Seed dormancy and germination of *Ficus lundellii* and tropical forest restoration

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Summary We investigated seed dormancy and germination in *Ficus lundellii* Standl. (Moraceae), a native species of Mexico’s Los Tuxtlas tropical rain forest. In an 8-h photoperiod at an alternating diurnal (16/8 h) temperature of 20/30 °C, germination was essentially complete (96%) within 28 days, whereas in darkness, all seeds remained dormant. Neither potassium nitrate (0.05–0.2%) applied continuously nor gibberellic acid applied either continuously (10–200 ppm) or as a 24 hour pre-treatment (2000 ppm) induced germination in the dark. Germination in the light was not reduced by a 24-h hydrochloric acid (0.1–1%) pretreatment, but it was reduced both by a 24-h pretreatment with either H₂O₂ (0.1–5 M) or 5% HCl, or by more than 5 days of storage at 40 °C (4.5% seed water content). In a study with a 2-dimensional temperature gradient plate, seeds germinated fully and rapidly in the light at a constant temperature of 30 °C, and fully but less rapidly in the light at alternating temperatures with low amplitudes (< 12 °C) about the optimal constant temperature. The base, optimal and ceiling temperatures for rate of germination were estimated as 13.8, 30.1 and 41.1 °C, respectively. In all temperature regimes, light was essential for the germination of *F. lundellii* seeds.

Keywords: alternating and constant temperature, hemiepiphyte, strangler fig, temperature gradient plate.

Introduction

Strangler fig, *Ficus lundellii* Standl. (Moraceae), inhabits Los Tuxtlas tropical rain forest (southeast Mexico) where it plays an important role in the ecological interactions between animals and plants. It is a hemiepiphyte whose seeds germinate in the upper canopy of a host tree. Howler monkeys (*Alouatta palliata mexicana*) are important dispersers of *F. lundellii* seeds (Serio-Silva and Rico-Gray 2002), feeding on the fruits and dispersing the seeds in their feces, which may be lodged in branch crotches or knotholes of host tree trunks, where seeds germinate and seedlings become established. Seedling roots elongate rapidly, growing downward in close contact with the bark of the host tree. Once roots reach the ground, the plant grows rapidly. Common host trees for strangler figs include *Diospyros dygina* Jacq. (Serio-Silva and Rico-Gray 2002), *Quercus virginiana* Mill. and *Sabal palmetto* (Walt.) Lodd. ex Schult. & Schult.f. (Bessey 1908), *Vitex altissima* L.f. Tree, *Diospyros bourdilloni* Brandis and *Eugenia* spp. (Athreya 1999), palms (Swagel et al. 1997) and dipterocarps (Laman 1996). Good hosts must have knotholes, branch crotches, or leaf bases (palms) large enough to accumulate significant amounts of water-holding leaf litter or other organic detritus (Athreya 1999, Doyle 2000).

*Ficus* species are some of the most abundant in the seed rain within Los Tuxtlas, but are seldom found in the soil seed bank (Vázquez-Yanes et al. 1996), implying limited seed dormancy. In contrast, although temperature, light and humidity are the main factors influencing strangler fig seed germination and establishment (Bessey 1908, Ramirez 1976, Serio-Silva and Rico-Gray 2002), the presence of the viscid seed coat is thought to hinder germination (Ramírez 1976). For example, soaking seed in hydrochloric acid is reported to promote germination (Vázquez-Yanes et al. 1996). Moreover, seeds collected from feces show either higher germination than seeds extracted from fresh fruits (Midya and Brahmachary 1991, Figueiredo and Perin 1995, Serio-Silva and Rico-Gray 2002) or only slightly reduced germination (Lisci and Pacini 1994).

We investigated several approaches to breaking dormancy in seeds of *F. lundellii* with two objectives. First, to determine how best to promote the germination of seeds used in the artificial propagation of the species. Second, to explain the paucity of *F. lundellii* in the soil seed bank.

