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THE EFFECTS OF TEMPERATURE AND STORAGE ON *CONOCARPUS ERECTUS* (COMBRETACEAE) GERMINATION

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ABSTRACT

This study examined the effects of seasonal temperature regimes on Buttonwood [*Conocarpus erectus* L. (Combretaceae)] seed germination and how well seeds store under refrigeration vs. ambient temperature. There was no difference in imbibition between scarified and non-scarified seeds, so non-scarified seeds were used for the germination experiments. Seeds were growth chamber germinated to determine which season is most conducive to Buttonwood germination. The growth chamber was set at a day/night (12 hour day/12 hour night) temperature regime for each of 3 trials: 28°C/14°C, 31°C/16°C, and 33°C/20°C representing the months of January, April, and July, respectively, on San Salvador Island. Germination ranged from 0 to 0.12%, while Timson's values ranged from 0 to 1.43 out of a possible score of 50. Seeds germinated best at 28°C/14°C (January, the coolest month) with 0.12% germination and a 1.1 Timson's value. Seeds stored at ambient temperature and refrigerated at 4°C were germinated at the 28°C/14°C regime, and it was found that refrigerated seeds had the best germination.

INTRODUCTION

Buttonwood, *Conocarpus erectus* L. (Combretaceae) is a tropical shrub native to the New World tropics and subtropics and West Africa (Francis, 2007). It is salt tolerant (Tomlinson, 1994) and grows landward of true mangroves above the high tide mark and along beaches. Buttonwood grows in the open or in co-dominant stands of plants of similar size; however, it will not grow in the understory because it is shade intolerant (Francis, 2007). Although Buttonwood is sometimes called Button Mangrove (Francis, 2007), it lacks specialized structures such as pneumatophores and prop roots (Tomlinson, 1994). For these and other reasons it is often not considered a "true" mangrove species (Tomlinson, 1994).

In Florida, Buttonwood blooms begin in March and end in September; in contrast, in Mexico and The Bahamas, Buttonwood blooms throughout the year (Francis, 2007; Kass et al., 2007). Buttonwood flowers are green to white (Francis, 2007), 2 mm long (Kass et al., 2007), and in clusters of 25 or more (Tomlinson, 1994). Flowers are likely wind-pollinated (Kass et al., 2007). The sexual system of Buttonwood has only recently been studied in any detail. It was originally thought that Buttonwood was dioecious (plants with either staminate or pistillate flowers); however, a study by Kass et al. (2007) examined over 1840 flowering Buttonwood plants on San Salvador Island, The Bahamas, and found that 11% of morphological males produced some fruit. They termed such "male" flowers cryptically andromonoecious, therefore rendering the Buttonwood population on San Salvador polygamous (Kass et al., 2007).

Buttonwood produces small winged drupes arranged in globular, button-like cones (Kass et al., 2007) that are about 1 cm in diameter (Tomlinson, 1994). *Conocarpus erectus* derives the common name Buttonwoood from these button-like infructescences (Guppy, 1917) that shatter when fruits are mature (Kass et al, 2007). Although observed to be a copious seed producer, Buttonwood seedlings are rare in comparison with associated mangrove species (Tomlinson, 1994).

There have been very few studies on Buttonwood germination (Kass et al., 2007). Guppy (1917) found that on Turks Island, The Bahamas only 3-4% of fruits produced by Buttonwood had seeds in them, and in Jamaica only 10% of the fruits had seeds in them. Francis and Rodriguez (1993) reported 12% germination from a Puerto Rican population. Experimental studies by Kass et al. (2007) found seed germination to be almost 10% lower than previous studies. In these studies, seeds were germinated at ambient temperature and there was no attempt to account for day/night temperature changes or seasonal variation. In addition, seeds produced by cryptically andromonoecious flowers germinated at the same rate as seeds produced by plants with only staminate flowers (1.3-1.7%) (Kass et al., 2007).

