Seed Germination Ecology of Hawaiian Montane Species: A Continuation of Efforts to Acquire, Organize, and Share Data to Facilitate Propagation and Restoration Efforts

Carol C. Baskin, Jerry M. Baskin, Alvin Yoshinaga, Susan Cordell,

Donald Drake, Sean Gleason, and Patti Welton

Final report, September 2004

The purpose of this report is to up-date and supplement our previous report (2003) on seed germination ecology of Hawaiian montane species, giving information on each of our four specific aims.

Aim 1

Compile a data base of seed dormancy classes in Hawaiian montane species

Using data from studies done on seed storage at the University of Hawaii Seed Conservation Laboratory and those done on seed dormancy in the Baskin laboratory in Kentucky and information in the book by J. L. Culliney and B. P. Koebele (1999) entitled "A native Hawaiian Garden" (University of Hawaii Press, Honolulu), the class of dormancy (or in some species the presence of nondormancy) has been determined/inferred for 163 species from the montane zone in Hawaii (Table 1).

In addition to information on the dormancy state of fresh seeds, data have been obtained on the optimum temperature for dormancy break and/or germination and on the time required for dormancy break and/or germination for 58 species.

Table 1. Nondormancy or class of dormancy in Hawaiian montane[a] species. CD = conditional dormancy, MD= morphological dormancy, MPD = morphophysiological dormancy, ND= nondormant, ND/PD = part at the seeds were nondormant but others had physiological dormancy, PD = physiological dormancy, PY = physical dormancy, PY + PD = combination of physical and physiological dormancy, ? = further verification is needed, * = species has been (or is currently being) studied in the Baskin lab. (M) = indicates that a move along experiment has been (or is being) done.

Genus and species	Nondormancy or class of dormancy	Optimum germination temperature (ºC)[b]	Time for 50% germ. or maximum germ. % and time
Abutilon menziesii	PY		
Abutilon sandwicense	PY		

**Acacia koa*	PY	28/20	
**Achyranthes splendens*	ND, PD(CD)	25/15	10 w
**Alphitonia ponderosa*	PY + PD	25/15	
Alsinidendron obovatum	ND		
Alsinidendron trinerve	ND/PD		
**Alyxia oliviformis*	ND/PD PD	20/10	8%, 40 w
**Antidesma platyphyllum*	PD(CD)	25/15	6 w
**Argemone glauca* (M)	MPD	20/10	3%, 8 w
**Astelia menziesiana* (M)	MPD	20/15	14 w
Bidens asymmetrica	ND?		
Bidens sandvicensis	ND		
* *Bidens torta* (M)	ND?	20/15	about 4 w
Bobea elatior	PD		
* *Boehmeria grandis*	ND	25/15	2w
**Broussaisia arguta* (M)	PD(CD)	25/15	11 w
Caesalpinia kavaiensis	PY		
Canavalia hawaiiensis	PY		
**Carex alligata* (M)	PD(CD)	25/15	12w
Carex meyenii	PD		
Carex wahuensis	PD		
Cenchrus agrimonioides	PD		
Chamaesyce celastroides	PD		
Chamaesyce herbstii	PD		
Charpentiera ovata	seeds not viable		
Charpentiera tomentosa	PD		
Cheirodendron platyphyllum	MPD		
**Cheirodendron trigynum* (M)	MPD	20/10	12 w
**Chenopodium oahuense*	ND?	15/6	(were seeds fresh? need to repeat)
Clermontia clermontioides	ND/PD		
Clermontia drepanomorpha	PD		
**Clermontia fauriei* (M)	MPD, MD	25/15	3w
**Clermontia hawaiiensis* (M)	MPD, MD	25/15	3w
**Clermontia kakeana* (M)	MPD, MD	25/15	3w
Clermontia montis-loa	ND/PD		
Clermontia oblongifolia	PD		
Clermontia parviflora	ND		
Clermontia persicifolia	PD		
**Clermontia pyrularia* (M)	MPD	15/6	7w
**Colubrina oppositifolia*	ND, PY[c]	28/20	19 days
**Coprosma cymosa*	PD	15/6, 20/10	8w
* *Coprosma montana* (M)	PD(CD)	20/10	5w
**Coprosma ochracea* (M)	PD	15/6, 20/10	8w
Coprosma rhynchocarpa	PD		
**Cynaea angustifolia* (M)	MPD, MD	25/15	3w
Cyanea grimesiana	ND		

