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Germination of *Tabebuia heterophylla* seeds (Bignoniaceae) from a wet and dry forest of Puerto Rico

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Abstract: Seed germination response of the Puerto Rican wet and dry forest populations of *Tabebuia heterophylla* trees was tested using a gradient of osmotic potentials from 0 to -1.5 MPa. Morphological comparisons were also made from adult specimens. Dry forest trees showed smaller leaves, fruits, and seeds, and greater specific leaf weight. Dry forest fruits produced smaller seeds than wet forest fruits when similar small fruits were compared. Germination percentage was strongly reduced as osmotic potential decreased, and was significant in both seed origins. This reduction was greater on seeds from the wet forest population. Days for germination showed the same response but this was less evident in the seeds from the dry forest. Osmotic potential lower than -0.9 MPa. completely arrested seed germination in both populations. No significant difference in final germination percentage and days to germinate exist at high osmotic potentials in both seed populations. This physiological capacity along with the morphological modifications indicates why this species can maintain viable populations in the contrasting environmental conditions of the dry and moist forest habitats.

Key words: osmotic stress, phenotypic variation, PEG, seed size, seed germination, *Tabebuia.*

Because morpho-physiological variations of tropical plants have been related to the existence of water gradients, in which plants resist or avoid moisture stress (Medina 1983), we expected that some morphological differences within species existed in response to stressing environments. Traits such as the presence of sclerophyllous leaves, deep root systems, deciduousness, microphylly and compound leaves, greater belowground biomass, and tissue osmotic adjustment (Sobrado and Cuenca 1979, Medina 1983) are common adaptations to water stress, but they have been mostly treated at the interespecific level. At the intraspecific level, phenotypic plasticity has been demonstrated among populations (Schlichting 1986), but not many of the studies include tropical tree forms (Stemmerman 1983, Abrams *et al.* 1992). At the seed level, sensitivity of seed germination to water stress has been reported among and within temperate tree species (Vuillemin 1982, Falusi *et al.* 1983). The importance of germination time for the establishment and growth of seedlings in a semideciduous tropical forest is known (Augspurger 1979, Garwood 1986), and underscores how seed level processes related to water stress are of primary ecological interest. Vegetative variability among *Tabebuia* species of West Indies has been noted, and is often associated with edaphic specialization (Gentry 1990).

Tabebuia heterophylla (DC). Britton (Bignoniaceae) is no exception (Longwood 1971, Gentry 1990). In Puerto Rico, vegetative variations of *T. heterophylla* seem to be correlated with the amount of precipitation, which defines a strong north-south gradient across the island. We studied the seed germination of two *T. heterophylla* populations when subjected to a gradient of water potentials. We also present some morphological traits related to the two contrasting subtropical forests where this tree species occurs. *T. heterophylla* is a shrub or secondary tree up to 20 m in height. It flowers mainly in early spring and sporadically through the year (Little and Wadsworth 1989). The natural range of *T. heterophylla* includes Hispaniola, Puerto Rico, Virgin Islands through the Lesser Antilles to Grenada and Barbados. In Puerto Rico, it grows from sea level to approximately 1000 m (Longwood 1971).

MATERIAL AND METHODS

Seeds were collected from El Verde and Guánica. El Verde in the Luquillo Experimental Forest (northeastern Puerto Rico, 65°49'W, 18°20'N) has a mean annual precipitation of 3920 mm. The forest is in the subtropical wet forest life zone (Ewel and Whitmore 1973), and the soil water content is maintained during most of the year. Guánica Commonwealth Forest and Biosphere Reserve in the southwestern part of Puerto Rico (65°52'W, 17°52'N) presents a subtropical dry forest life zone (Ewel and Whitmore 1973). Soil moisture deficits can occur 10 months a year, and a strong dry season commonly exists from January to May (Lugo *et al.* 1978). Rainfall varies among 600 to 1100 mm annually (Ewel and Whitmore 1973), but there are two well-defined rainfall peaks in April and September (Lugo, *et al.* 1978). Long-term precipitation patterns show strong unpredictability in annual precipitation and its distribution in Guánica (Medina and Cuevas 1990).

