

EFFECTS OF ACID AND HOT WATER PRETREATMENTS AND SEED BURIAL  
ON THE GERMINATION OF TROPICAL MOIST FOREST SEEDS<sup>1</sup> /

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Resumen

*Para determinar si las cubiertas duras de algunas semillas del bosque tropical húmedo fueron un factor causante de las demoras largas en su germinación, se escogieron 39 especies de Panamá y Costa Rica y se suavizaron artificialmente las cubiertas de las semillas con ácido sulfúrico o agua caliente. De las 29 especies que germinaron, se observó que el ácido o el agua caliente aceleró la germinación en seis, pero la entorpeció en 14 especies. Las especies en las cuales fue acelerada la germinación no fueron necesariamente las que suelen durar más tiempo en germinar. Por ende el hecho de tener una cubierta de semilla dura no parece ser un factor determinante en la lenta germinación de algunas especies del bosque tropical húmedo. Los efectos de los tratamientos sobre las semillas variaban según la edad de éstas. No se observaron diferencias entre especies que colonizan zonas perturbadas o quemadas vs especies que colonizan lugares no perturbados, ni entre especies dispersadas por el viento y las dispersadas por animales.*

Introduction

Seed dormancy is usually maintained by the structures surrounding the seed (the "seed coat") in most species (2, 8). Hard "seed coats" frequently delay germination because they are impermeable to water or gases, mechanically restrain the embryo, or prevent the release of inhibitors. Germination often occurs after the seed coat is softened naturally by mechanical abrasion, microbial degradation, temperature fluctuations or passage through animal guts.

The primary objective of this study was to determine whether hard seed coats are an important cause of delays in germination in tropical species.

Delays in germination are frequent in species from the tropical moist forest of Barro Colorado Island (BCI), Panama; mean time until germination ranged from two to 370 days and was greater than four weeks in over half of 185 species tested by Garwood (4). If hard seed coats cause delays in germination, artificial softening of the seed coat should decrease the length of the dormant period and/or increase percent germination; this effect should be most pronounced in species which require longest to germinate without treatment.

Acid or hot water pretreatments, commonly used to break dormancy in hardcoated seeds (8), were used to soften the seed coat in 32 species from BCI and seven species from eastern Panama and Costa Rica (five of which also occur on BCI). In addition, the effect of seed burial on length of dormancy was examined in six species from BCI to determine whether the improved moisture environment of buried seeds and/or the action of soil microbes would promote germination.

Hard-coated seeds are considered frequent in a) the Leguminosae, b) species which colonize disturbed or burned areas, and c) species whose seeds are passed through vertebrate guts during dispersal (8, 12). These generalizations are primarily based on temperate species, although some tropical species are known

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in each category. If these generalizations are also true for tropical species, artificial softening of the seed coat should promote germination more frequently in these three groups of species than in species which are not legumes, not colonizers or not dispersed through animal guts.

### Materials and methods

Two sowing conditions (peat pots and petri dishes) and three seed treatments (acid, hot water and burial) were used.

**Sowing conditions.** 1) **Peat pots** Seeds were collected on BCI, treated, then sown in soil-filled peat pots kept in a screened growing house on BCI (see 4) Seeds collected in 1976-1977 were treated with hot water or buried; those collected in 1978 were treated with acid 2) **Petri dishes.** Seeds were collected from eastern Panama (Darien Province) and Costa Rica (Guanacaste Province) in 1983 and from BCI in 1975 or 1984. Seeds were sown on moist filter paper in petri dishes at  $\sim 25^{\circ}\text{C}$  on a 12-hour light/dark cycle

For both sowing conditions, seed collection and preparation methods follow Garwood (4). Control and treated seeds were put into separate peat pots or petri dishes; one pot was used for small seeds, several pots for larger seeds. When not planted immediately after collection, air-dried seeds were stored in cellophane envelopes at room temperature; seed age is given. Germination was followed until all seeds germinated or rotted or, in 1978, until four months had passed; seeds were kept moist during this period.

**Seed treatments.** 1) **Acid.** Seeds were covered with concentrated sulfuric acid, shaken, soaked for five minutes, then rinsed five times with water and air-dried. This is a mild acid treatment: the *Woody Plant Seed Manual* (13) recommends soaking in acid for 15-20 minutes. 2) **Hot water.** Seeds were covered with hot water ( $\sim 80-85^{\circ}\text{C}$ ), soaked for 30 seconds (peat pot experiments) or 10 minutes (petri dish experiments), rinsed in cool water, then air-dried. Thirty seconds is a mild hot water treatment: Vazquez-Yanes (11) found that maximum percent germination of *Ochroma lagopus* occurred after boiling seeds 15-120 seconds. 3) **Burial.** Seeds were planted 1 cm below the soil surface; control seeds were planted on the surface.

