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Germination of drupelets in multi-seeded drupes of the shrub *Leptecophylla tameiameiae* (*Ericaceae*) from Hawaii: a case for deep physiological dormancy broken by high temperatures

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Abstract

This study addressed the difficulty of germinating drupelets (hereafter seeds) in the multi-seeded stony dispersal units (drupes) of *Leptecophylla tameiameiae* (*Ericaceae*). Embryos in fresh seeds were 77% the length of the endosperm, and seeds inside the intact drupes imbibed water. We monitored germination at 15/6, 20/10 and 25/15°C for 162 weeks, after which each drupe was cut open and ungerminated seeds counted. Drupes contained 1–6 seeds, and the total number of seeds in all treatments and controls was 1977, with 20, 29, 25, 18, 7 and <1% of them occurring in one-, two-, three-, four-, five- and six-seeded drupes, respectively. The percentage of seeds germinating in one-, two-, three-, four-, five- and six-seeded drupes was 74, 66, 65, 72, 56 and 0, respectively. Neither warm nor cold stratification for 6 or 12 weeks significantly increased germination percentages, compared to controls incubated continuously at 25/15°C for 162 weeks, where 72% of the seeds in the drupes germinated. At 25/15°C, 24–49 weeks were required for 20% of the seeds to germinate. Warm followed by cold stratification did not promote germination, and there was no widening of the temperature range for germination. Like seeds of other species known to have deep physiological dormancy (PD), those of *L. tameiameiae* required extended periods of time (16 to ≥ 162 weeks) to come out of dormancy and germinate, gibberellic acid (GA_3) did not promote germination and excised embryos failed to grow. Thus, we conclude that seeds of *L. tameiameiae* have deep PD. However, unlike seeds of other species with deep PD, those of *L. tameiameiae* required an extensive period of warm rather than of cold stratification to come out of dormancy. It is suggested that a subtype a (seeds require a long period of cold stratification to come out of dormancy) and a subtype b (seeds require a long period of exposure to warm stratification to come out of dormancy) of deep PD be recognized in the Nikolaeva formula system for classifying seed dormancy.

Keywords: deep physiological dormancy, *Ericaceae*, Hawaii, *Leptecophylla tameiameiae*, multi-seeded drupes, seed dormancy, stony endocarps, warm stratification

Introduction

One of the goals of studies on the world biogeography of seed dormancy is to determine if there are differences in relative proportions of non-dormancy and of each of the five classes of dormancy (*sensu* Baskin and Baskin, 2004) among the major vegetation regions on Earth. Information on the world biogeography of seed dormancy could provide insight on: (1) how timing of germination is controlled in different kinds of climates; and (2) the phylogeny of seed dormancy, e.g. has seed dormancy within a lineage changed through time, as species have migrated and/or the climate has changed? Tropical montane zones are of special interest from a seed dormancy perspective because these areas not only support a rich flora, but they are islands. In continental tropical areas, the montane zone on different

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mountains is separated by a 'sea' of lowland tropical vegetation. However, tropical mountains on islands, e.g. the Hawaiian Islands, are separated by water and, to some extent, by lowland vegetation, if the islands are large enough to have more than one mountain. Many species in tropical montane zones belong to genera that also have species growing in temperate regions (Carlquist, 1974), and for montane zones on islands, e.g. the Hawaiian Islands, the species are derived from ancestors that immigrated to the islands (Wagner *et al.*, 1999). Thus, information on the biogeography of seed dormancy of tropical montane species will help us determine if changes have occurred in lineages. However, such studies require a complete understanding of seed dormancy and germination of the various species included in the lineage, especially of those growing in different kinds of habitats.

