

Increasing Seed Germination of *Bursera graveolens*, a Promising Tree for the Restoration of Tropical Dry Forests

Michael Morgan and Shibu Jose

Agroforestry Research Specialist, University of the Virgin Islands, Kingshill, USVI; Director of the Center for Agroforestry, University of Missouri, Columbia, MO

Abstract

This article describes a method to increase the germination rate of *Bursera graveolens*, a socially, commercially, and ecologically valuable tree species in southwest Ecuador and northwest Peru. The species suffers from (regional) unsustainable harvesting for its aromatic resin. Increased propagation success could lead to increased use of the tree in reforestation, restoration, and social forestry projects and on industrial plantations. Ecological observations of *B. graveolens* were made while the primary author managed the reforestation program and tree nursery in the Cerro Blanco Protected Forest in Guayaquil, Ecuador (2°11' S. and 79°53' W.), from 1995 to 2006.

Introduction: The Need for Restoration of Tropical Dry Forests

Worldwide, tropical dry forests and woodlands are characterized by annual precipitation between 10 and 40 in (250 and 2,000 mm) (Holdridge 1978), frost-free conditions year round with a mean annual temperature of 62.6 °F (17 °C), and an evaporation rate that exceeds precipitation (Holdridge 1978, Murphy and Lugo 1986). Holdridge (1978) defines tropical dry forests as having 40 to 80 in (1,000 to 2,000 mm) of annual precipitation, very dry tropical forests as having 20 to 40 in (500 to 1,000 mm) of annual precipitation, and tropical thorn scrubs (which is, in essence, degraded tropical dry forest woodland) as having 10 to 20 in (250 to 500 mm) of annual precipitation.

Subtropical and tropical dry forests of the world are quickly disappearing under human pressure. According to Janzen (1988), the tropical dry forest is the tropical lowland habitat most threatened with destruction—not the tropical rain forest. In Ecuador, less than 1 percent of the original dry forest currently exists (Neill and Nunez 1996, Sánchez and others 2006). Murphy and Lugo (1986) state various reasons for the destruction of tropical dry forests, one being that more dry forest just was, and is, available to destroy than was rain forest.

Of the Earth's tropical landmass, 40 percent is dominated by forest, of which 42 percent is dry forest, 33 percent is moist forest, and only 25 percent is wet or rain forest.

Tropical dry forests worldwide have been severely impacted by human settlement for thousands of years. People prefer tropical dry forests because they are healthier places to reside compared with tropical humid forests, and they are easier to clear for agriculture because of shorter trees and a favorable climate for raising livestock. Dry forest soils tend to be more fertile than wet or rain forest soils due to less nutrient leaching by rain. In addition, many valuable hardwood species, such as teak (*Tectona grandis* L. F.) and caoba (*Swietenia macrophylla* King), grow in dry forests. In fact, Murphy and Lugo (1986) suggest that scientists may never know the former true extent of tropical and subtropical dry forests because many savannas, scrubs, or thorn woodlands are thought to be originally dry forests degraded beyond recognition.

Although severely reduced in extent, tropical dry forests in Ecuador and coastal Peru are biologically diverse. These forests are part of the Tumbesian ecoregion and cover approximately 70,625 mi² (113,000 km²) along the Pacific coast of South America from northern coastal Ecuador to just north of Lima, Peru (Stattersfield 1998). The ecoregion is named for the Peruvian city of Tumbes, located on the Pacific coast close to the border of Ecuador and Peru. These Tumbesian tropical dry forests support many plant and animal species, including 313 woody species, 66 of which are endemic to the region. This high level of endemism is most likely due to their isolation from the tropical dry forests of the Pacific coast of Central America and the Brazilian Cerrado (Neill and Nuñez 1996, Sánchez and others 2006).

Worldwide, there is increasing interest in restoring degraded lands back to the ecological community that existed on site before degradation. That ecological community ideally includes both plants and animals. Because reintroducing animal species to areas of their former range can be

difficult, restorationists tend to focus initial efforts on plant communities. After a suitable habitat is created, the hope is that native animals will recolonize the now-restored site (Hobbs and Harris 2001, Ruiz-Jaen and Aide 2005).