Cyanea membranacea	ND/PD		
Cyanea superba	ND/PD		
**Cyrtandra cordifolia* (M)	ND	25/15	3w
Cyrtandra grandiflora	ND		
**Cyrtandra lessoniana* (M)	ND	25/15	3w
**Cyrtandra longifolia*	ND	25/15	3w
Delissea rhytidosperma	ND/PD		
Delissea subcordata	ND/PD		
Delissea undulata	PD		
**Deschampsia nubigena* (M)	PD(CD)	25/15	6w
**Dianella sandwicensis* (M)	PD	20/10	12w
**Diospyros sandwicensis*	PD	25/15	25%, 12 w
**Dodonaea viscosa*	PY	25/15, 30/15	
Dubautia herbstobatae	PD		
Dubatia latifolia	PD		
**Dubatia menziesii* (M)	PD(CD)	25/15	10%, 8w
Elaeocarpus bifidus	PD		
**Eragrostis atropioides* (M)	PD(CD)	25/15	4w
Erythrina sandwicensis	PY		
Euphorbia haeleeleana	ND?		
Fluggae newowawraea	PD		
**Gahnia beecheyi* (M)	PD	no germination at three temps. after 64w	
**Gahnia gahniiformis* (M)	PD	no germination at three temps. after 64 w	
Gardenia brighamii	ND/PD		
**Haplostachys haplostachya*(M)	PD	25/15	8%, 40w
Hedyotis acuminata	ND/PD		
Hedyotis centranthoides	PD		
**Hedyotis terminalis* (M)	PD(CD), ND	25/15	2w
Hibiscus arnottianus	PY		
Hibiscus brackenridgei	PY		
Hillebrandia sandwicensis	PD		
**Ilex anomala* (M)	MPD	25/15	24w
Joinvillea ascendens	PD		
**Kokia drynarioides*	PY	28/20	
**Labordia tinifolia*	PD	20/15	6w
Lipochaeta tenuifolia	PD		
Lobelia dunbarii	ND		
Lobelia hypoleuca	ND/PD		
Lobelia niihauensis	PD		
Lobelia oahuensis	ND/PD		
**Lysimachia remyi* (M)	ND	25/15, 20/15	4w
**Machaerina angustifolia* (M)	PD(CD)	25/15	8w
Machaerina mariscoides	PD		
Mariscus hillebrandii	PD		
**Melicope knudsenii*	PD	20/10	22%, 18w; 37%, 46w
Metrosideros polymorpha	ND		

Metrosideros tremuloides	ND		
**Myoporum sandwicense* (M)	PD	25/15	15 of 27 (total) seedlings by 28 w
**Myrsine lanaiensis*	PD	25/15	14w
Myrsine lessertiana	ND/PD		
**Nestegis sandwicensis*	PD	20/10	25%, 22w
Neraudia angulata	PD		
Nothocestrum breviflorum	ND		
Nototrichium humile	PD		
Nototrichium sandwicense	PD		
**Osteomeles anthyllidifolia*	PD	20/10	22w
Panicum pellitum	PD		
Panicum tenuifolium	PD		
Peperomia latifolia	ND/PD		
Peperomia leptostachya	PD		
**Peperomia membranacea* (M)	PD(CD)	25/15	5w
Peperomia tetraphylla	ND/PD		
Perrottetia sandwicense	ND/PD		
Phyllostegia grandiflora	PD		
Phyllostegia stachyoides	PD		
Phyllostegia velutina	PD		
**Pipturus albidus*	PD(CD), ND	25/15	6w
Pisonia brunoniana	PD		
Pisonia sandwicensis	PD		
Pisonia umbellifera	PD		
Pittosporum flocculosum	MPD		
Pittosporum hawaiiense	MPD		
Plantago princeps	PD		
**Pleomele hawaiiensis*	PD	25/15	25%, 6w
Pouteria sandwicensis	PD		
Pritcharida remota	MPD		
Psychotria hathewayi	PD		
Psychotria hawaiiensis	PD		
Psychotria kaduana	PD		
Psychotria mariniana	PD		
**Psydrax odorata*	ND/PD, PD	25/15	5%, 30w
Remya kauaiensis	PD		
Reynoldsia sandwicensis	MPD		
**Rhus sandwicensis*	PY	25/15	
Rumex albescens	ND/PD		
Sanicula mariversa	MPD		
Santalum ellipticum	MPD		
Santalum freycinetianum	MPD		
**Sapindus oahuensis*	PD	28/20	10w
**Sapindus saponaria*	PD	28/20	2w
Scaevola gaudichaudiana	PD		