Leptecophylla [including *Styphelia* (in part) *sensu* Quinn *et al.* (2003)] is one of several genera in the Hawaiian Islands that has affinities with Australia, New Zealand and other Pacific regions (Wagner *et al.*, 1999). As a contribution to understanding seed dormancy and germination in this genus, we have studied *Leptecophylla tameiameiae* (Cham. & Schlechtend.) C. M. Weiller [= *Styphelia tameiameiae* (Cham. & Schlechtend.) F. v. Muell. (*Epacridaceae*), but now placed in *Ericaceae*, *sensu* APG-II (Angiosperm Phylogeny Group, 2003)]. *L. tameiameiae* is found on all the main Hawaiian Islands, except Ni'ihau and Kaho'olawe, and in the Marquesas Islands (Wagner *et al.*, 1999). Our study involved only material from the Hawaiian Islands. The elevation range of *L. tameiameiae* in Hawaii is from 15 to 3230 m, and it can be found in a variety of habitats including 'mesic forest to open areas of low elevation or montane wet forest, fogswept alpine shrubland, and bogs, rarely windward coastal sites' (Wagner *et al.*, 1999, p. 591). The wide distribution of *L. tameiameiae* in montane and subalpine zones makes it a highly desirable species to use in habitat restoration projects.

The ovary of *Leptecophylla* has 2–10 locules, with one seed in each locule, and each seed is covered by stony endocarp, with the endocarps of adjacent locules fused to each other. The dispersal unit is a multi-seeded drupe with a soft mesocarp and thin exocarp. Seeds are very difficult to germinate. Even after various treatments, such as cracking the endocarp and soaking in water or in acetic acid, many months may be required for only about half the seeds to germinate (Stratton *et al.*, 1998). Further, treatment of fresh *L. tameiameiae* drupes with gibberellic acid (GA_3) did not significantly increase germination percentages compared to water controls. Seeds in GA_3-treated drupes began to germinate on day 107 and those in the water controls on day 197; however, germination of seeds extended from day 107 to day 412 in GA_3-treated drupes, but from day 197 to day 381 in the water controls (Yoshinaga, unpublished). Criley (1999) concluded that an immature embryo was not the reason why freshly harvested seeds failed to germinate.

In view of the previous difficulties encountered in germinating seeds in drupes of *L. tameiameiae*, our purpose was to identify dormancy-breaking and germination requirements for this species. Such information would greatly facilitate studies on other species in the genus, increase our knowledge of the germination ecology of multi-seeded, stony dispersal units and enhance the propagation of an important species in the Hawaiian montane/subalpine zones. The specific purposes of our studies on *L. tameiameiae* were to determine: (1) if seeds have underdeveloped embryos; (2) if seeds inside intact drupes imbibe water; (3) the effects of cold and of warm stratification on germination; (4) effect of warm followed by cold stratification on germination; (5) effect of GA_3 on germination of excised seeds; and (6) if excised embryos would grow.

Materials and methods

Mature fruits of *L. tameiameiae* were collected at an elevation of 300–360 m on Loa Ridge on the island of Oahu in Hawaii on 1 November 2001. The mesocarp was removed from around the stony endocarp (central part), and drupes were dried for 10 d prior to being air-mailed to the University of Kentucky. Germination studies were initiated on 21 November 2001 and terminated on 2 January 2005, after a total of 162 weeks.

Embryo characteristics

To determine if fresh seeds of *L. tameiameiae* have underdeveloped embryos, i.e. growth of embryo would be required before seeds could germinate (*sensu* Nikolaeva, 1969), one seed was excised from each of 15 drupes under a dissecting microscope, using a razor blade. Seed length was measured using a micrometer, and then the embryo was excised from the seed and measured. Also, during the germination studies seven drupes were found in which one drupelet had split open but the radicle had not yet emerged. Seed and embryo lengths were determined for each of these seeds.

Imbibition studies

Since lack of germination of seeds in the drupes of *L. tameiameiae* could be due to failure of dispersal units to imbibe water, imbibition studies were conducted on

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intact drupes. Three replications of 50 drupes each were carefully inspected under a dissecting microscope to ensure that all the mesocarp had been removed. Drupes were placed on filter paper moistened with distilled water and after 0, 2, 4, 6, 8 and 24 h and 7 and 14 d they were removed from the wet paper, gently rolled on dry filter paper to remove any excess water and weighed.

However, even if drupes imbibe water, this does not prove that the seeds themselves are imbibed. To address this question, intact seeds were excised from drupes that had been on wet filter paper for 162 weeks; seeds were excised using a razor blade and a dissecting microscope. Three replicates of 25 intact seeds were excised, and each was quickly weighed. Then seeds were dried at 70°C for 48 h and reweighed.