This paper presents quantitative data on Buttonwood seed germination in four related experiments. We believe it is one of the first detailed ecological studies of the germination of this species. We looked at seasonal variation with day/night temperature regimes, the potential for seed coat dormancy, the effects of seasonal temperature on germination, and how seed storage may affect seed germination.

Hypotheses

This study tested three separate hypotheses related to Buttonwood germination with four different tests. The first hypothesis was that a broken seed coat will allow scarified seeds to take up more water and at a faster rate than unscarified seeds. The second hypothesis was that seeds germinated at the lowest temperature will have the highest percent germination and fastest germination rate because this is when heat stress and salinity would be at their lowest in a tropical saline environment like Fresh Lake where many of our seeds were collected. And lastly, storing buttonwood under refrigeration will reduce percent germination as well as germination rate because the cool temperature may damage this tropical species.

MATERIALS AND METHODS

This study entailed four experiments. The first experiment tested seed coat dormancy of Buttonwood. The second experiment examined germination in response to seasonal temperatures. The third study compared germination of seeds that were stored unrefrigerated at room temperature to seeds that were stored refrigerated at 4°C. In all three studies seeds were placed in 15 x 100mm Petri dishes on 9mm no. P5 Fisherbrand© filter paper moistened with 10mL of de-ionized water. The water layer just covered the top of the seeds. The forth study used tetrazolium to test seed viability.

Seeds for the first two experiments were collected along Fresh Lake causeway (N. 24° 6.011'; W. 74° 26.844') from several Buttonwood shrubs with ripe infructescences on 1 May 2005. Seeds for the third experiment were collected on the access road to the AV pond (N. 23° 59' 4.5", W. 74° 29' 8.7") from a single female tree on 26 June 2005. Seeds for the tetrazolium test used the above groups of seeds as well as freshly collected seeds (23 June 2007) from the Fresh Lake causeway.

Seed Coat Dormancy

Baskin and Baskin (1998) found that when studying the germination biology of a species for which little is known, it is best to determine if an unscarified seed will imbibe water. Francis and Rodriguez (1993) state in their germination study of several tropical species that many of the hard coated seeds were nicked with a knife or file prior to germination; however, they do not specify if Buttonwood received this treatment in their germination trial.

To determine if the seed coat was inhibiting germination, an experiment comparing scarified seeds with unscarified seeds was performed. The experimental group of Buttonwood seeds were scarified between 2 pieces of 150 grit aloxite sand paper and were abraded just enough to break the seed coat. The control group contained unabraded seeds. Seeds were weighed dry, then placed in 10 mls of reverse osmosis water on filter paper in a Petri dish. Seeds were allowed to soak for 1, 2, 19, and 32 hours, then blotted dry on paper towels and weighed. There were three sets of 50 seeds for the control group and each experimental group. Data are presented as 1-(mass @ time n/original dry weight) vs. time (hours) (Fig. 1). Data were graphed in this manner to represent imbibition as an increase in a type II graph. Scarification did not increase imbibition, and may have slightly hindered it; therefore, non-scarified seeds were used for the temperature and seed storage studies.

Temperature Effect

Very little is known about seasonal variation and how that influences Buttonwood germination. We wanted to determine if seed germination was influenced by season. To represent the full range of possible temperature regimes on San Salvador Island, we replicated average documented temperatures in a growth chamber from January, April, and July from 2002. The three different day/night temperature regimes chosen were 28°C/14°C (January), 31°C/16°C (April), and 33°C/20°C (July) (Gamble 2002). Five Petri dishes containing 50 seeds per dish on moist filter paper were monitored. De-ionized water was replenished every three to four days, as needed. Each temperature trial ran until seeds stopped germinating (approximately 28 days). Percent germination as well as relative germination rates for each treatment were calculated using a modified Timson's Index (Timson, 1965; Khan and Ungar, 1984) in which the sum of germinated seeds for each day at the end of the germination period was divided by the total number of seeds. Because each replicate contained 50 seeds, the highest possible Timson's Index value would be 50 if all of the seeds germinated after one day.

