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Spore germination of eight homosporous ferns in a temperature gradient

S. Juárez-Orozco [a], A. Orozco-Segovia [b,*], A. Mendoza-Ruiz [c], B. Pérez-García [c,**]

[a] Facultad de Ciencias, Universidad Nacional Autónoma de México, Departamento de Ecología y Recursos Naturales, Laboratorio de Procesos e Interacciones Ecológicas, Mexico
[b] Instituto de Ecología, Universidad Nacional Autónoma de México, Departamento de Ecología Funcional, Av. Universidad 3000, Apartado Postal 70-275, Ciudad Universitaria, C.P. 04510 México, D. F., Mexico
[c] Universidad Autónoma Metropolitana-Iztapalapa, Departamento de Biología, San Rafael Atlixco 186, C.P. 09340 México, D. F., Mexico

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ABSTRACT

Temperature determines the germination percentage and germination rate of non-dormant fern spores and is known to be a crucial factor for breaking or inducing dormancy in seeds. The objective of this study was to determine the effect of optimal, supraoptimal and suboptimal temperatures on spore germination of eight species of terrestrial homosporous ferns. Spores were incubated for one month in a temperature gradient from 15 to 35 °C in either light or dark. Thereafter, the spores were transferred to 25 °C and incubated in the light. The optimal temperature for germination varied among species, and germination inhibition and possible secondary dormancy were induced at both suboptimal and supraoptimal temperatures. At 35 or 30 °C, with light, spores of most species did not germinate or exhibited low germination percentages, owing to thermoinhibition or possible thermodormancy. In the dark, the spores did not germinate at any of the temperatures tested. After the spores were transferred to the light at 25 °C, a high percentage of spore germination was observed. Incubation in the dark at different temperatures also promoted high germination or dormancy. Temperatures that promoted germination might be related to the season that the different species germinate rather than their habitat, as a number of species within the same habitat had different optimum temperature requirements. Differences in temperature requirements may help to form a soil spore bank. It is necessary to determine whether secondary dormancy was induced by supraoptimal and suboptimal temperatures to further understand spore germination and colonisation in the field.

1. Introduction

Fern spores and angiosperm seeds share the two main groups of storage behaviour (short or extended longevity, Pérez-García et al., 1994) and requirements for germination, although the presence of dormancy has been rarely documented for fern spores (McLetchie, 1999). Light and temperature are the main environmental factors that regulate germination in both groups of plants (Baskin and Baskin, 2001; Raghavan, 1989), and in seeds these factors have been identified as environmental cues that largely determine germination timing in seasonal and disturbed environments (Vázquez-Yanes and Orozco-Segovia, 1994; Pérez-García et al., 2007).

Temperature is also one of the most important factors that limit the latitudinal and elevational distribution of plants (Thompson, 1970a, b; 1973, 1975). However, few studies have investigated fern spore germination in temperature gradients. Some studies have focused on several fern species growing in the same plant community, such as five Cyatheaceae species growing in a montane cloud forest in Puebla, Mexico (Pérez-García and Riba, 1982) and eight species from a tropical semideciduous mesophytic forest in Sao Paulo, Brazil (Ranal, 1999). Other studies have focused on individual species, including *Cheilanthes feei* T. Moore (Pteridaceae) growing in limestone crevices in areas ranging from Southwestern Canada to Central Mexico, *Rumohra adiantiformis* (Forst.) Ching (Dryopteridaceae) from the Peruvian Andes (Brum and Randi, 2002) and *Polypodium lepidopteris* (Langsd. & Fisch.) Kunze (Polypodiaceae) from the Brazilian Caatinga (Viviani and Randi, 2008). Despite all these studies differing in the number of temperatures included in the gradient and in the interval between temperatures, it was concluded that there is a wide variation in temperature requirements between and within each species, which may be related to the species distribution.