Further, observations were made on seeds to supplement the information obtained from imbibition studies. Seeds excised from drupes that had been on wet filter paper for 162 weeks were cut open so the relative softness of the endosperm could be evaluated. Also, excised seeds were gently pressed with forceps to see if water exuded from them.

Effect of warm and cold stratification on germination

Drupes were given a moist cold- or moist warm-stratification treatment and then incubated at three temperature regimes. Drupes to be given a cold stratification treatment were kept at a constant temperature of 5°C (14 h daily photoperiod) for 6 or 12 weeks. After each of the two cold stratification treatments, they were transferred to 15/6, 20/10 and 25/15°C and incubated in light. Drupes to be given a warm stratification treatment were kept at 25/15°C for 6 or 12 weeks. After each of the two warm stratification treatments, they were transferred to 15/6 and to 20/10°C and incubated in light. Drupes receiving either warm or cold stratification and then incubated at 15/6°C remained at this regime for 44 weeks. After 44 weeks, they were moved to 20/10°C for 44 weeks and then to 25/15°C for the remainder of the study. Drupes receiving either cold or warm stratification and then incubated at 20/10°C remained at this regime for 44 weeks, after which they were moved to 25/15°C for the remainder of the study. If drupes were tested for germination at 25/15°C after receiving 6 or 12 weeks of cold stratification, they were at 25/15°C for 156 or 150 weeks, respectively. Controls (drupes receiving no cold or warm stratification) were maintained at 15/6, 20/10 and 25/15°C for the entire 162 weeks of the study.

Three replications of 30 drupes each were used for each treatment and control. All drupes were placed in 5.5-cm Petri dishes on two sheets of Whatman No. 1 filter paper moistened with distilled water; water was added as needed throughout the study to keep the drupes and paper moist. At 15/6, 20/10 and 25/15°C, drupes were exposed to a 12/12 h daily alternating temperature regime and a 14-h daily photoperiod (40 μmol $m^{-2} s^{-1}$, 400–700 nm, cool white fluorescent light). Lights came on in the incubators 1 h before the beginning of the daily high temperature period and remained on for 1 h after the beginning of the low temperature period each day.

All drupes were checked for germination at 2-week intervals for the first 2 years of the study but at 4-week intervals (with a few exceptions) during the third year. As seeds germinated, the number of seedlings was recorded and seedlings discarded. We kept track of the number of seedlings produced by each drupe. When a drupe produced the first seedling, the drupe was moved to a new dish labelled 'one seedling'. When a second seed germinated in a drupe, the drupe was moved to a dished labelled 'two seedlings'; five was the maximum number of seedlings produced by a drupe. For each of the original 39 dishes (treatments + controls), there were five additional dishes to receive the drupes that had produced 1–5 seedlings; original and additional dishes were kept at the same conditions. No attempt was made to record the dates when individual drupes produced a seedling. However, on any recording date the number of drupes with one, two, three, four or five seedlings (in each treatment and control) was determined.

After 162 weeks, the number of ungerminated seeds in all drupes was determined by cutting open each drupe (using a single-edge razor blade) under a dissecting microscope. The number of ungerminated seeds was recorded for each category of drupes, i.e. drupes with zero, one, two, three, four or five seeds. The ungerminated seeds were white and firm, and the embryo tested positive with tetrazolium.

Final germination percentages in all treatments and controls were arcsin transformed and analysed using a one-way analysis of variance (ANOVA), followed by a Tukey honestly significant difference (HSD) test. All analyses were carried out with SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA).

Effect of warm followed by cold stratification on germination

The purpose of this experiment was to determine if warm followed by cold stratification would promote germination, in which case it would provide evidence for the presence of intermediate physiological dormancy (*sensu* Nikolaeva, 1969) in seeds of *L. tameiameiae*. Drupes were collected on Oahu on 1 November 2001, and the experiment was initiated on 21 November 2001 and terminated on 2 January 2005.