Description and Ecology of *Bursera graveolens*

Bursera graveolens (Kunth) Triana and Planch, known as palo santo, is a deciduous tree species native to the tropical dry forests of Ecuador and Peru. The wood has been traditionally burnt as incense and mosquito repellent because it possesses aromatic resins and oils (Soudkoup 1970, Valverde 1990). In recent years, these resins and oils are being extracted from the wood by the perfume industry (Yukawa and Iwabuchi 2004). *B. graveolens* is a relatively fast-growing species that colonizes rocky outcrops. Not only does the tree produce a commercially valuable product, it has potential for use in the ecological restoration of tropical dry forests and minelands.

B. graveolens is common in dry tropical forests from the Yucatan Peninsula of Mexico, south to Peru, and on the Galapagos Islands of Ecuador (Valverde 1998). *B. graveolens* grows from sea level near the equator up to elevations as high as 5,000 ft (1,500 m), particularly in the Andes of Southern Ecuador and northern Peru (Colter and Maas 1999, Sánchez et al. 2006). *B. graveolens* grows on rocky, arid, and nutrient poor soils (Clark and Clark 1981, Guerrero and López 1993). In the driest areas that support tropical thorn scrub or very degraded dry forest, *B. graveolens* is found on a wide variety of soils, and is generalized throughout the landscape. In somewhat moister landscapes, such as dry and very dry tropical forests, *B. graveolens* occurs on xeric sites such as rocky slopes, ridge tops, and abandoned quarries instead of growing through the landscape (figure 1).



Figure 1. Leafless trees of *Bursera graveolens* during the dry season. (Photo by Eduardo Jaime Arias)

B. graveolens grows to a mature height of 24 to 50 ft (8 to 15 m) and a diameter at breast height of 12 to 24 in (30 to 50 cm). The leaves are compound leaves and the bark is smooth and gray, streaked with white, where resin drips down from cuts or abrasions (figure 2). *B. graveolens* has tiny, white unisexual flowers (Valverde 1990) (figure 3). Many taxa of the Burseraceae family are dioecious (Daly 1993, Opler and



Figure 2. Trunk of *Bursera graveolens* with resin streaks. (Photo by Eduardo Jaime Arias)

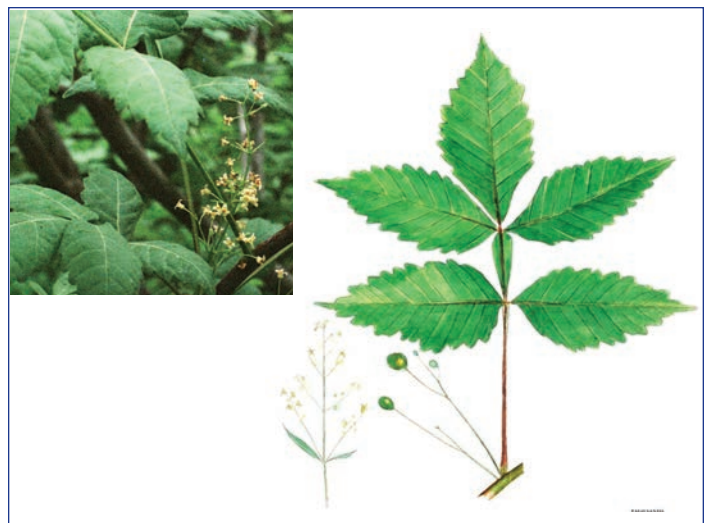


Figure 3. Watercolor of leaves, fruit, and flowers of *Bursera graveolens* and photo inset of *B. graveolens* leaves and flowers. (Art and photo by Eduardo Jaime Arias)

Bawa 1978), though it is not clear whether *B. graveolens* is or not because there is no obvious visible difference in male or female plants other than the presence or absence of fruit. Recent communication with New York Botanical Garden taxonomists inclines toward a belief that the species is monocious (Cornejo, personal communication 2007; Daly, personal communication 2007).

The fruit of *B. graveolens* is an aril: a small black seed, covered by a red pulp, contained in a green capsule one-half in (1.2 cm) long by one-fourth in (0.6 cm) wide, attached to a stalk (figure 4). The two halves of the capsule fall off when the fruit is ripe. The aril is rich in lipids, which makes it attractive to ants, rodents, and birds (Daly 1993). Lone individuals, and particularly groups of trees, emit an odor similar to anise (Guerrero and López 1993, Valverde 1990).