Differences in time until germination between treated and control seeds were tested with a two-tailed Wilcoxon two-sample test; differences in percent germination were tested with a 2 x 2 test of independence using the G-statistic and Williams' correction (10). Nomenclature follows Croat (3)

and Janzen and Liesner (6). Full species names are given in Tables 1 and 2; generic names are used in the text except where there are congeners.

Not included in Table 1 are six species (*Heisteria concinna*, *Guarea glabra*, *Piper cordulatum*, *Adenopodia polystachya*, *Desmopsis panamensis*, and *Tetragastris panamensis*), in which the two-to three-month-old seeds in the control and treatment all decayed; *Coccoloba* sp., in which neither treatment germinated; and three species (*Sapium caudatum*, *Cephaelis ipecacuanha*, and *Psychotria emetica*) in which neither control nor treatment seeds germinated within four months, but untreated seeds were known to require  $> 160$  days to germinate (4)

### Results and discussion

The results from seeds sown in peat pots are presented in Table 1. The results from seeds sown in petri dishes are presented in Table 2.

Mild acid and hot water pretreatments significantly promoted germination (increased percent germination and/or decreased time until germination) in the young seeds ( $< 3$  months old) of only six of the 29 species which germinated, significantly decreased germination in 14 species, and had no effect in nine species. The mean time until germination of untreated seeds of species in which germination was promoted was not significantly greater than that of species in which germination was decreased (1-tailed Wilcoxon 2-sample test,  $p > .1$ ), or that of species that were not effected ( $P > .1$ ). In addition, treated seeds of three species known to require  $> 160$  days to germinate did not germinate in four months. Thus, hard seed coats were not a frequent mechanism causing delays in germination in seeds of the species tested and were not more frequent in species with longer delays in germination.

Artificial softening of the hard seed coats common in the Leguminosae usually breaks dormancy (8). Of the hard-seeded legumes in this sample, acid treatment promoted germination in *Ormosia macrocalyx*, three-month-old seeds of *Cassia* (at the 1% level only), but not 12-month-old seeds, and 12-month-old seeds of *Erythrina*, but not three-month-old seeds; treatment did not significantly promote germination in *Ormosia coccinea* (but sample sizes were very low). In other species with hard (although not necessarily impermeable) seed coats, acid or hot water treatments promoted germination in *Ochroma*, *Luehea*, *Luffa*, and *Guazuma*, but did not in *Diospyros*, *Annona*, *Guatteria*, and *Tupinia*. In species which had thick, but not necessarily hard seed coats, acid or hot water treatments decreased germination (*Prionostenma* and *Thevetia*) or had

Table 1. Effects of acid and hot water pretreatments and seed burial on percent germination and mean number of days until germination ( $\bar{X} \pm \text{SD}$ ) of seeds sown in peat pots in the BCI growing house.