Scaveola gaudichaudii	PD		
**Scaveola procera* (M)	PD(CD)	25/15	8w
**Senna gaudichaudii*	PY	28/20	
Sesbania tomentosa	PY		
Sicyos anunu	PY		
Sida fallax	PY		
Silene lanceolata	PD		
**Solanum imcompletum* (M)	PD	15/6	12w
Solanum sandwicense	PD		
**Sophora chrysophylla*	PY	25/15	
Stenogyne angustifolia	PD		
**Styphelia tameiameiae* (M)	PD	25/15	16w before any seeds germinated; germination still continuing after 148w
Syzygium sandwicensis	PD		
Tetramolopium filiforme	PD		
Tetramolopium humile	ND		
Touchardia latifolia	ND/PD		
**Trematolobelia macrostachys*(M)	PD, MPD?	25/15	7w
Urera kaalae	ND		
Vaccinium calycinum	PD		
**Vaccinium reticulatum* (M)	PD(CD)	25/15	7w
**Viola chamissoniana* (M)	PD	15/6	11w
Wikstroemia oahuensis	PD		
Wikstroemia phillyreifolia	PD		
Wikstroemia sandwicensis	PD		
Xylosma hawaiiense	PD		

[a]If the lower limit of the montane zone in Hawaii is defined as 500 m, all 163 species occur in this zone. However, a dozen or so of the species just barely reach 500 m, and seeds used in this study were collected below 500 m.
[b]Seeds were tested at 15/6, 20/15, 25/15º, and sometimes also at 20/15 or 28/20ºC.
[c]Some seeds exhibited physical dormancy for up to 3 weeks.

Previously, a version of Table 1 was posted on the web. However, now that the time required for germination for the various species we have studied has been added to Table 2, we would like to post this table. We think the time required for germination would be useful information for people attempting to germinate seeds of these species.

Aim 2

Determine the dormancy breaking and germination requirements of species in the montane

We have conducted (or are in the process of conducting) studies on 60 species (see Table 1).

A manuscript has been written on germination of six lobelioid species (*Clermontia fauriei*, *C. hawaiiensis*, *C. kakeana*, *C. pyrularia*, *Cyanea angustifolia*, and *Trematolobelia macrostachy*), and the abstract is presented below.

ABSTRACT. The purpose of this study was to investigate seed dormancy-breaking and germination requirements of six Hawaiian endemic lobelioids native to the montane zone. Fresh seeds of *Clermontia pyrularia* and *Trematolobelia macrostachys* were dormant and did not germinate during 4 w of incubation in light at 15/6, 20/10, or 25/15EC, whereas those of *C. fauriei*, *C. hawaiiensis*, *C. kakeana*, and *Cyanea angustifolia* germinated to 61-85%, but only at 25/15EC. Since seeds of the latter four species eventually germinated to 84-100% when incubated for 12-36 w at the three temperature regimes, fresh seeds were conditionally dormant. Seeds of *T. macrostachys* also came out of dormancy (and germinated to ∃90%) during 18 w of incubation at each of the three temperatures regimes, but those of *C. pyrularia* did so only at 15/6EC. Simulated seasonal temperature variations did not promote dormancy-break and germination in any species except *C. pyrularia*. Seeds of *C. pyrularia* exposed to 25/15EC for 12 w germinated to 90% when moved to 20/10EC, whereas controls kept at 20/10 and 25/15EC germinated to 8 and 0%, respectively. Seeds of all species had an absolute light requirement for germination. Thus, high germination percentages of seeds of these six lobelioids can be obtained with relatively long periods of incubation in light at either high (25/15EC) or low (15/6EC) temperatures, depending on the species. See Figure 1, below.