Chazdon and others (1996) treat the congener *Bursera simaruba* (L) Sarg., as an early successional species. *B. graveolens* could also be considered an early successional species or long-lived pioneer that establishes in a forest opening and persists in the overstory for many years. In fact, growth rings of *B. graveolens* have been used for dendro-chronical studies in Peru to record changes in precipitation and occurrences of El Niño Southern Oscillation Event (ENSO) events over a 47-year period from 1954 to 2001 (Rodríguez and others 2005).

Flowering and pollination of *B. graveolens* occurs during the transitional period between the absolute drought of the dry season and the abundant downpours of the rainy season. Opler and others (1976) found that light rains in a Venezuelan tropical dry forest triggered *B. graveolens* flowering in anticipation of the heavier rains that will follow. Around the city of Guayaquil (2°11' S. and 79°53' W.), *B. graveolens* flowering occurs soon after the first light rains start around the middle of December (weather data from Instituto Nacional

de Meteorología en Hidrología de Ecuador [INAMHI]), 1995 through 2006). Pollination is ambophilous (achieved by both insects and wind). Wind pollination is favored by the absence of rain, low relative humidity, and good air movement. These are common conditions in tropical dry forests (Bullock 1994). Flores (2002) reported that the congener *B. simaruba* is pollinated by wasps, which coincide with observations by the primary author of small wasps visiting the scentless, white flowers of *B. graveolens*.

Ripe fruit begin to appear in the last week of April and continue to ripen until the first week of June. The fruits do not all ripen at once but rather in ones and twos (Guerrero and López 1993, Valverde 1990). The seed capsules dehisce leaving the fruit attached to a stalk and hanging from the branch. The fruit either fall to the ground or are eaten by birds. These birds either consume the seeds or disperse them by defecation or regurgitation after digesting the red pulp that surrounds the seed.

In the Cerro Blanco protected forest and the Guayaquil area, there are 220 species of birds (Berg 1994, Pople and others 1997, Sheets 2004). Some are exclusively frugivorous; many more are partially frugivorous (or omnivorous) like the tyrant-flycatchers (of which 29 species are in Cerro Blanco) or the yellow-rumped Cacique (*Cacicus cela* L.) (figure 5). Some are granivores, totally or partially, like the finches, grosbeaks, and the aptly named seedeaters of the genus *Sporophila*. All these species could be seed dispersers, predators, or both. The primary author was unable to determine which bird species are consumers and which are dispersers of *B. graveolens* seed.



Figure 4. Closed and opened seed capsules of *Bursera graveolens*. Note the black seed surrounded by a red pulp. (Photo by Eduardo Jaime Arias)



Figure 5. Yellow-rumped Cacique (*Cacicus cela* L.), one of many bird species that disperse seeds of *Bursera graveolens*. (Photo by Eduardo Jaime Arias)

Research in the Galapagos Islands found dispersal and predation of *B. graveolens* seeds by the Galapagos dove (*Zenaida galapagoensis* Gould), the Galapagos mockingbird (*Nesomimus parvulus* Gould) (Clark and Clark 1981), and four species of Darwin's finches (*Geospiza* spp Gould) (Grant and Grant 1980). In Mexico, the white-eyed vireo (*Vireo griseus* Boddaert) and the grey catbird (*Dumetella carolinensis* C.T. Wood) ate the fruits and dispersed seeds of *Bursera fagaroides* (Kunth) Engl., while the white-tipped dove (*Leptotila verreauxii* Bonaparte) consumed the seeds (Ortiz-Pulido and Rico-Gray 2006). It is obvious that the Galapagos bird species are not present on the mainland of South America or the Cerro Blanco Protected Forest where the seeds were collected for the experiment described in this article, but their congeners are. Both the Pacific or West Peruvian dove (*Zenida meloda* Tschudi) and the long-tailed mockingbird (*Mimus longicaudatus* Tschudi) occur along the landward edge of mangroves, in areas of tropical thorn scrub that contain trees of *B. graveolens*, and in areas of very dry tropical forest that grade into tropical thorn scrub. Interestingly enough, these two species do not occur in the high hills or low mountains of the Cerro Blanco Protected Forest but rather in the nearby plains that extend to the Pacific Ocean. The hills are somewhat moister than the plain. Within the Cerro Blanco Protected Forest, the white-tipped dove (*Leptotila verreauxii*), which eats seeds of *B. fagara* in Mexico, is common. Also, another 11 species of dove or pigeon are present. Instead of the white-eyed vireo (*Vireo griseus*) that consumes *B. fagara* fruit in Mexico, there is the red-eyed vireo (*Vireo olivaceus* Linnaeus).