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As described previously, three replicates of 30 drupes each were used for each treatment and control, and all drupes were placed on moist filter paper in 5.5 cm Petri dishes and exposed to a 14-h daily photoperiod.

Drupes were given 6 weeks of warm stratification at 25/15°C, followed by 12 weeks of cold stratification at 5°C. After 12 weeks at 5°C, they were transferred to 15/6, 20/10 or 25/15°C. After 40 weeks, no seeds had germinated at 15/6°C, so drupes were moved to 20/10°C. After 40 weeks at 20/10°C, the drupes were moved to 25/15°C for 64 weeks, i.e. until the end of the experiment. After 40 weeks, none of the seeds initially incubated at 20/10°C had germinated, so drupes were moved to 25/15°C for 104 weeks, i.e. until the end of the experiment.

Controls were drupes incubated continuously at 15/6, 20/10 and 25/15°C for 162 weeks. As previously described, all drupes were checked for seedlings at 2- to 4-week intervals and, at the end of the experiment, all drupes were cut open to determine the number of ungerminated, viable seeds they contained.

Effect of GA_3 on germination of excised seeds

Seeds excised from drupes that had been incubated for 138 weeks on moist filter paper at 25/15°C were used to test the effect of GA_3 on germination. Three replications of 25 excised seeds each were placed on filter paper in 10-cm glass Petri dishes moistened with 5 ml of 100 mg l^{-1} of GA_3 (K-GA_3) dissolved in distilled water, and three replicates were placed on filter paper moistened with distilled water. Dishes were wrapped with plastic film, and seeds were incubated in light at 25/15°C and checked for germination at weekly intervals for 6 weeks.

Germination of excised embryos

Embryos were excised from drupes/seeds that had been incubated on filter paper moistened with water at 25/15°C for 138 weeks. Two replications of 10 embryos each were placed on filter paper moistened with distilled water, and two replicates on filter paper moistened with 5 ml of 100 mg l^{-1} of GA_3. Embryos were incubated in light at 25/15°C and checked for radicle elongation and greening of the cotyledons at weekly intervals for 6 weeks.

Results

Embryo characteristics

Mean (± SE) length of fresh seeds was 1.33 ± 0.04 mm, and mean embryo length was 1.00 ± 0.05 mm; thus, embryos were 75.2% the length of the seeds. Embryos were linear and about five times longer than wide. Each embryo was surrounded by a thin layer of white endosperm, and seeds were covered by a thin translucent seed coat. In the seven drupelets with a split endocarp, seed and embryo lengths were 1.65 ± 0.08 and 1.24 ± 0.06 mm, respectively, and thus the embryos were 75.2% the length of the seed.

Imbibition

Percent (mean ± SE) increase in mass after 2, 4, 6, 8 and 24 h and 7 and 14 d of incubation on moist filter paper was 14.9 ± 0.8, 16.8 ± 0.4, 17.4 ± 0.5, 17.5 ± 0.5, 19.5 ± 0.5, 22.6 ± 0.3 and 23.2 ± 0.5, respectively. Mean (± SE) mass of seeds excised from drupes that had been on wet filter paper for 162 weeks was 0.0176 ± 0.0010 g, and it decreased 31.63 ± 2.05% during drying for 48 h. Seeds excised after 162 weeks on wet filter paper were very soft, and a little pressure on the seeds resulted in exudation of water from them.

Effect of warm and cold stratification on germination

The presence of seeds in *L. tameiameiae* drupes can not be detected unless seedlings emerge or a drupe is cut open to expose the ungerminated seeds. Thus, an important part of the results on seed germination is how many seeds were actually used in the study, and what proportion of them germinated. Mean percentage of drupes (based on results for each replicate in each treatment and control = 39 dishes of drupes) with one or more seeds was 83 ± 1, and mean number of seeds per dish was 47 ± 8. The total number of seeds in all replicates was 1977, and 20, 29, 25, 18, 7 and <1% of them were in one-, two-, three-, four-, five- and six-seeded drupes, respectively. Only one six-seeded drupe was found. When the percentage of seeds germinating in each size category of drupes was calculated, control drupes incubated continuously at 15/6 and 20/10°C for 162 weeks were not included, because few or no seeds had germinated. In one-, two-, three, four-, five- and six-seeded drupes, 74, 66, 65, 72, 56 and 0% of the seeds, respectively, germinated.