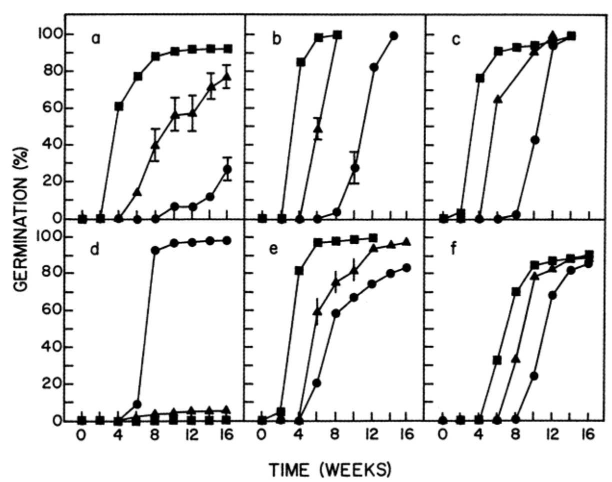

Figure 1. Germination percentages (mean ± SE, if ∃5%) of fresh seeds of (A) *Clermontia fauriei*, (B) *C. hawaiiensis*, (C) *C. kakeana*, (D) *C. pyrularia*, (E) *Cyanea angustifolia*, and (F) *Trematolobelia macrostachys* incubated in light at 15/6E (X), 20/10E (B), and 25/15EC (>) for 16 w.

However, after the manuscript was written we made the very exciting discovery that seeds of some taxa of the Campanulaceae have underdeveloped embryos, and these embryos must grow to some critical length before the seeds can germinate. Thus, these taxa of the Campanulaceae have morphophysiological dormancy (MPD). Seeds in the Campanulaceae usually are ≤2 mm in length, and Martin (1946, *Amer. Midl. Nat*. 36: 513-660) placed this family into his "dwarf-seed" category; to our knowledge this is the first time MPD has been documented in dwarf seeds. We found MPD in seeds of five of the six lobeloids and have studies in progress on the sixth species. Upon completion of studies on the sixth lobeloid, we will revise the manuscript and submit it for publication.

Aim 3

Develop a "move-along experiment" for species from the montane of Hawaii

A move-along experiment now has been conducted on seeds of 32 species (Table 1). In no species, have we been able to document a significant improvement in germination as a consequence of seeds having been shifted from lower to higher or from higher to lower temperatures *versus* seeds being kept continuously at 15/6, 20/10, 20/15, or 25/15ºC. The best temperature for continuous incubation varied with the species. Of the 32 species subjected to a move along, 62 % of them germinated to the highest percentages at 25/15ºC, 6% at 20/15, 13% at 20/10, 13% at 15/6ºC, and 6% (the two species of *Gahnia*) thus far have failed to germinate at any temperature (Table 1).

At first glance, these results are difficult to understand. Although we do not have detailed temperature measurements in the microhabitat of various species, temperature data from weather stations do indicate that there is seasonal variation in the montane zone. Thus, temperatures do not remain relatively constant for extended periods of time. However, our results indicate that exposure of seeds to high or low (depending on the species) temperatures may "prime" the seeds for faster germination at a different (subsequent) temperature regime.

A case in point is seen in our results for *Dianella sandwicensis.* After 24 weeks of continuous incubation at 15/6, 20/10, and 25/15EC, dormancy- break and germination had occurred in 73-100% of the seeds, with 20/10EC being the optimum temperature regime (Table 2). Germination was very slow at the three temperature regimes, and 22 weeks were required for all the seeds kept continuously at 20/10EC to germinate. However, for seeds first given 12 weeks at 25/15EC and then moved to 20/10EC, all the seeds germinated after only 8 weeks at 20/10ºC.

Table 2. Germination of *Dianella sandwicensis* seeds incubated in light.

	Germination percentage at various temperature regimes			
Time (weeks)	15/6	20/10	25/15	25/15
2	0	0	0	0
4	0	0	0	0
6	0	0	0	0
8	0	0	0	0
10	0	3	0	0
12	7	33	1	10
				↓ 20/10EC
14	27	67	7	17
16	27	73	10	40
18	57	97	10	77
20	57	97	43	100
22	73	100	73	100
24	73	100	73	100

What do these data mean? Dormancy-break and germination of *Dianella* seeds can occur over a wide range of temperatures, but these processes are slow, especially at high temperatures. Although seeds germinated to the highest percentage in the least amount of time at 20/10EC, there is no way that 24 weeks of relatively low temperatures would occur in the montane zone. However, seeds responded relatively quickly to low temperatures, if they previously had been imbibed for a long period of time at a high temperature. Thus, the high temperatures that seeds receive during summer "primes" them for germination during the low temperature period in winter.