After the bird-dispersed or fallen seed is on the ground, it stays in the leaf litter for approximately 6 months until the rainy season begins again before germinating. During that period, some seed is subject to predation by ants and rodents as observed by the primary author and described by Daly (1993).

In addition to providing food and habitat for birds, *B. graveolens* also provides overstory conditions favorable for other forest tree species to colonize a site. Observations of forest changes over 18 years in the Cerro Blanco Protected Forest showed that plots along three ascending gradients of moisture, elevation, and successional status with an overstory of *B. graveolens* had saplings of *Simira ecuadorensis* (Standl.) Steyer. and *Capparidastrum petiolare* (Kunth) Hutch. developing underneath the canopy, two species representative of the next phase of forest succession (Morgan, unpublished data 1995–2006).

Study Objectives

One obstacle to the wider adoption of *B. graveolens* for ecological restoration projects is its low germination rate and, by extension, availability as planting stock. For example, informal germination tests performed in the tree nursery of the Cerro Blanco Protected Forest found, at best, germination rates of 8 percent. The objectives of this study were to (1) determine the most effective treatment to increase seed germination and (2) determine if there is a required period of seed dormancy that can be met through seed storage. We hypothesized that a pretreatment that mimics the passage of a seed through the digestive tract of a bird will increase seed germination, and that a time period equal to the length of the coastal Ecuadorian dry season must pass before the seeds germinate.

Materials and Methods

This study has an interesting aspect to it because research and observations were performed over various years and in two locations. Field observations were made over the course of 11 years in Ecuador, as were some informal germination trials. However, the formal experiments were performed at the University of Florida.

Seed Collection

Seeds for this experiment were obtained from the Cerro Blanco Protected Forest, more commonly known by its Spanish name, Bosque Protector Cerro Blanco. It is located outside Guayaquil, Ecuador's largest city and port with close to 2.5 million inhabitants (Instituto Nacional de Estadística y Censo del Ecuador, 2010). Cerro Blanco, which means *white mountain* or *white hill*, is a private forest reserve of approximately 15,000 ac (6,000 ha) of very dry tropical forest. Elevations range from nearly sea level to 1,696 ft (514 m). It is administered by the Ecuadoran nongovernmental organization Fundación ProBosque, which employed the primary author for 11 years managing the tree nursery and reforestation program.

The climate is tropical with an average annual temperature of 77 °F (25 °C), ranging from minimums of 57 °F (14 °C) and maximums of 99 °F (37 °C). Average annual precipitation is 39.52 in (988 mm) and is concentrated in the wet season months of December through May. It is supplemented by fogs or "garuas" in the summer months of the dry season. Fogs are not an insignificant source of precipitation, although difficult to quantify (Bonifaz and Cornejo 2004). Ecuador's Pacific coast is periodically subject to the ENSO where the amount

of rainfall can be double, triple, or quadruple that of a normal year (weather data from INAMHI 1995 through 2006, BBC 2008).

Seed for this experiment was collected from a small stand of 30 *B. graveolens* trees on a rocky slope that was quarried approximately 40 years ago for limestone before the establishment by government decree of the Cerro Blanco Protected Forest in 1989. The stand is at an elevation of 240 to 330 ft (80 to 100 m) above sea level. The nursery staff of Fundación Pro-Bosque collected fruits every day or two from April 2006, when the fruits started to ripen, until June. The staff collected fruits on the lower branches by hand and those on higher branches with a pole-mounted pruning shear. Because fruits do not ripen all at once, but rather in ones and twos, it was necessary to return every day or two to collect seeds. Collected fruits were put on a table in a shed to dry for 1 week and the seeds were removed from the fruit capsules as they opened. In 2007, a second batch of seeds was obtained fortuitously from former co-workers of the primary author and was used for an additional trial, the fourth and final one.