Species	Seed age (mon)	Treatment	Percent germination <sup>a</sup>		Days until germination <sup>a</sup>	
			Control	Treatment	Control	Treatment
<i>Ormosia macrocalyx</i> <sup>c</sup>	*	Acid	26 (27)	96 (26)***	47 ± 28	18 ± 18**
<i>Ormosia coccinea</i> <sup>c</sup>	*	Acid	50 ( 4)	75 ( 4) <sup>ns</sup>	58 ± 62	16 ± 2
<i>Luehea seemannii</i> <sup>c</sup>	3	Acid	10 (50)	23 (40) <sup>ns</sup>	12 ± 15	4 ± 1**
<i>Mouriri myrtilloides</i> <sup>c</sup>	3	Acid	0 ( 5)	40 ( 5) <sup>ns</sup>	—	18 ± 5
<i>Tetrayvlacion johansenii</i> <sup>c</sup>	*	Acid	39 (75)	49 (47) <sup>ns</sup>	10 ± 3	10 ± 3 <sup>ns</sup>
<i>Didymopanax morototoni</i> <sup>c</sup>	2	Acid	12 (25)	4 (25) <sup>ns</sup>	36 ± 5	31 ± 0
<i>Calophyllum longifolium</i>	*	Acid	100 ( 5)	60 ( 5) <sup>ns</sup>	6 ± 1	11 ± 3*
<i>Lantana camara</i> <sup>c</sup>	3	Acid	92 (13)	46 (13)**	4 ± 1	6 ± 8 <sup>ns</sup>
<i>Casearia arborea</i>	*	Acid	36 (75)	0 (37)***	30 ± 12	—
<i>Jacaranda copaia</i>	*	Acid	53 (47)	0 (37)***	25 ± 6	—
<i>Lycianthes maxonii</i>	1	Acid	80 ( 5)	0 ( 5)*	20 ± 3	—
<i>Prionostemma aspera</i>	3	Acid	70 (10)	0 (10)*	79 ± 22	—
<i>Thevetia ahouai</i>	*	Acid	83 (24)	0 (13)***	55 ± 23	—
<i>Tocovena pittieri</i>	*	Acid	83 (18)	0 (18)***	58 ± 12	—
<i>Turpinia occidentalis</i> <sup>c</sup>	*	Acid	12 (91)	0 (86)***	74 ± 8	—
<i>Psychotria limonensis</i>	*	Hot water	20 (10)	0 (10) <sup>ns</sup>	41 ± 0	—
<i>Annona spraguei</i>	*	Hot water	56 (50)	0 (50)***	222 ± 12	—
<i>Apeiba tibourbou</i>	*	Hot water	23 (30)	0 (30)***	15 ± 4	—
<i>Guatteria dumetorum</i>	*	Hot water	100 ( 5)	0 ( 3)***	51 ± 12	—
<i>Zuelania guidonia</i>	*	Hot water	80 (10)	0 (10)***	9 ± 1	—
<i>Annona spraguei</i>	*	Burial	56 (50)	26 (50)*	222 ± 12	231 ± 10**
<i>Mouriri myrtilloides</i>	*	Burial	100 ( 8)	100 ( 2) <sup>ns</sup>	92 ± 9	53 ± 3*
<i>Spondias mombin</i>	*	Burial	44 (16)	31 (16) <sup>ns</sup>	264 ± 16	263 ± 36 <sup>ns</sup>
<i>Spondias radlkoferi</i>	*	Burial	100 ( 5)	17 ( 6)*	163 ± 20	172 ± 0
<i>Zanthoylum sctulosum</i>	*	Burial	12 (50)	37 (30)*	260 ± 19	240 ± 11 <sup>ns</sup>
<i>Psychotria horizontalis</i>	*	Burial	7 (100)	44 (50)***	164 ± 8	168 ± 7 <sup>ns</sup>

a Significance levels: \*, 5%; \*\*, 1%; \*\*\*, 0.5%; ns, not significant; blanks indicate that sample sizes were too small to detect a significant difference

b Sample size for percent (%) germination is N; sample size for days until germination is % × N

c Germination possibly incomplete when trial ended after four months

d \* indicates that seed age is < days

no effect (*Mouriri*, *Calophyllum*, and *Odontadenia*). Thus, acid and hot water pretreatments did not uniformly promote germination in all species that had hard seed coats, including those in the Leguminosae.

If hard seed coats are more frequent in species of disturbed or burned areas, softening of the seed coat would be expected to promote germination more frequently in species known to establish in light-gaps in the forest (*Annona*, *Luehea*, *Casearia*,

*Jacaranda*, *Apeiba membranacea*, *Cissus*), in larger disturbances (*Ochroma*, *Didymopanax*), and in areas frequently burned (*Apeiba tibourbou*, *Guazuma*, *Lantana*) (Garwood, personal observations), than in those that establish in the undisturbed, shaded understory of forests. Of these species, temperature fluctuations are known to promote germination in *Ochroma* and *Didymopanax* (see 12), although two-month-old seeds of the latter were unaffected by acid treatment here. To determine whether acid or hot water treatment promoted germination more

Table 2. Effects of acid and hot water pretreatments on percent germination and days until germination of seeds sown in petri dishes. Seed sources: Barro Colorado Island, Panama (BCI); eastern Darien Province, Panama (Panama) and lowland Guanacaste Province, Costa Rica (Costa Rica).