Seed Storage

The temperature effect study indicated that the best germination temperature regime for Buttonwood was 28°C/14°C (January). This temperature was then used to determine if seeds were best stored at room temperature or refrigerated at 4°C.

Five Petri dishes (n=5) containing 50 seeds per dish were germinated on moist filter paper. Each Petri dish contained 10mL of deionized water. Water was replenished every three to four days, as needed. The trial ran approximately 28 days, until seeds stopped germinating. Germination percent and relative rates were calculated. Relative germination rate of each treatment was calculated using a modified Timson's Index (Timson, 1965; Khan and Ungar, 1984) as previously described. Because each replicate contained 50 seeds, the highest possible Timson's Index value would be 50 if all of the seeds germinated after one day.

Tetrazolium Test for Viability

Seeds collected on 1 May 2005 were stored at 4°C. Seeds collected on 26 June 2005 and 23 June 2007 were stored at ambient temperature. Ten seeds from each of the above groups were tested for viable embryos via a tetrazolium test. A 2,3,5-triphenyltetrazolium chloride solution was used to test for dehydrogenase (respiratory) enzymes. In live seeds these enzymes reduce tetrazolium to a red color (Bonner, 1974; Baskin and Baskin, 1998). Seeds were soaked in reverse osmosis water overnight then dissected from their achene and placed in a solution of 0.5% solution tetrazolium for six days. Field corn seeds cut in half were used as a control for solution concentration.

RESULTS AND DISCUSSION

With such extremely low germination, many Petri dishes did not have any seeds that germinated, and it would require counting several thousand seeds in order to have meaningful statistical analyses. Germinating and counting so many seeds is beyond any reasonable expectation. Although statistical analyses could not be performed, this study is important because now we have documented germination conditions that are favorable to Buttonwood.

Seed Coat Dormancy

Both scarified and unscarified seeds took up water at about the same rate (Fig. 1). Interestingly, the seeds with their seed coats intact took up slightly more water than scarified seeds, but these differences were minimal. The mean seed weight (n=3 for Petri dishes of 50 seeds each) before imbibition for unscarified seeds was 0.12g, and mean weight for scarified seeds was 0.14g. After 32 hours, unscarified seeds weighed 0.28g, while scarified seeds weighed 0.26g. This represents a 225% increase in weight for unscarified seeds, but only a 190% increase for the scarified seeds.

Hypothesis number one was not supported because both intact and scarified seeds absorbed water at roughly the same rate, and exogenous dormancy did not occur (Baskin and Baskin, 1998). Results of the tetrazolium test demonstrated that none of the seeds tested had a viable embryo, and we can rule out other reasons or dormancy mechanisms for preventing germination.

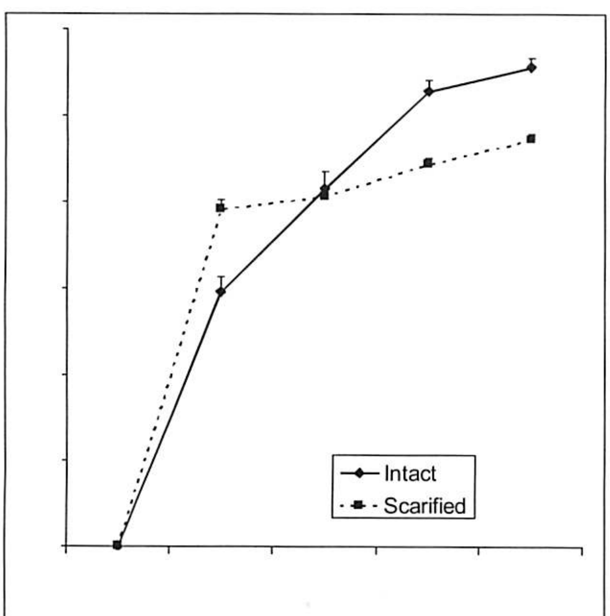

Figure 1. Mean (± S.E.) water uptake (g) by scarified and non-scarified Conocarpus erectus *seeds over time (0-32 hours).*