Seeds were washed in a 10-percent household bleach solution (3 to 6 percent sodium hypochlorite, NaOCl) to disinfect pathogens and remove the pulp that surrounds the seeds. Seeds were then air-dried and stored in sealed jars in a dry, dark place for approximately 2 months. Seeds were transported to the University of Florida in Gainesville where germination trials were conducted.

Germination Trials

In the first trial (March 16 through May 7, 2007), 6-month-old seeds were subjected to four treatments: untreated control; physical scarification with sandpaper; 4-minute soak in 95 percent pure sulfuric acid (H_2SO_4) (figure 6); and a hot water treatment. The hot water treatment consisted of placing seeds in water heated to 122 °F (50 °C) and allowing them to soak for 24 hr as the water cooled. Seeds were sown into commercially available trays of pressed peat pellets (36 pellets per tray, 1.44 in [36 mm] diameter, Jiffy brand). One seed was sown per pellet and one tray comprised a single treatment replication; there were three treatment replications total. The trial was performed in a growth chamber illuminated for 12 hr daily and maintained at 77 °F (25 °C). Pellets were kept moist at all times.

Three subsequent germination trials were conducted in a greenhouse. The substrate used for those trials was a 1:1 mixture of sand and vermiculite in 4-in (100-mm) diameter petri dishes. As in the first trial, the substrate was kept moist at all times. Temperatures in the greenhouse ranged between 77 °F (25 °C) and 104 °F (40 °C).

Treatments applied to 1-year-old seeds in the second trial (June 8 through August 11, 2007) included four sulfuric acid treatments, three hot water treatments, and an untreated control. The acid treatments consisted of immersing the seed for 1, 2, 3, or 4 min in 95-percent pure H_2SO_4 (figure 6) and thoroughly rinsing the seeds with water upon removal from the acid bath. Seeds treated with hot water were soaked for 24 hr in water heated to 122 °F (50 °C), 140 °F (60 °C), or 158 °F (70 °C); thereafter, the water was allowed to cool to room temperature. Each treatment had four replications with 15 seeds in each replication.

The third trial (August 17 through October 10, 2007) consisted of immersing relatively fresh seeds into six hot water treatments: 122 °F (50 °C), 140 °F (60 °C), 158 °F (70 °C), 176 °F (80 °C), 194 °F (90 °C), and 212 °F (100 °C). After immersion, the water was allowed to cool down and the seeds were soaked for 24 hr. In addition to using the hot water treatments, the third trial included a control treatment. The seeds were collected in 2007 from the same stand of trees as before and were approximately 2 months old at the time of the trial. Each treatment had four replications (petri dishes) with 15 seeds in each with the exception of the 212 °F (100 °C) treatment. Because it was expected that the 212 °F (100 °C) water treatment would destroy the seed embryo, that treatment had only one replication with 11 seeds.

Because of contradictory results of the third trial, a fourth trial was conducted November 1, 2008, through February 1, 2009, using 8 replications of 15 seeds. Seeds were subjected to the same gradient of water temperature treatments as used in the third trial (122 °F [50 °C] to 212 °F [100 °C]). In addition, some seeds were soaked in an acid bath for 1, 2, 3, 4, or 5 min.



Figure 6. Seeds were subjected to acid scarification treatments by placing them in this spoon-shaped container for loose tea leaves and soaking in 95 percent pure sulfuric acid for 1, 2, 3, 4, or 5 min. (Photo by Michael Morgan)

Percent seed germination was evaluated at the end of each trial. Data were normalized by calculating the square root of the proportion of germinated seeds and then multiplying by the arcsine. Transformed data were subjected to analysis of variance. Treatments were compared with Tukey Post-Hoc tests (Chen and Maun 1998, Longnecker and Ott 2004, Pereira de Souza and Valio 2001). Each trial was analyzed separately to compare differences among treatments. To see if storage time affected germination, the germination rates of the control seeds in the four trials were compared, because the seeds in each trial had been stored for different periods.