Species	Seed source	Seed age (mon) <sup>b</sup>	Treatment	Percent germination <sup>a</sup> (N) <sup>c</sup>		Days until germination <sup>a</sup> X ± SD <sup>c</sup>	
				Control	Treatment	Control	Treatment
<i>Ochroma pyramidale</i>	BCI	1	Hot water	10 (20)	70 (20)***	38 ± 42	13 ± 15 <sup>ns</sup>
<i>Apeiba membranacea</i>	BCI	10Y	Hot water	70 (10)	80 (10) <sup>ns</sup>	28 ± 8	24 ± 8 <sup>ns</sup>
<i>Guazuma ulmifolia</i>	Costa Rica	3	Acid	0 (25) <sup>d</sup>	48 (25)***	—	98 ± 55
	Costa Rica	12	Acid	0 (14)	18 (11) <sup>ns</sup>	—	42 ± 14
	Costa Rica	12	Hot water	—	0 (17) <sup>ns</sup>	—	—
<i>Cassia fruticosa</i>	Panama	3	Acid	30 (10)	70 (10) <sup>—</sup>	8 ± 3	5 ± 2 <sup>ns</sup>
	Panama	12	Acid	30 (10)	30 (10) <sup>ns</sup>	8 ± 3	7 ± 2 <sup>ns</sup>
	Panama	12	Hot water	—	40 (10) <sup>ns</sup>	—	15 ± 12 <sup>ns</sup>
<i>Erythrina costaricensis</i>	Costa Rica	3	Acid	20 (10) <sup>d</sup>	40 (10) <sup>ns</sup>	128 ± 0	32 ± 14 <sup>ns</sup>
	Costa Rica	12	Acid	0 (5)	80 (5)**	—	18 ± 9
	Costa Rica	12	Hot water	—	0 (5) <sup>ns</sup>	—	—
<i>Luffa aegyptiaca</i>	Costa Rica	3	Acid	53 (15)	80 (10) <sup>ns</sup>	204 ± 36	46 ± 42***
	Costa Rica	12	Acid	70 (10)	20 (10)*	20 ± 40	34 ± 8 <sup>—</sup>
	Costa Rica	12	Hot water	—	44 (9) <sup>ns</sup>	—	8 ± 4 <sup>ns</sup>
<i>Odontadenia</i> sp.	Panama	3	Acid	40 (10)	80 (5) <sup>ns</sup>	179 ± 59	118 ± 9 <sup>ns</sup>
<i>Diospyros nicaraguensis</i>	Costa Rica	3	Acid	100 (5) <sup>d</sup>	100 (5) <sup>ns</sup>	20 ± 13	28 ± 23 <sup>ns</sup>
<i>Cissus sicyoides</i>	Panama	3	Acid	60 (10)	0 (5)*	21 ± 2	—

a Significance levels: —, 10%; \*, 5%; \*\*, 1%; \*\*\*, 0.5%; ns, not significant; a blank indicates that sample sizes were too small to detect significant differences.

b Y = years

c Sample size for percent (%) germination is N; sample size for days until germination is %X N.

d Seeds rinsed in bleach five minutes before sowing

frequently in the above species compared to those of the remaining species, which included species that establish in the shaded understory and species whose establishment requirements are unknown, the number of species in which the treatments promoted, had no effect on, or inhibited germination was compared using a 2 x 3 test of independence: the results were not significant ( $p > .1$ ).

If seeds that are dispersed through the guts of animals have harder, more resistant seed coats than species that are not passed through the gut, artificial softening of the seed coat should promote germination more frequently in the former species. However, only wind- and animal-dispersed species could be compared because it was not known whether seeds

of these animal-dispersed species are typically passed through the gut or regurgitated. In the five wind-dispersed species, acid or hot water pretreatments promoted germination in two species (*Luehea* and *Ochroma*), decreased germination in two species (*Jacaranda* and *Prionostemma*), and did not effect *Odontadenia*. The remaining 24 species, with the possible exception of *Luffa*, are animal-dispersed (3): germination was promoted in four species, decreased in 13 and not affected in seven species. There were no significant differences between the wind- and animal-dispersed species in the number of species in which the treatments promoted, inhibited or had no effect on germination (2 x 3 test of independence,  $p > .5$ ). Thus, the seed coats of the animal-dispersed species were not more resistant to treatments than the wind-dispersed species.

Since seeds of *Tocoyena* are known to germinate after passing through white-faced monkeys (9), and only small differences in germination between gut-passed and control seeds were seen in several other species (5), perhaps even mild acid or hot water treatments are more severe than passage through the digestive tract of many tropical dispersers. However, some hard-seeded species survive passage through the long, severe guts of large tropical mammals (1, 6)

Treatment effects were age-dependent in the four species whose seeds were tested when three and 12-months old. Acid treatment promoted germination in young, but not old seeds of *Guazuma*, *Cassia*, and *Luffa*, and of old, but not young seeds of *Erythrina*. Thus, the germination requirements of seeds change with age even in dry seeds; whether this involves changes in the seed coat is unknown.