Morphological measurements: The plant material was collected from ten trees at each site during July 1991. The length and width of the largest leaflet, specific leaf weight (SLW, g/cm2), fruit length, number of developed seeds per fruit, and seed and seed plus wing width were measured. Data on fruit length, number of seed per fruit, and seed weight (calculated from the weight of 25 seeds) were taken from collected fruits of at least 18 trees during late May 1992.

Germination experiment: A pooled sample of seeds was obtained from available dry fruits arbitrarily collected in June 1991 from 16 trees at each forest. Small and damaged seeds were eliminated from the samples. Seeds were treated with solutions of polyethylene-glycol (PEG, molar mass 6000, Sigma Co.), with osmotic potential of -0.1, -0.3, -0.5, -0.7, -0.9, -1.2 and -1.5 MPa, at 23 C, following the procedures and conditions previously considered (Cordero and Di Stéfano 1991, and references there in). Twenty-five seeds were put on Petri dishes with 10-15 ml of PEG solutions or distilled water (e.g. control), and maintained on a table under laboratory conditions (25 C ambient temperature and a 50% relative humidity). Treatments were replicated four times. Seed germination was recorded every day during 28 days. PEG solutions were renewed every eight days, as well as the control. Number of days for initial germination was recorded, and the final germination percentages were calculated. Viability of seeds (tetrazolium test) collected during May 1995 was not different to the seed germination in destilled water under the same conditions as above, but a little higher than the 1991 seeds.

Statistical analyses: Morphological traits were compared using the non parametric Rank Sum Test of the Statistix Program, due to non homogeneity of variances in some of the compared characters. The regression coefficients between fruit length and seed number were compared for the two populations using a modified Student "t" test. Final germination percentages were normalized with the arcsine transformation for the statistical analysis. Two ways ANOVA with seed source (populations) and treatments (osmotic solutions) as factors was performed as suggested by Scott et al. (1984). Backtransformed values are reported. The same analysis was performed for the days to initial germination, eliminating those treatments where no germination occurred. Mean differences between treatments were compared by using the Tukey test. Statistical differences were considered significant at P<0.05.

RESULTS

Morphological traits: Leaf morphology showed strong differences among populations (Table 1). Leaflet dimensions were larger and specific leaf weights were lower at El Verde population, indicating bigger and thinner leaves in the wet forest. A tendency to reduce the number of leaflets (such as producing simple leaves) is remarkable in the Guánica trees. Strong differences in fruit and seed variables were obtained among populations. El Verde fruits were larger than Guánica fruits. Seed size at El Verde was twice larger than Guánica.

Also, significant differences were found on seed width and seed plus wing's width (Table 1). Guánica seeds were four times lighter than El Verde seeds (Rank Sum test, P<0.001). In general, seed traits were less variable in the fruits from the dry forest trees. On the fruits collected in 1991, mean number of seeds per fruit from Guánica population was slightly greater than ones in the El Verde population. This tendency changed for 1992 fruits, but differences among populations were not significant in any of the two years.

The relationship and its regression slope between fruit length and number of seeds per fruit (Fig.1) were different among populations (P<0.001)..

TABLE 1

Mean and standar error (SE) of some morphological traits measured on adult T. heterophylla *trees from a wet forest (El Verde) and a dry forest (Guánica) in Puerto Rico.*

	Wet forest			*Dry forest*			
	Mean	*SE*	*n*[3]	*Mean*	*SE*	*n*[3]	*P*[1]
Leaflet length (cm)	14.14	0.58	30	3.45	0.15	38	**
Leaflet width (cm)	5.69	0.192	30	1.51	0.075	38	**
Leaflet area (cm^2)	55.52	4.56	30	5.51	0.45	26	**
Specific leaf weight (g/cm^2)	95.11	1.86	30	104.6	2.24	26	*
Fruit length 1991 (cm)	19.17	0.79	27	9.26	0.38	30	**
Fruit length 1992 (cm)	15.35	0.67	32	9.33	0.31	30	**
Seeds per fruit 1991	68.2	9.29	10	74.53	3.56	30	NS[2]
Seeds per fruit 1992	79.4	5.9	32	71.6	2.63	30	NS[2]
Seed + wings (cm)	2.73	0.05	40	1.85	0.04	40	**
Seed width (cm)	1.21	0.02	40	0.633	0.07	40	**
Seed weight 1992 (g)	0.0174	3.7E-4	36	4.0E-3	4.3E-4	30	**