Results

Results from all four seed germination trials are shown in table 1.

In the first trial, physically scarified seeds had significantly higher germination than control seeds ($p = 0.019$) (figure 7). Physical scarification, however, was considered too labor-intensive for practical application in large nurseries.

The second trial showed no significant differences among the acid treatments and the control. Seeds treated with the 70 °C hot water treatment had the highest average germination (53 percent, $p = 0.016$), suggesting that hot water treatments are effective at breaking the seed coat and promoting germination.

In the third trial, germination tended to increase as water temperature increased, then declined and ceased ($p = 0.00018$) as follows: started at 122 °F (50 °C), increased at 140 °F (60 °C), peaked at 158 °F (70 °C), dropped sharply at 176 °F (80 °C), and ceased at 194 °F (90 °C). It was unexpected however, to find that the control seeds had the highest germination (20 percent). If we exclude the results of seeds subjected to the 90 °C treatment, in which no seeds germinated, the p value is 0.22, indicating no statistical difference among treatments. Because these results were unexpected, the fourth trial was conducted with identical hot-water treatments. It was surprising to find that the control seeds again had the highest mean germination (18 percent). As with the second trial, acid treatments did not increase average germination relative to the controls.

No significant differences existed in germination among the control seeds of the four trials, indicating that neither seed age nor storage duration was a factor in the germination of these seeds (figure 8). These results also indicate that no physiological seed dormancy exists for this species; after the seed coat is broken and the seed embryos absorb water, the seeds can germinate. Tropical seeds tend to have a short

Table 1. Percent germination of seed for each trial and treatment. Within each trial, means followed by the same letter do not differ significantly at $p \leq 0.05$.

TRIAL	TREATMENTS	MEAN	MIN	MAX	SE
1	Control	24.07 b	19.44	30.56	2.72
1	Acid 4 min	23.15 b	33.33	25.00	0.76
1	Sandpaper	34.26 a	33.33	36.11	0.76
1	Hot water 50 °C	28.70 ab	25.00	30.56	1.51
2	Control	23.25 b	13.00	33.00	4.33
2	Acid 1 min	23.33 b	12.50	33.33	4.45
2	Acid 2 min	20.00 b	6.25	31.25	5.63
2	Acid 3 min	11.67 b	6.67	18.75	2.94
2	Acid 4 min	21.67 ab	12.50	33.33	4.42
2	Hot water 50 °C	21.67 b	0.00	37.50	7.81
2	Hot water 60 °C	36.67 ab	20.00	56.25	8.07
2	Hot water 70 °C	53.33 a	33.33	75.00	8.85
3	Control	20.00 a	12.50	31.25	4.43
3	Hot water 50 °C	11.75 a	7.00	20.00	3.09
3	Hot water 60 °C	13.25 a	7.00	20.00	2.66
3	Hot water 70 °C	19.75 a	13.00	33.00	4.71
3	Hot water 80 °C	11.50 a	0.00	20.00	4.17
3	Hot water 90 °C	0.00 b	0.00	0.00	0.00
3	Hot water 100 °C	0.00 b	0.00	0.00	0.00
4	Control	18.00 a	6.67	20.00	3.5
4	Acid 1 min	5.00 b	0.00	13.33	2.7
4	Acid 2 min	14.00 a	0.00	26.67	3.4
4	Acid 3 min	6.6 a	0.00	20.00	2.1
4	Acid 4 min	10.00 a	6.67	20.00	1.7
4	Acid 5 min	10.00 a	6.67	13.33	3.3
4	Hot water 50 °C	17.50 a	6.67	33.33	3.7
4	Hot water 60 °C	12.50 a	0.00	33.33	4.6
4	Hot water 70 °C	16.20 a	0.00	33.33	9.4
4	Hot water 80 °C	5.00 b	0.00	13.33	1.6
4	Hot water 90 °C	0.00 c	0.00	0.00	0.00
4	Hot water 100 °C	0.00 c	0.00	0.00	0.00



Figure 7. Seeds of *Bursera graveolens* germinating. (Photo by Michael Morgan)