Materials and methods

Mature syconia (i.e., fruits) of *Ficus lundellii* were collected in March 2003 from several individuals at the Estación Biológica de Los Tuxtlas (18°27′ N, 95°13′ W and 300 m a.s.l.), Veracruz, Mexico. The seeds were extracted by hand, air-dried and stored at 5 °C for about 40 days until shipped by air to Reading, where they were re-dried (12% relative humidity at 15 °C) for 2 days, cleaned in an air column to remove empty seeds.
were placed on two 50 × 50 mm pieces of Whatman 3MM chromatography paper, moistened with 1.5 ml of deionized water. The cells were covered by a well-insulated lid lined with moistened chromatography paper. Temperature was adjusted to 15 and 43 °C at opposite edges of the plate, with a 16/8 h diurnal cycle providing 13 constant (across the diagonal) and 156 alternating temperature regimes. Temperatures at the four corner cells and one at the center of the plate were measured and recorded every 5 min with a data logger (TempScan/1000A, Iotech, OH). The apparatus was kept in an air-conditioned room (22–25 °C), with continuous white light of 0.2 µmol m⁻² s⁻¹ at the seed level. Germination was assessed as radicle emergence (≥ 1 mm) every 2 days for 28 days. Germination isopleths were mapped using SigmaPlot 7.0.

Chemical treatments
We investigated the effects of the following chemical treatments on germination: gibberellic acid (GA₃), applied continuously at concentrations of 10, 50, 100 and 200 ppm, or during a 24-h pretreatment at a concentration of 2000 ppm; potassium nitrate (KNO₃) applied continuously at concentrations of 0.05, 0.1 or 0.25%; hydrochloric acid (HCl) applied during a 24-h pretreatment at concentrations of 0.1, 0.5, 1 and 5%; and hydrogen peroxide (H₂O₂) applied during a 24 h pretreatment at concentrations of 0.1, 0.25, 0.5, 1, 2, 3 and 5 M. For each treatment, there were four replicates of 50 seeds imbibed on two filter papers (Whatman Grade 181) moistened with 4.5 ml of deionized water (control) or treatment solution. For the pretreatments, seeds were soaked in deionized water (as a control) or the appropriate test solution at 20 °C (200 seeds in 8 ml) for 24 h. Seeds pretreated with HCl or H₂O₂ were rinsed in deionized water. Germination tests were carried out at an alternating diurnal (16/8 h) temperature of 20/30 °C in white light (from cool white fluorescent lamps) (8 h day⁻¹ at 1.6 µmol m⁻² s⁻¹). Germination was assessed every 2 or 3 days for 35 days. The criterion for germination was normal seedling development (ISTA 2005).

Dry storage
Ten sealed glass vials (0.5 cm diameter, 5 cm tall), each containing 200 seeds with a water content of 4.5%, were stored in an incubator at 40 ± 0.5 °C for 0, 1, 2, 3, 4, 5, 7, 10, 14 or 21 days. One vial was withdrawn after each period and the seeds tested for germination on filter papers moistened with deionized water in an 8-h photoperiod at an alternating diurnal (16/8 h) temperature of 20/30 °C (as above).

Constant and alternating temperature regimes
A 2-dimensional temperature gradient plate was used to investigate the effects of alternating and constant temperatures on seed germination. The system comprised a horizontal 650 × 650 mm copper plate on which seeds were set to germinate, covered with one sheet of Whatman 3MM chromatography paper (Whatman, Maidstone, Kent, UK) moistened with deionized water in troughs at the edge of the plate (Murdoch et al. 1989). On top of the chromatography paper, a 13 × 13 cell Perspex (Plexiglas) matrix provided 169 test cells, each with a 50 × 50 × 40 mm polystyrene box. Within each box, 50 seeds were placed on two 50 × 50 mm pieces of Whatman 3MM chromatography paper, moistened with 4.5 ml of deionized water. The cells were covered by a well-insulated lid lined with moistened chromatography paper. Temperature was adjusted to 15 and 43 °C at opposite edges of the plate, with a 16/8 h diurnal cycle providing 13 constant (across the diagonal) and 156 alternating temperature regimes. Temperatures at the four corner cells and one at the center of the plate were measured and recorded every 5 min with a data logger (TempScan/1000A, Iotech, OH). The apparatus was kept in an air-conditioned room (22–25 °C), with continuous white light of 0.2 µmol m⁻² s⁻¹ at the seed level. Germination was assessed as radicle emergence (≥ 1 mm) every 2 days for 28 days. Germination isopleths were mapped using SigmaPlot 7.0.