P[1]*: indicates significant difference between means using Sum Rank Test*
NS[2]*: no significant, P > 0.05, (*) P < 0.01, and (**) P< 0.001,*
n[3]*: sample size.*

TABLE 2

Number of days for initiation of germination of T. heterophylla *seeds under several osmotic water potentials treatments. Seeds came from a wet forest (El Verde) and a the dry forest (Guánica) of Puerto Rico. Four replications per treatment. Statistical analysis explained in the text. No germination occurs in some treatments.*

	Wet forest		*Dry forest*	
Treatment	*Mean*	*S.E.**	*Mean*	*S.E.**
Control	5.50a	0.86	2.25a	0.25
-0.1 MPa	5.50ab	0.96	3.75ab	0.63
-0.3	4.50ab	0.75	4.75bc	0.48
-0.5	7.00ab	0.91	5.00bc	0.41
-0.7	11.0b	1.22	7.00c	0.41
-0.9	15	-	6.00bc	0.71
-1.2	-	-	15	-
-1.5	-	-	-	-

**SE, mean standard error.*
Different letter indicates significant differences between means of the Tukey test at 0.05 level..

Germination experiment: A two-way ANOVA for the days to germination showed significant differences due to the osmotic potential treatments ($p<0.001$, Table 2). A marginal population effect ($p<0.059$), and a non significant interaction ($p<0.201$) were obtained. Due to this, a separate one way ANOVA was done for each population, which reveled a strong treatment effect on seeds from both El Verde ($p<0.001$) and Guánica ($p<0.0001$). In general, a delay in the time for initiation of germination on El Verde seeds was observed. In addition, lower variation in this parameter in the Guánica seeds indicates an even germination in every treatment (Table 2). Seeds from El Verde were less able to germinate under low water potentials, producing a delayed germination with an increased variance among treatments (Fig. 2). At the end of the 28 days' germination period, seeds from the dry forest showed fewer differences in the total germination percentage among osmotic treatments than those from the wet forest (Fig. 2). The osmotic stress on *T. heterophylla* seeds (see Fig. 2) produced a significant reduction in final germination percentage as osmotic potential decreased (two way ANOVA, treatment effect, $p<0.0001$). This response occurred in both seed provenances (population effect, $p<0.0001$).

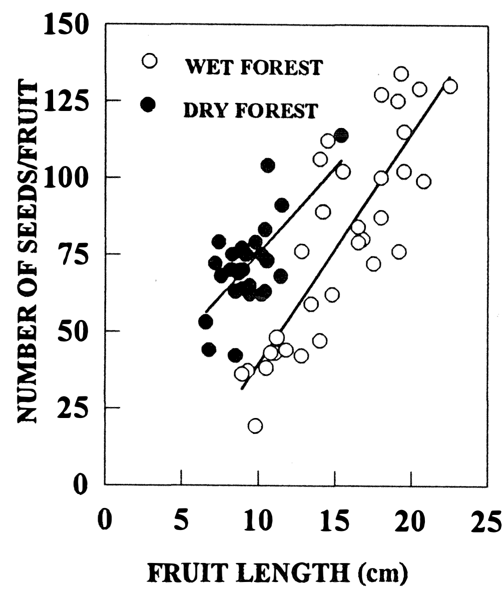

Fig. 1. Fruit length versus seed number linear regression analysis of *T. heterophylla* fruits from Guánica dry forest (filled circles) and from the El Verde wet forest (open circles) from Puerto Rico.