The one-way ANOVA on the whole (arcsin transformed) data set was highly significant ($P < 0.001$), but the Tukey HSD test could distinguish only two homogeneous sets of data at the $P < 0.05$ level. One set was controls (a) and (b), and the other was control (c) and treatments (d)–(m) (Table 1). Thus, regardless of length of warm or cold stratification period, or initial incubation temperature after a treatment, final germination was not significantly different from control seeds kept continuously at 25/15°C for 162 weeks. The highest germination

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Table 1. Mean (±SE) percentage of the total number of *Leptecophylla tameiameiae* seeds that germinated in control drupes and all treatments. Percentages with same superscript are not significantly different at $P < 0.05$ (one-way ANOVA, followed by Tukey HSD test). Untransformed data are shown, but the analysis was conducted on arcsin transformed data

Controls/treatments	Mean (±SE) % of total number of seeds germinated
(a) 15/6°C 162 weeks	0^a
(b) 20/10°C 162 weeks	3 ± 2^a
(c) 25/15°C 162 weeks	72 ± 9^b
(d) 25/15°C 6 weeks → 15/6°C 44 weeks → 20/10°C 44 weeks → 25/15°C 68 weeks	62 ± 5^b
(e) 25/15°C 6 weeks → 20/10°C 44 weeks → 25/15°C 112 weeks	82 ± 3^b
(f) 25/15°C 12 weeks → 15/6°C 44 weeks → 20/10°C 44 weeks → 25/10°C 62 weeks	62 ± 2^b
(g) 25/15°C 12 weeks → 20/10°C 44 weeks → 25/15°C 106 weeks	80 ± 6^b
(h) 5°C 6 weeks → 15/6°C 44 weeks → 20/10°C 44 weeks → 25/15°C 68 weeks	69 ± 1^b
(i) 5°C 6 weeks → 20/10°C 44 weeks → 25/15°C 112 weeks	81 ± 3^b
(j) 5°C 6 weeks → 25/15°C 156 weeks	89 ± 4^b
(k) 5°C 12 weeks → 15/6°C 44 weeks → 20/10°C 44 weeks → 25/15°C 62 weeks	58 ± 4^b
(l) 5°C 12 weeks → 20/10°C 44 weeks → 25/15°C 106 weeks	80 ± 6^b
(m) 5°C 12 weeks → 25/15°C 150 weeks	67 ± 7^b

obtained in the study (89%) was for seeds receiving 6 weeks' cold and then incubated at 25/15°C for the remaining 156 weeks of the study. However, 89% germination was not significantly higher than that obtained for seeds receiving 6 weeks cold, 12 weeks cold, 6 weeks warm or 12 weeks warm and initially incubated at 20/10°C (and subsequently at 25/15°C).

Although warm and cold stratification had no effect on final germination percentages (compared to control at 25/15°C), the initial incubation temperatures following treatment had an impact on time required for germination (Fig. 1). Using 20% as an arbitrary indication that germination had begun, most seeds did not germinate until drupes were moved to 25/15°C, regardless of warm or cold stratification treatment, or if drupes were initially incubated at 15/6 or 20/10°C after a treatment. In all treatments (and the control at 25/15°C), time at 25/15°C required for 20% of the seeds to germinate ranged from 21 to 48 weeks. Drupes receiving 6 or 12 weeks warm stratification

