germination by treatment was noted 12 days after sowing (p = 0.04), indicating that seeds in all treatments, with the exception of the hot-water treatment, had begun to germinate. Average seed germination ranged from 6 to 44 percent across treatments. Note, however, that much variation occurred within treatment groups. For example, the three trays of 12 seeds assigned to the sandpaper treatment had 25, 33, and 75 percent germination (figure 6).

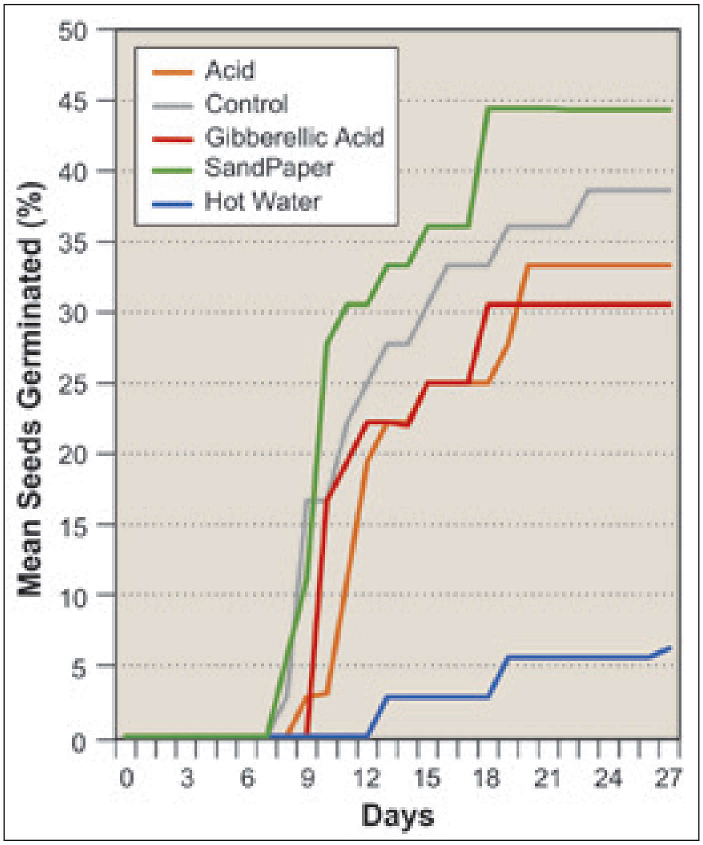

Figure 4. Average germination of *Bursera simaruba* seeds for each scarification treatment over time

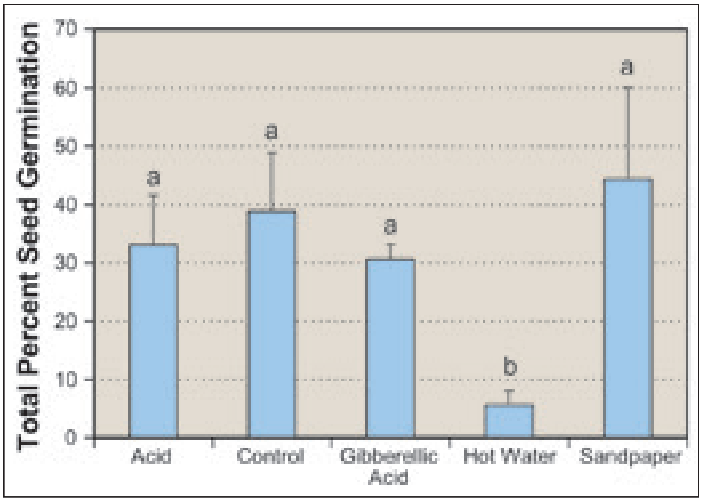

Figure 5. Total seeds germinated in each scarification treatment at the end of the study (30 days).

We suspect that the seeds in the hot-water treatment may have been exposed to too much heat, thus reducing the viability of the seeds. This result was surprising because a previous study with *Bursera graveolens* found that seeds immersed briefly in hot water (158 °F [70 °C]) had improved germination compared with untreated seeds (Morgan and Jose 2013), while those immersed in water heated to 219 °F (90 °C) were killed.

In the second trial, with seeds collected from the ground and sown untreated, germination began 5 days after sowing and ceased after 20 days, with a mean of 50 percent total germination. According to Navarrete-Tindall and Orellana-Nuñez (2002), 80 to 100 percent of *Bursera simaruba* seeds should germinate without any pregerminative treatment.

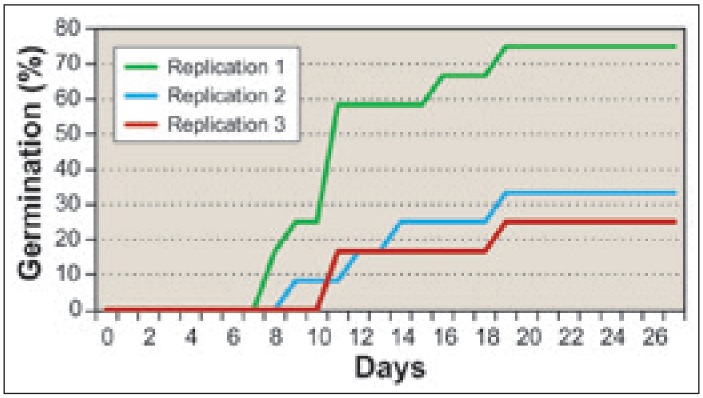

Figure 6. Germination was quite variable within treatments. This example shows germination among replications of sandpaper-scarified seed varying from 25 to 75 percent.

Our results using both fresh and older fresh seeds contradict this finding, with untreated seeds having 39 and 50 percent germination, respectively.

Murray and Russell (1994) conducted a study to determine if fruits of another bird-dispersed tree species (*Witheringia* spp.) had a laxative effect on the black-faced solitaire (*Myadestes melanops* Salvin), a type of thrush, while increasing seed germination. They found that the longer the seed was in the bird's digestive tract, the less likely it was to germinate. Of seeds that passed through the bird's stomach, however, 62 percent germinated compared with 51 percent germination for mature seeds just picked off the tree.

Although birds consume *Bursera* spp. fruit, it appears that this action serves primarily as a means of dispersal. In the trial with *B. graveolens* (Morgan and Jose 2013), germination of control seeds averaged 21 percent across four trials and did not differ greatly from the scarification treatments. Ortiz-Pulido and Rico-Gray (2006) similarly found that 17 percent of *B. fagaroides* (Kunth) Engl. seeds germinated if eaten and defecated by gray catbirds (*Dumetella carolinensis*) but no seeds germinated if eaten and defecated by white-eyed vireo (*Vireo griseus*), both of which were lower than the rate observed for seeds without any treatment (20 percent).

Conclusions

Sandpaper scarification treatment increased *Bursera simaruba* seed germination relative to the untreated control treatment. This result was not statistically significant, however, and notable variation was observed among replications. Because sanding each seed is labor intensive, the best method to propagate seedlings from seed is to sow the seeds without treatment after the seedcoat has been sterilized. Given these results and those of others, it appears that consumption of the aril and deposition of the seeds by birds is more important as a dispersal mechanism than as a scarification effect. Vegetative propagation is also an option for this species. At the University of the Virgin Islands, small cuttings of *B. simaruba*, 0.5 in (1 cm) in diameter and 12 in (30 cm) long, sprout new leaves and roots when placed in a container full of planting substrate and adequately watered (M. Morgan, personal observation).

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