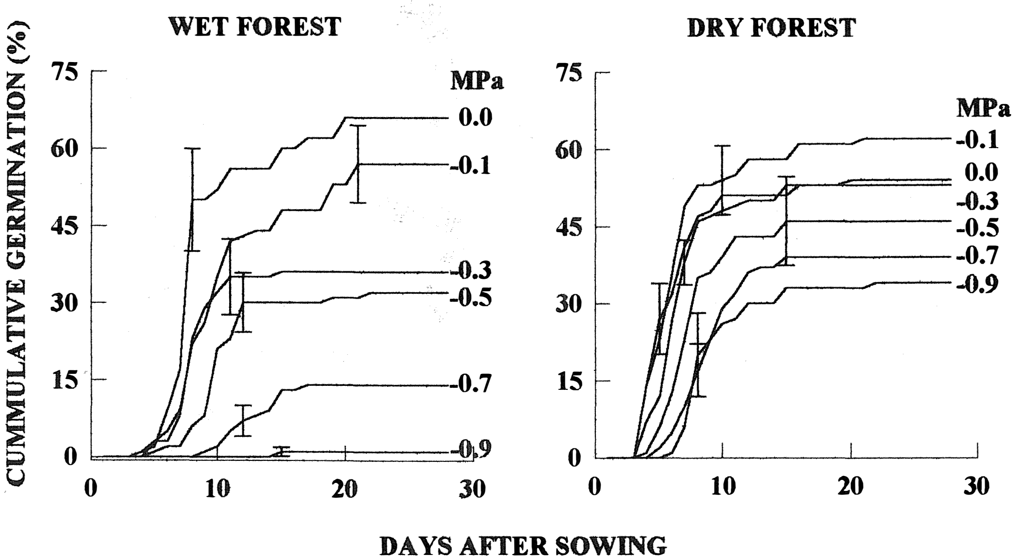

Fig. 2. Cumulative germination curves for two populations of *Tabebuia heterophylla* seeds under a gradient of osmotic solutions of PEG. Error bars indicates the greater standard error of four replications. Treatments at -1.2 and -1.5 MPa completely inhibited germination.

An interaction effect ($p<0.000$) indicated that plastic response to treatments by seed origins occurs in a different degree for each population (Fig. 3). Osmotic potentials above -0.1 MPa produces similar germination percentage in El Verde and Guánica seed populations. Osmotic potentials below -0.9 MPa completely inhibited seed germination in both populations (Fig. 3).

DISCUSSION

Morphological traits: There was a significant difference in eight out of nine (leaf and reproductive) morphological variables among wet and dry forest trees. Dry forest trees presented smaller values in all size traits, and a greater specific leaf weight. This response was expected due to the strong effect on cell enlargement and division of water stress during plant growth (Bradford and Hsiao 1982). Also, phosphorus limitation and other soil characteristics at Guánica dry forest (Lugo and Murphy 1986), could be interacting with low soil moisture availability (Lugo *et al.* 1978; Medina *et al.* 1990) for determining foliar sclerophylly and reduced sizes on this species. It has been postulated that seed size is a trait normally under strong selection. This is supported by the fact that in many species, seed variation is lower than other plant traits (Harper 1977). However, we found differences in seed dimensions and weight among populations of *T. heterophylla*, as it has been reported in other species (Melzack and Watts 1982). In *Desmodium paniculatum*, within plant variability in seed size is reduced under limited nutrients (Wulff 1986). In addition, maternal environment influenced seed size (Gutterman 1980-81, Wulff 1986). On the other hand, there were no differences in the number of seeds per fruit among populations, but dry forest fruits have more and small seeds per fruit when small fruits are compared (Fig. 1). These results suggest a compromise among seed quality (size, seed resources) and seed number. We think that in a very unpredictable environment (like this dry forest) (Medina and Cuevas 1990), where environmental stresses could restrict seed quality (size) and its variation, selection might favors for augmenting dispersal chance by increasing the number of less expensive propagules.

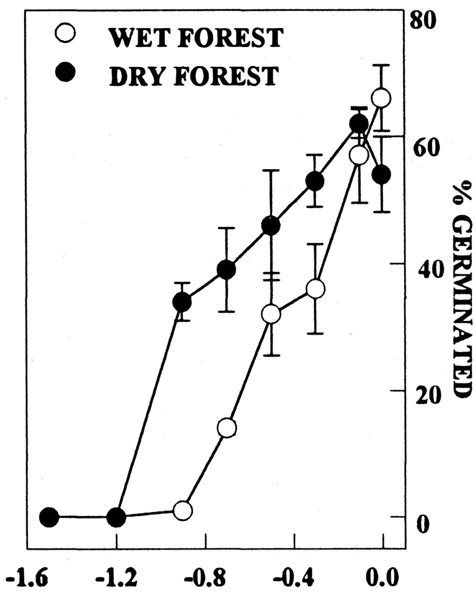

Fig. 3. Relationship between osmotic potential of germination treatment and the final germination percentage of *T. heterophylla* seeds. Two populations were studied: Guánica dry forest (filled circles) and El Verde wet forest (open circles).