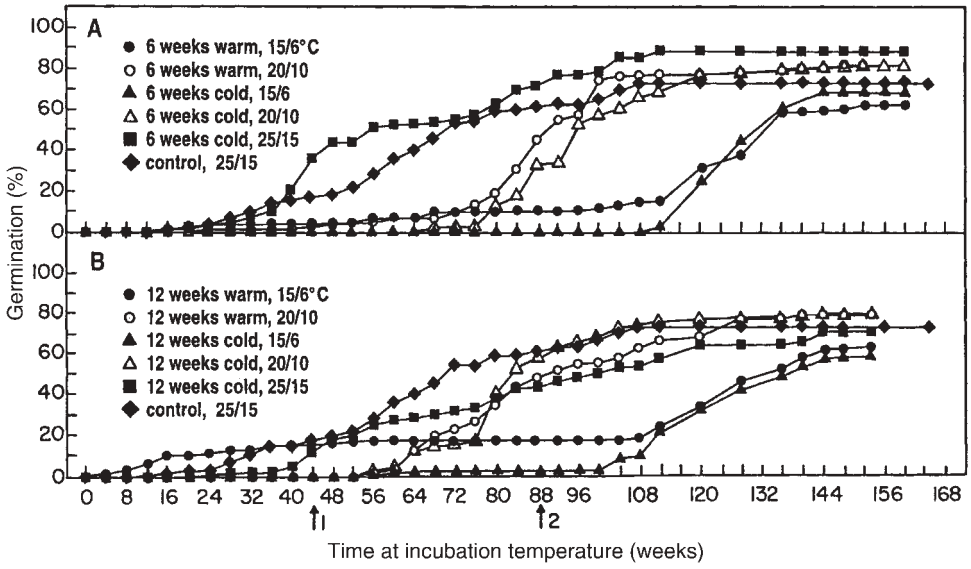

Figure 1. Effect of warm or of cold stratification for 6 (A) or 12 (B) weeks on germination of *Leptecophylla tameiameiae* seeds. Controls were kept continuously at 25/15°C for 162 weeks, and data are shown in both A and B for ease of interpretation. Data for controls at 15/6 and 20/10°C are not shown because few or no seeds germinated at these regimes. ↑1 indicates the time when drupes initially incubated at 15/6°C (after warm or cold stratification) were moved to 20/10°C, and the time when drupes initially incubated at 20/10 were moved to 25/15°C. ↑2 indicates the time when drupes initially incubated at 15/6°C (after warm or cold stratification) were moved from 20/10 to 25/15°C.

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and then incubated at 15/6 or 20/10 required only 21–36 weeks at 25/15°C for 20% of the seeds to germinate. However, if the 6 or 12 weeks of warm stratification is added to the 21–36 weeks at 25/15°C, the length of exposure to 25/15°C required for 20% germination increases to 33–42 weeks. Germination of seeds in drupes receiving 6 or 12 weeks cold and initially incubated at 15/6°C reached 20% germination at 25/15°C after 29 and 24 weeks, respectively; however, maximum germination was only 69 and 58%, respectively.

Effect of warm followed by cold stratification on germination

In drupes receiving warm followed by cold stratification, 0, 0 and 1 ± 1% of the seeds had germinated after 40 weeks of incubation at 15/6, 20/10 and 25/15°C, respectively. After the 40-week test period, drupes at 15/6°C were moved to 20/10°C and those at 20/10°C were moved to 25/15°C; after 40 weeks at 20/10°C, drupes (initially at 15/6°C) were moved to 25/15°C. At the end of the experiment, 36 ± 6, 41 ± 2 and 81 ± 7% of the seeds in drupes receiving warm followed by cold stratification and then initially tested at 15/6, 20/10 and 25/15°C, respectively, had germinated. However, it should be noted that in drupes receiving warm plus cold stratification and then initially incubated at 15/6, 20/10 and 25/15°C, the total number of weeks of exposure to 25/15°C by the end of the experiment was 70, 120 and 150, respectively. In controls kept continuously at 15/6, 20/10 and 25/15°C, 0, 2 ± 1 and 28 ± 4% of the seeds, respectively, had germinated after 40 weeks, and 0, 3 ± 2 and 71 ± 9% of the seeds, respectively, had germinated after 162 weeks. A Mann–Whitney U-test at the 5% level of significance showed that the germination percentages of seeds in drupes placed directly at 25/15°C after the warm plus cold stratification treatments was not significantly different from that of seeds in drupes incubated continuously at 25/15°C for 162 weeks.

Effect of GA_3 on germination of excised seeds

After 6 weeks, only one of the 75 seeds treated with GA_3, and only two of the 75 in the water-controls had germinated.