The trends presented here should be viewed cautiously. First, the species tested here do not comprise a large percent or a random sample of all forest species from BCI. Therefore, the results presented here do not necessarily represent community-wide trends in germination in this forest. Second, it is not known whether acid or hot water treatments effectively mimic natural conditions, such as microbial degradation or digestion, which naturally break dormancy.

There were no consistent trends among the six species in the burial treatment, although all species have been seen germinating from buried seeds in the forest. Burial significantly decreased percent germination in two species, increased it in two species and had no effect on another. Burial significantly increased time until germination in one species, and decreased it in another.

### Summary

The seed coats of 39 species from Panama and Costa Rica were artificially softened with sulfuric acid or hot water to determine whether hard seed coats were a factor causing the long delays in germination noted in tropical moist forest species from Panama. Of the 29 species which germinated, acid or hot water promoted germination in only six species but decreased germination in 14 species. Species in which germination was promoted were not necessarily those that required longer to germinate. Thus, hard seed coats are unlikely to be a primary mechanism causing delays in germination in tropical moist forest species. The effects of treatment varied with seed age. No differences were found between

species which colonize disturbed or burned areas vs. species which colonize undisturbed areas nor between species which are wind-dispersed vs. those that are animal-dispersed.

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## Reseña de libros

GRIST, D.H. Rice 6a ed. Tropical Agriculture Series 1986. 599 p.

Esta nueva edición de uno de los libros dedicados a la producción de arroz está dividido en cuatro partes. La primera habla sobre la planta de arroz e incluye seis capítulos. En el primero el autor nos relata el posible origen de *Oryza sativa* L., el inicio de su cultivo en el sureste de Asia y su introducción en Europa, África, América y Australia. En el segundo y tercer capítulos menciona los diversos componentes del clima y el suelo, así como su efecto sobre la planta. En el cuarto trata a profundidad los diversos aspectos de la relación entre la planta y el agua, mientras que en el quinto ofrece una descripción minuciosa de los diversos órganos y la anatomía del arroz, algunos aspectos de su fisiología, otras características y las fases de su crecimiento. En el sexto menciona generalidades de las numerosísimas variedades de *O. sativa* y algunos sistemas propuestos para su clasificación a nivel mundial.

La segunda parte consta sólo de dos capítulos, los que se refieren a los aspectos genéticos y de mejoramiento del cultivo. En el séptimo el autor se refiere a la citología y herencia de varios caracteres anatómicos de la planta, mientras que en el octavo describe varios procedimientos usados para el mejoramiento de las variedades y otros aspectos relacionados con este tema.

La tercera parte del libro está dedicada a aspectos de producción e incluye nueve capítulos. En ellos el autor describe sucesivamente los diversos métodos

de producción del cultivo, el arroz inundado, el arroz de secano, el arroz de aguas profundas, la mecanización del cultivo, los fertilizantes y enmiendas, las principales malezas, la producción concomitante de peces en arrozales inundados y las principales plagas y enfermedades del cultivo.

La cuarta parte está dedicada al grano y consta de cinco capítulos; en ellos el autor se refiere al almacenamiento, con todos sus problemas, las diversas modalidades de su procesamiento industrial, los productos derivados del grano y la planta, su valor alimentario y, finalmente, los aspectos económicos de su producción.

El libro incluye además cuatro apéndices, una extensa bibliografía y un índice alfabético de temas. En general, la información presentada es muy valiosa y permite tener un panorama muy amplio sobre las diversas condiciones en que este importante cereal es cultivado en todo el mundo: sin embargo, algunos aspectos tratados están desactualizados, v.g., la terminología usada para describir los diversos tipos de suelos usados para la producción del cultivo y los productos químicos mencionados para el combate de algunos insectos y nematodos. Un análisis de la extensa bibliografía consultada (1 026 citas) reveló que sólo un 10% de las mismas (103 citas) habían sido publicadas en 1976 o más recientemente. Dado que ésta es una nueva edición, se esperaba que muchos aspectos fueran actualizados, lo que desafortunadamente no ocurrió. A pesar de esto, la información contenida en el libro es básica para entender el cultivo y lo mantiene en la categoría de los clásicos para el arroz.

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