Seed Germination: The arrested germination produced as the osmotic potential decreases is well known from cultivated plants (Bodsworth and Bewley 1981), and in tropical tree species (Cordero and Di Stéfano 1991). This kind of response might be selected in areas with sporadic rains and great variation of quantity of rain (Gutterman 1980/81). Our results could also be due to the physics of water imbibition as affected by seed size, but the general appearance of seeds indicated that they were well imbibed, which suggested that radicle growth was the arrested process, and not the seed imbibition. The most relevant result from this study lies on the differences in response among dry and wet forest Tabebuia tree seeds. This confers an ecological importance to the ability of seeds to germinate under moisture stresses, as the one adduced to the habitat distribution of two *Quercus* species (Vuillemin 1982), and to some populations of *Pinus* (Falusi *et al.* 1983).

Several factors probably reduce soil water potentials at Guánica. This dry forest presents shallow soils with frequent limestone outcropping, where rain water rapidly percolates, runs off, or evaporates (Lugo *et al.* 1978).

Then, biophysical properties of soil, high temperature and rapid use of available soil water by plants could favor the maintenance of reduced low soil water potentials, especially during dry to wet season transition, when deciduous species are resprouting and increasing the water absorption again. We consider that the higher germination rate of small seeds may confer advantages in both greater imbibition and seedling survival under low water potentials, helping to maintain the population under reduced moisture availability. In some species, seedling survival is also greater for plants from small seeds under short-term drought (Wulff 1986, Hendrix and Trapp 1992). Under that situation, we hypothesize that the irregular and unpredictable rainfall pattern during the dry to wet season transition in the dry forest of Guánica (Medina and Cuevas 1990), produce a preconditioning effect of the germination response of seeds.

Enhanced and faster germination after preconditioning treatments has been obtained for a tropical tree (Cordero and Di Stéfano 1991) and others cultivated plants (Bodsworth and Bewley 1981). Also, this preconditioning effect could explain the absence of differences in germination and seedling survival to watering regimes found in other studies (Blain and Kellman 1991). This mechanism would be irrelevant in wet forests where less if any soil moisture deficit occurs. In ecological terms, it is predicted that preconditioned seeds will have a more homogeneous germination and emergence through the dry to wet season transition. Thus, the differences in physiological phenotypic plasticity of both seed populations and their environmentally correlated morphological traits in the natural populations are in concordance with the ability of *T. heterophylla* to survive and maintain viable populations in these two contrasting subtropical forests. Plant demography, seed size variation among populations and its maternal effects and the extent to which these variations will affect seedling traits in longer periods would produce different results (Hendrix and Trapp 1992).

RESUMEN

El efecto de la tensión hídrica sobre la germinación de las semillas de *Tabebuia heterophylla* (Bignoniaceae), procedentes de un bosque seco y otro muy húmedo en Puerto Rico, se estudió utilizando un gradiente de potenciales osmóticos (0 a -1.5 Mpa). Algunas comparaciones morfológicas fueron hechas de individuos adultos de ambas poblaciones. Los árboles del bosque seco presentaron hojas, frutos y semillas más pequeñas y el peso foliar específico fue mayor. Los frutos del bosque seco contenían mayor cantidad de semillas más pequeñas que los frutos de tamaño similar de árboles del bosque muy húmedo. El porcentaje de germinación se redujo drásticamente conforme el potencial osmótico fue más negativo en ambas poblaciones, aunque fue más acentuado en las semillas del bosque húmedo. El número de días para el inicio de la germinación presentó la misma respuesta, pero ésta fue menos marcada en las semillas de árboles del bosque seco. La germinación fue inhibida completamente a potenciales osmóticos menores que -0.9 MPa, y no fue diferente entre los potenciales osmóticos más altos, en ambas poblaciones respetivamente. Estos efectos sobre la germinación junto con las modificaciones morfológico-ambientales parecen capacitar a esta especie a mantener poblaciones viables en estos ambientes tan contrastantes.

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