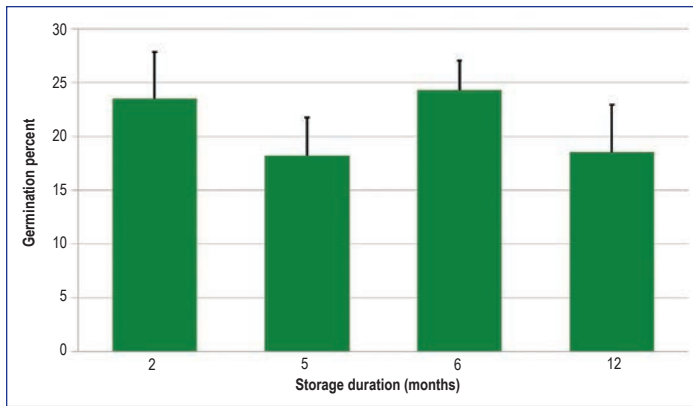


Figure 8. Effect of seed age and seed storage on germination (\pm SE); there was no difference in germination among untreated (control) seed in the four trials.

storage period, because the higher temperatures of the tropics result in faster chemical reactions, such as respiration and photosynthesis, than in cooler climates. Hence, refrigeration and cold rooms are essential for seed storage. These trials demonstrate that *B. graveolens* seeds can be stored for up to a year without affecting germination.

Discussion

The results of this experiment bring up some interesting points about frugivory, seed dormancy, and pregerminative treatments of seeds. In general, plants reproduce themselves by exchanging genetic material in the form of pollen with other plants of the same species. From the successful exchange of genetic material, seeds are formed. The seeds, once in the soil, start to germinate when there is sufficient light, air, and moisture.

Seeds, once fully formed, need to get from the branch to the soil. The most obvious way is to fall off the plant onto the ground below. This option, however, is not necessarily the best for the seed. Many seeds and seedlings will not germinate under the shade of its parent(s). The shady microsite underneath the parent might favor the development of pathogens that prey on seeds and seedlings. Or, a concentration of succulent seedlings will attract herbivorous predators. Therefore, many plants have evolved or devised ways for their offspring to be transported away from the parent and to a (hopefully) suitable site for germination, establishment, growth, and future reproduction. Dispersal distances can vary widely. Many trees produce light, windborne seeds that are carried away in the wind some distance. Two examples of trees with wind-dispersed seeds are the ashes (*Fraxinus* sp) in the temperate regions of the world and laurel (*Cordia alliodora* [Ruiz and Pav.] Oken), from the neotropics. On the other hand, red mangroves (*Rhizophora mangle* L.) and coconuts (*Cocos nucifera* L.) seeds float on water and use the

ocean waves and currents to transport the seeds to a suitable site. Other species use animals to transport the seeds. Some seeds are sticky such as beggartick (*Bidens frondosa* L.) and attach themselves to animals' fur to carry them away. Other plants use edible fruits to attract dispersing animals. The seeds of algarrobo (*Prosopis juliflora* [SW] DC) are contained within a sweet pod that ruminant animals, such as cows, eat. The seeds are either spit out while the animal chews its cud or defecated later. Walnuts (*Juglans* spp) produce big nuts that are collected and cached by squirrels (*Sciurus* sp) to eat later some distance away from the parent tree. Sometimes the animal does not return for its seeds and a seedling sprouts from the forgotten cache. Birds also disperse many seeds. They are attracted to the ripe fruits and either defecate or regurgitate the seeds. Some birds, such as parrots, and the appropriately named seedeaters consume or predate upon seeds if they are not poisonous. For this reason, seeds of many species are poisonous to avoid predation.

Some fresh seeds have physiological and/or physical dormancy and do not germinate when planted in conditions with appropriate light, moisture, temperature, and aeration. Physiological (or chemical) dormancy avoids having the seed germinate during a brief window of favorable conditions only to result in the tender seedling being killed when conditions revert to being too dry or too cold. The classic example of chemical dormancy is that of acorns from oak (*Quercus* sp). These seeds overwinter under the snow and/or leaf litter where it is cool and damp before they germinate in the spring. In fact, these seeds will not germinate in a nursery if planted immediately or soon after collection; they must first be stored some weeks in a refrigerator in a plastic bag full of wet leaves. In the case of *B. graveolens*, we would expect that if there is a seed dormancy period, the best seed germination would happen approximately 6 months after fruiting and seed fall, at the start of the rainy season. Results of this experiment, however, disproved the need to break chemical dormancy. Most tropical seeds can germinate readily after seed fall provided there is sufficient moisture available (Smith and others 2002).