Germination of excised embryos

The excised embryos remained white, and the radicles did not elongate, regardless of whether they were incubated on filter paper moistened with the GA_3 solution or with distilled water.

Discussion

Seeds requiring more than about 4 weeks to germinate are usually considered to be dormant (Baskin and Baskin, 1998). Sixteen weeks passed before the first seeds germinated in the control drupes at 25/15°C; thus, fresh seeds of *L. tameiameiae* were very dormant. Since little or no embryo growth occurs in *L. tameiameiae* seeds before the radicle pushes through the endosperm, the cause of dormancy can not be attributed to an underdeveloped embryo, i.e. seeds did not have morphological or morphophysiological dormancy. Likewise, detailed measurements of embryos in seeds of *Empetrum hermaphroditum* Hagerup [*Empetraceae*, but now *Ericaceae sensu* APG-II (Angiosperm Phylogeny Group, 2003)], which individually are covered by a stony endocarp, revealed that embryo growth was not a prerequisite for dormancy break (Baskin *et al.*, 2002).

The imbibition of water by drupes and by individual seeds indicates that physical dormancy (i.e. failure to take up water) is not the cause of dormancy in *L. tameiameiae*. When one seed in a drupe germinated, the drupe frequently broke into two (or more) parts. Thus, if lack of water uptake had been the cause of dormancy in seeds of *L. tameiameiae*, it seems reasonable that all the remaining seeds in a drupe would have germinated immediately after the first one in a drupe had done so. However, after the first seed germinated, there was often a long period of time before the second one germinated. For example, in the first three drupes to produce a seedling in the controls at 25/15°C, the time required for the second seed to germinate was 10, 16 and 36 weeks. Also, only three of the 150 excised seeds germinated, indicating that something other than physical dormancy was preventing germination.

If seeds of *L. tameiameiae* do not have morphological, morphophysiological or physical (and thus not combinational) dormancy, the only other kind (class) of dormancy they could have is physiological dormancy (PD). PD is due to a physiological-inhibiting mechanism in the embryo, and Nikolaeva (1969) distinguished three levels of PD: non-deep, intermediate and deep. Characteristics of the three levels of PD and the data we obtained for seeds of *L. tameiameiae* are compared in Table 2. When seeds with non-deep PD are subjected to cold or to warm stratification, depending on the species, a relatively short period of time is required for dormancy to be broken. Thus, seeds of *L. tameiameiae* clearly do not have non-deep PD. Further, warm followed by cold stratification did not break dormancy in seeds of *L. tameiameiae*, so they do not have intermediate PD. With the exception of the long cold stratification requirement for dormancy break, seeds of *L. tameiameiae* fit the characteristics of deep better than those of non-deep or intermediate PD.

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Table 2. Dormancy and germination characteristics of *Leptecophylla tameiameiae* seeds compared to those of seeds with non-deep, intermediate and deep physiological dormancy (PD)

Characteristic	Non-deep PD	Intermediate PD	Deep PD	*L. tameiameiae*
GA_3 promotes germination	Yes	Yes or no	No	No
Excised embryos produce normal seedlings	Yes	Yes	No, or dwarf	No growth
Time required for dormancy break	Few days to few months	Several months (1–6)	Several months to 1–2 years	4–40 months or longer
Temperature requirement for dormancy break	W or C	C or W + C	C	W
Widening of temperature range for germination as dormancy is broken	Yes	Yes	No	No
Dormancy cycling	Yes	No	No	Not known

W, warm stratification; C, cold stratification.