Temperature Effect

Timson's Index values and percent germination were much higher for the 28°C/14°C (January) temperature regime than the 31°C/16°C (April) and 33°C/20°C (July) temperature regimes (Figs. 2 and 3). Timson's Index values and percent germination for seeds germinated at the two warmer temperature regimes were very similar. Unfavorable environmental conditions like temperature may be what caused April and July's germination rates to be low (Baskin and Baskin, 1998). Therefore, hypothesis number two was supported because the 28°C/14°C (January) temperature regime had the highest percent germination, Timson's Index value, and fastest germination velocity. This is consistent with Kass et al. (2007) who found a major flowering peak in December (as well as June). However, Tomlinson (1994) reports that in south Florida populations of Buttonwood have a prolonged winter dormancy.

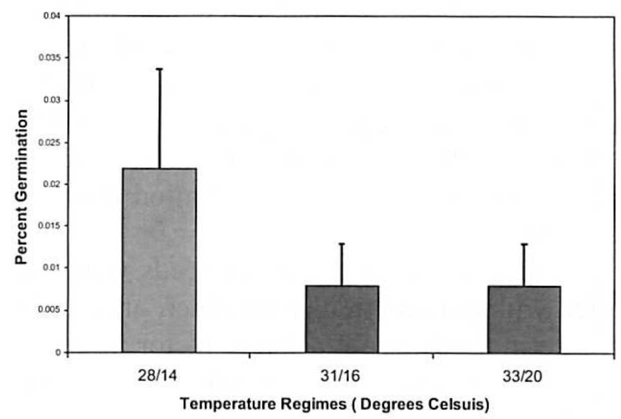

Figure 2. Mean (± S.E.) percent germination for Conocarpus erectus *at three temperature regimes: 28°C/14°C (January), 31°C/16°C (April), and 33°C/20°C (July).*

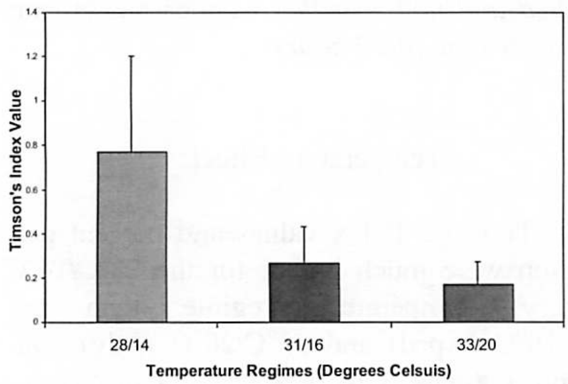

Figure 3. Mean (± S.E.) Timson's Index Values for Conocarpus erectus *seeds germinated at 28°C/14°C (January), 31°C/16°C (April), and 33°C/20°C (July).*

Seed Storage

The last study examined germination of unrefrigerated seeds in the best temperature regime for germination as determined by the second study (i.e., 28°C/14°C). We wanted to determine if the temperature regime that provided the highest germination rate for refrigerated seeds would also result in similar germination rates and velocities for unrefrigerated seeds.

Hypothesis number three was not supported because the refrigerated seeds had a higher Timson's Index (0.77 ± 0.43 vs 0.33 ± 0.20) and a higher percent germination (0.02% ± 0.01 vs. <0.01% ± 0.00). Therefore, storing buttonwood seeds under refrigeration does not harm the seeds.

Because both groups of seeds were collected within a month of each other, an age difference in seeds is not a likely factor in germination variability, especially since seeds stored under refrigeration were collected sooner.

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