Physical (or seed coat) dormancy requires that the seed coat be broken to allow the entrance of water, so that the seed embryo can imbibe water and start metabolizing. For those species that are animal dispersed, the seed coat protects the seed embryo, while the overlying fruit is being consumed, allowing for later dispersal. We know that *B. graveolens* produces a flesh-covered seed that is defecated by frugivorous birds. This implies that the seed coat needs to be broken before the seeds can germinate (Smith and others 2002). Not only do birds have stomach acid, they have rough gizzards,

sometimes filled with stony grit to help them grind up and digest their food (Gill 1990); the seed coat allows the plant embryo to survive passage through the gastro-intestinal tract.

Various scarification techniques have been used to break physical seed dormancy. One way is to use sandpaper on a seed until it loses its shine, because the oily lipids that seal the seed have been abraded away. Another is to crack the seed with a hammer (Smith 2002). Acid baths and hot water soaks are often used to imitate stomach acids (Smith 2002). These treatments also allow scarification of many seeds at once. Problems associated with this method are that the seed embryo can be damaged or killed by soaking too long (i.e., be cooked) and that handling hot water and acid is potentially dangerous to personnel. A safer method is to soak seeds in cool water for several hours or days (changing the water regularly to remove leachate and/or pathogens) so that chemicals that inhibit germination leach out and the seeds can then imbibe water. The drawback with this method is that seeds can rot if soaked too long. For example, Cascol (*Caesalpinia paipai* Ruiz Lopez and Pavon) is a hard-coated seed from a tropical dry forest tree. The seed is found in woody pods eaten by ruminants such as cattle or deer. One would think that soaking overnight would be an appropriate pretreatment. Soaking for more than 4 hr, however, leads to rotting seeds (Morgan, unpublished data 1995–2006). Some more unconventional seed 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; allowing ants to eat the pericarp; and treating seeds with fungal spores (Centro Agrícola Tropical de Investigación y Enseñanza, 2000).

In this study, *B. graveolens* responded to seed scarification (albeit inconsistently). In other seed germination research, *Bursera simaruba*, a congener of *B. graveolens*, is dispersed by both birds and monkeys and had germination between 80 and 100 percent without scarification (Navarette-Tindall 1990). Murray and others (1994) experimented with the bird dispersed tree species *Witheringia* spp.) and the black-faced solitaire (*Myadastestes melanops* Salvin) to determine if the fruit of *Witheringia* had a laxative effect, while increasing seed germination. They found that the longer the seed was in a bird's digestive tract, the less likely it was to germinate; however, 62 percent of the seeds passed through a bird's stomach germinated, as opposed to 51 percent of mature seeds just picked off the tree.

Perhaps in some cases, frugivory is more important as a means of seed dispersal than as a pregerminative treatment. Ortiz-Pulido and Rico-Gray (2006) found that 17 percent

of *B. fagaroides* seeds germinated if eaten and defecated by *Dumetella carolinensis* and 0 percent germinated when eaten and defecated by *Vireo griseus*. These rates were actually lower than the germination rate observed for seeds without any treatment (20 percent).

Conclusions

Results suggest that mechanical scarification with sandpaper improved germination of *B. graveolens* seeds. It is unfortunate that this method is too laborious for the production of large quantities of seedlings. Further results suggest that immersing *B. graveolens* seeds in 158 °F (70 °C) hot water and allowing them to soak for 24 hr increase germination. The average germination of three trials with this treatment was 30 percent. Exposure to hot water temperatures greater than 158 °F (70 °C) resulted in reduced, or no, germination. Although birds consume fruit from this species, it appears that this action serves primarily as a means of dispersal, because germination of control seeds averaged 21 percent across four trials and did not differ greatly from several of the scarification treatments.

Address correspondence to:

Michael Morgan, Care of UVI-AES, RR#1, Box 10,000, Kingshill, VI, 00850; e-mail: mikeboskey@hotmail.com.

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