Combined effects of light, chemicals and temperature
We tested the effects on germination of a factorial combination of two chemicals (0.1% KNO₃ applied continuously and GA₃ applied as a pretreatment at 2000 ppm) in the light (8-h photoperiod, 1.6 µmol m⁻² s⁻¹) or darkness at either a constant (30 °C) or an alternating day/night temperature (20/30 °C, 16/8 h). For the dark treatment, each petri dish was wrapped in two layers of aluminum foil. Germination was assessed as normal seedling development after 14 and 28 days.

Results
Chemical treatments
In the control treatment, germination was virtually complete (96%) after 28 days in the light, whereas no germination occurred in the dark (results not shown). Neither KNO₃ nor GA₃ induced germination in the dark. A 24-h pretreatment with 0.1 to 1% HCl had no effect on germination in the light, whereas light germination was reduced by a 24 h pretreatment with 0.1–5 M H₂O₂ or 5% HCl (results not shown).

Dry after-ripening
Up to 5 days of storage at a water content of 4.5% and a temperature of 40 ± 0.5 °C had no significant effect on germination in the light (P > 0.25), but longer durations reduced germination (results not shown).

Constant and alternating temperatures
Germination was first detected at an alternating diurnal temperature of 32/27 °C (16/8 h) and neighboring temperature regimes (Figure 1A). By 21 days, 100% of the seeds in most alternating temperature regimes within 5 °C of a constant 30 °C had germinated (Figure 1C). At 28 days, 100% germination was observed in constant and alternating (16/8 h) temperature regimes within the range 23–36 °C (Figure 1D). Seeds germinated most rapidly at a constant 30 °C (Figure 2A). Relationships between rate of germination and constant temperature were linear above and below the optimum temperature of 30 °C (Figure 2B). Extrapolation suggests base and ceiling temperatures of 13.8 and 41.1 °C, respectively.
Combined effects of light, chemicals and temperature

During the first 14 days in the germination tests, no seeds in any dark treatment germinated (Figure 3A); however, after 28 days in the dark, 1% of seeds in the GA\textsubscript{3} treatment germinated (Figure 3B). Germination in an 8-h photoperiod was considerable and more rapid at 30 °C than at an alternating diurnal (16/8 h) temperature of 20/30 °C (\(P < 0.005\)). Although KNO\textsubscript{3} and GA\textsubscript{3} promoted more rapid germination of \textit{F. lundellii} in an 8-h photoperiod at 30 °C (\(P < 0.005\)), there was no effect of KNO\textsubscript{3} or GA\textsubscript{3} (\(P > 0.25\)) or of temperature regime (\(P > 0.05\)) on germination after 28 days in light (Figure 3B).

Discussion

Comparison of germination in light and dark regimes demonstrated the existence of photo-dormancy in \textit{Ficus lundellii}.

Figure 1. Progress of germination of \textit{Ficus lundellii} seeds in continuous light in response to constant and alternating diurnal (16/8 h) temperatures provided by a 2-dimensional temperature gradient plate after 7 (A), 14 (B), 21 (C) and 28 days (D) (isopleths drawn by SigmaPlot 7.0).

Figure 2. Response of mean germination rate (day\(^{-1}\)) of \textit{Ficus lundellii} seeds in continuous light to constant and alternating diurnal (16/8 h) temperatures as isopleths (A), and in response to a constant temperature (B).
seeds (Figure 3). However, we obtained no evidence of other dormancy mechanisms. About 3–4% of seeds remained viable but dormant after 28 days in an 8-h photoperiod at an alternating diurnal (16/8 h) temperature of 20/30 °C. In a subsequent experiment, 100% germination occurred in continuous light over a wide range of temperature regimes (Figure 1).