Warm stratification was the only treatment that broke dormancy in seeds of *L. tameiameiae*. Regardless of the length of warm or cold stratification period, or of initial incubation temperatures (15/6, 20/10 or 25/15°C) following these treatments, germination did not exceed 20% until after drupes had been at 25/15°C for 24 or more weeks (Fig. 1). Similar results were obtained for drupes of *L. tameiameiae* collected in the montane zone on the island of Hawaii and in the subalpine zone on the island of Maui (Baskin *et al.*, unpublished data). Thus, the seeds of this species have a high-temperature requirement for dormancy break. Twelve weeks of warm stratification did not promote germination at 15/6 or 20/10°C, and few or no seeds germinated in drupes incubated continuously at these regimes for 162 weeks (Fig. 1). Thus, we found no evidence of lowering of the temperature required for germination. Like seeds with deep PD that come out of dormancy in response to long periods of cold stratification and germinate to high percentages at the dormancy-breaking temperature, seeds of *L. tameiameiae* also germinated to high percentages only at the optimum temperature (25/15°C) for dormancy break. Dormancy in *L. tameiameiae* seeds is long persisting, and some seeds were dormant (viable and ungerminated) after 162 weeks at 25/15°C, failing to germinate even after they were excised from the drupes. Our conclusion is that dormancy in *L. tameiameiae* seeds matches that found in seeds with deep PD, with the exception that a long period of warm, rather than of cold, stratification is required to break dormancy in seeds of this species. However, just as non-deep PD can be broken by cold or warm stratification, depending on the species, we conclude that deep PD also can be broken by cold or warm stratification, depending on the species. To our knowledge, *L. tameiameiae* is the first species documented to have deep PD that is broken by warm stratification.

The presence of deep PD in *L. tameiameiae* seeds means that germination of seeds in a given cohort of drupes potentially could be spread over a period of 3 or more years. Part of this variation in time of germination is due to differences in rate of dormancy loss between drupes. In the controls kept continuously at 25/15°C, one drupe had produced a germinated seed after 16 weeks, while no seeds in 18 of the 90 drupes in the controls had germinated seeds at the end of the study; they contained from one to three seeds each. Also, within a single drupe, as already discussed, the appearance of a seedling does not mean that the other seeds will germinate immediately. Given the small seasonal variation in temperatures in its tropical habitat, the slow (steady) release of dormancy in a few seeds at a time indicates that some *L. tameiameiae* seeds could germinate at any time of the year. In contrast, seeds of three *Leucopogon* spp. (*Ericaceae*, formerly in the *Epacridaceae*) in New South Wales, Australia, exhibited a peak of germination in the field after fire, but germination was restricted to late autumn and winter (Ooi *et al.*, 2004). Further, if fire occurred in winter, seeds did not germinate until the next winter, but if fire occurred in summer, seeds germinated the subsequent winter; good rains followed fires in both seasons. Thus, there was an unidentified dormancy-breaking cue related to timing of fire. Perhaps, a warm, wet period is required to break PD of the seeds, and smoke compounds that remain in the soil from the fire promote germination when the cool, wet season begins. That is, seeds cannot respond to smoke until after PD has been broken.

Deep PD that is broken by long periods of warm stratification may be the reason why seeds of some tropical species are slow to start germinating after they are sown. For example, in Ng's (1991, 1992) studies on seed germination phenology of Malaysian trees, 28 species in 15 families did not begin to germinate until after 90 d, and some not until after more than 250 d. However, information on responses of seeds to GA_3 and behaviour of excised embryos is needed to determine if, like seeds of *L. tameiameiae*, seeds of these slow-germinating tropical species have deep PD.

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The discovery of a kind of deep PD that requires an extended period of warm stratification to be broken means that a new category needs to be added to the Nikolaeva formula system for classifying seed dormancy (Baskin and Baskin, 2004). Nikolaeva (2001) subdivided non-deep PD (symbolized by 'C_1') into C_{1a} for seeds requiring high temperatures to break dormancy, e.g. winter annuals, and C_{1b} for seeds requiring low temperatures to break dormancy, e.g. summer annuals. Following the Nikolaeva format, we propose to subdivide deep PD (symbolized by 'C_3') into C_{3a}, for seeds requiring long periods of cold stratification to break dormancy, and C_{3b}, for those requiring long periods of warm stratification for dormancy break, such as *L. tameiameiae*. Thus, following the Nikolaeva combined word-formula system, seeds with PD would be classified as follows: non-deep PD, subtype a (C_{1a}) and subtype b (C_{1b}); intermediate PD (C_2); and deep PD, subtype a (C_{3a}) and subtype b (C_{3b}).

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