Aim 4

Break physical dormancy by using simulated natural environmental conditions

Dodonaea viscosa

Studies on *Dodonaea viscosa* have been completed and a paper was published (Baskin et al. 2004. *Seed Science Research* 14: 81-90).

Rhus sandwicensis

A considerable amount of research has been done on “seeds” (seed + endocarp, hereafter seeds) of *Rhus sandwicensis*. Seeds are impermeable to water and thus have physical dormancy. Scarified seeds germinated to 100% at 25/15 and 30/15ºC within 1 week but germinated to 35 and 76% at 15/6 and 20/10ºc, respectively, after 4 week.

Dipping of seeds into boiling water is effective in making them water-permeable, but many seeds became permeable but fail to germinate. Also, after dipping into boiling water seeds imbibe water over a long period of time (Table 3).

Table 3. Imbibition and germination percentages of *Rhus sandwicensis* seeds after being dipped into boiling water for various periods of time and incubated in light at 25/15ºC for 70 days

Time in boiling water	Inbibition	Germination	Days to 50% of the seeds imbibed
0 seconds	11	3	--
5	22	13	--
10	54	37	--
15	85	65	28
20	77	65	28
25	86	51	66
30	88	41	--
40	91	29	--

Other treatments we have been tried on *Rhus* seeds include:

1) drying at 50ºC for 0, 1, 3, 4, and 6 days

2) drying at 60ºC for 1, 12, 24, and 36 hours

3) drying at 90 and 100ºC for 20 and 30 seconds

4) boiling for 30 seconds and dipping into ice water

5) drying at 60ºC for 3 hours → cooling over night at room temperatures → repeat; these cycles have been applied for 0, 1, 4, 8 and 12 times

Number of cycles	% imbibition	% germination
0	5	1
1	12	10
4	45	43
8	87	77
12	96	86

6) pouring 60ºC water over seeds in a beaker → cooling over night → repeat; these cycles have been repeated for 1, 1, 4, and 8 times

7) drying at 60ºC for 3 hours and then immediately pouring 60ºC water over seeds in a beaker → allow seeds to soak for 24 hours → remove imbibed seeds → repeat; these cycles have been repeated 0, 1, 4, and 8 times

Number of cycles	% imbibition	% germination
0	1	1
1	40	37
4	93	82
8	91	83

8) drying at 60ºC for 3, 6, 9, 12, and 24 hours → then immediately pouring 60ºC water over seeds in a beaker

The treatments that show the most promise for the ones numbered 5 and 7 above. However, treatment number 5 is the faster and easiest to apply, consequently this one has the most potential in terms of developing treatments that can be applied in the lab for seeds to be subsequently sown in the field. The next step is to test treatment number 5 with regard to how long pretreated seeds could be stored before they are sown in the field, and treated seeds still would germinate to high percentages.

Sophora chrysophylla

Seeds of *Sophora chrysophylla* have physical dormancy, but they imbibe water and germinate to about 90% within 2 weeks if a small hole is cut in each of them (mechanical scarification). The optimum temperature for germination of mechanically-scarified seeds is 25/15ºC. Seeds have been subjected to drying at 100ºC for periods of 1, 2, 4, 6, 8, and 10 minutes, and maximum germination (23%) was after 2 minutes of drying. However, after 10 minutes of drying 50% of the seeds imbibed, but apparently the heat damaged the embryo, and the imbibed seeds slowly decayed. Seeds of *Sophora* also have been dipped into boiling water for periods of 0, 5, 10, 15, 20, 25, and 30 seconds. Although 78% of the seeds imbibed after 30 seconds of boiling, only a maximum of 2% germination was obtained, after 10 second of boiling.

We would like to note that *Sophora* seeds used in these studies were collected from the Big Island. Seeds from the Big Island are golden in color and a golden pigment leaches from the seeds after they imbibe. Recently, *Sophora* seeds were obtained from Maui, and they are brownish in color. Further, no yellow pigment leaches from imbibed seeds from Maui.