Notwithstanding predation and loss of viability, seed germination as a result of the absence or loss of dormancy is the most common reason for the depletion of a species from the soil seed bank (Murdoch and Ellis 1992). *Ficus lundellii* seeds required light to break dormancy and temperatures close to 30 °C for rapid germination (Figure 3). There was no response to diurnal alternation of temperature. These findings are compatible with the reported limited soil seed bank for this species (Vázquez-Yanes et al. 1996).

The importance of light for seed germination of tropical forest species is well known (Vázquez-Yanes and Orozco-Segovia 1982, Metcalfe 1996). Among *Ficus* spp., there is variation in germination light requirements, with seeds of pioneer species being light-dependent, whereas seeds of other species can germinate in the dim light available on the forest floor (Vázquez-Yanes et al. 1990, 1996). Similarly, Metcalfe (1996) reported that seedlings of *F. chartacea* Wall. ex King, *F. fistulosa* Reinh. ex Bl. and *F. grossularioides* Burm. f. commonly occurred in the deep shade of rain forest floors. Our conclusion that light is the most important factor regulating germination of *F. lundellii* seeds is consistent with the observation that the seeds germinate better higher in the canopy (30 m aboveground) than lower in the canopy (20 m aboveground) (Serio-Silva and Rico-Gray 2002).

The climate in the Los Tuxtlas rainforest (18°27′ N, 95°13′ W, 300 m a.s.l.) where *F. lundellii* grows is wet (2000–4500 mm annual rainfall) and warm, with a mean annual temperature of 27 °C (minimum 16 °C in December and January, maximum 32 °C in July and August) (Ibarra-Manriquez and Sinaca-Colin 1987). Thus, for much of the year the temperature is suitable for rapid germination in the presence of light. Although howler monkeys appear to have no direct role in promoting *F. lundellii* seed germination, we conclude that they have an indirect role by depositing feces, and thus seeds, in permanently moist humus well above the forest floor, thereby creating an aerial seed bank. This is because successful seed germination and subsequent seedling growth in the wild require permanently moist humus in holes, pockets and cracks of tree trunks, stem bark and branch axils (Laman 1995, Swagel et al. 1997, Athreya 1999, Doyle 2000) high in the canopy.

We suggest that the role of howler monkeys in *F. lundellii* regeneration is important. The heavy use of fruit resources within tropical forests is believed to be a major factor in tropical vertebrate diversity (Willson 1993). Given year-round fruit production (Vázquez-Yanes et al. 1996), the howler monkey and the strangler fig probably rely heavily on each other. Based on the sensitivity of seed survival to the concentration of acid or peroxide (current results; Lisci and Pacini 1994), we posit that seed passage through the gut must be comparatively rapid. We conclude that assisted regeneration, or planting on cleared land, of tropical forests is feasible for *F. lundellii*, because seed germination can be promoted and seedlings can be grown successfully ex situ (data not shown), thereby bypassing the epiphytic stage of canopy development. Nevertheless, subsequent in situ regeneration of *F. lundellii* in such afforested areas is likely to be dependent on the frugivory of animal communities, as well as suitable host trees.

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**References**


**Figure 3.** Germination response (normal, %, angular-transformed scale) of *Ficus lundellii* seeds to an 8-h photoperiod or darkness in combination with potassium nitrate (KNO₃) (continuous, 0.1%), or gibberellic acid (GA₃) (pre-applied, 2000 ppm) at an alternating diurnal (16/8 h) temperature of 20/30 °C (solid histograms) or a constant temperature of 30 °C (open histograms) after 14 (A) or 28 days (B). Vertical bars on each histogram represent means ± SE. The least significant difference (lsd) (P = 0.05) is also shown.