Similar to seeds, the distribution of non-dormant spore germination in a temperature gradient reveals the temperature interval for germination and the optimal, suboptimal and supraoptimal temperatures for germination. For example, the optimal condition is the temperature(s) at which the germination percentage is high and germination occurs the fastest. Germination percentage and germination rate are reduced as the temperature moves away from the optimal condition toward suboptimal and supraoptimal temperatures. In dormant seeds, temperature can end primary dormancy, and in dormant and non-dormant seeds,

* Corresponding author. Tel.: +52 55 56229008; fax: +52 55 56228995.
** Corresponding author. Tel./fax: +52 55 50046458.
E-mail addresses: alma@ecologia.unam.mx (A. Orozco-Segovia), bpg@xanum.uam.mx (B. Pérez-García).

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temperature can promote and end secondary dormancy (Karssen, 1980–1981; Probert, 2000). In seeds, thermoinhibition and thermodormancy at supraoptimal temperatures have received special attention. Thermoinhibition is removed when seeds are transferred to favourable temperatures, and thermodormancy requires specific pretreatments, such as ethylene application, before germination can occur at adequate temperatures (Hills and van Staden, 2003). However, germination inhibition (conditional dormancy) and the enforcement of secondary dormancy occur at both suboptimal and supraoptimal temperature intervals. For example, for winter annual species, the high temperatures in summer release seeds from secondary dormancy, but low winter temperatures reinforce dormancy. As a result, secondary dormancy cycles occur in nature and are related to the life history of plants (Karssen, 1980–1981; Baskin and Baskin, 1985). Different mechanisms such as ABA exposure (Leymarie et al., 2008) and alterations in the permeability of the cellular membrane and respiratory metabolism are involved in promoting primary and secondary dormancy and in inhibiting germination at suboptimal and supraoptimal temperatures (Hilhorst, 1998; Hills and van Staden, 2003; Corbineau et al., 2007).

According to Vleeshouwers et al. (1995), primary dormancy differs physiologically from secondary dormancy. The same factors can break or induce both dormancy types. For example, low temperatures break primary dormancy in many species (Baskin and Baskin, 2001), but may induce secondary dormancy in species such as *Actinotus leucocephalus* (Apiaceae) and *Tersonia cyathiflora* (Gyrostemonaceae) which then require warm temperatures to break the secondary dormancy (Baker et al., 2005). In a study focused on the development of spore sensitivity to short light exposure during germination in a temperature gradient, the inhibitory effect of supraoptimal temperatures on the germination of *Onoclea sensibilis* L. (Woodsiaceae) spores from Michigan, USA, was also described (Towill, 1978).

To better understand the relationships between temperature and spore germination biology, and identify possible convergences between spores and seeds in the germination processes, we tested the effect of temperature on spore germination, germination inhibition and secondary dormancy in eight homosporous ferns. In this study, spores were incubated in a temperature gradient from 15 to 35 °C with either a 12/12 h light/dark photoperiod (i.e. with light) or in the dark. Following these treatments, ungerminated spores were transferred to 25 °C with light, and germination was analysed. The species used in this study were: *Argyrochosma formosa* (Liebm.) Windham (Pteridaceae), *Blechnum glandulosum* Willd. (Blechnaceae), *Dennstaedtia globulifera* (Poir) Hieron (Dennstaedtiaceae), *Hemionitis subcordata* (D. C. Eaton *ex* Davenp.) Mickel (Pteridaceae), *Plagiogyria pectinata* (Liebm.) Lellinger (Plagiogyriaceae), *Polystichum mickelii* A. R. Sm. (Dryopteridaceae), *Thelypteris glandulosa* (Desv.) Proctor and *Thelypteris serrata* (Cav.) Alston (Thelypteridaceae).

2. Materials and methods

2.1. Spore collection

Spores from *A. formosa*, *Blechnum appendiculatum*, *D. globulifera*, *H. subcordata*, *P. pectinata*, *P. mickelii*, *T. glandulosa* and *T. serrata* were collected in several locations and from different vegetation types in Mexico (Table 1). All of these species are terrestrial. Spores were collected from more than five individuals from mature pinnae. Pinnae were kept in paper bags and dried for 3 days at 25 ± 2 °C to favour the sporangia opening and subsequent spore release. The spores were separated from pinnae and sporangia fragments using a sieve with a mesh size of 0.074 μm.

2.2. Spore germination

Spores from the eight species were sown in sterile 5 cm diameter Petri dishes on 1% agar with five replicates per treatment. All of the dishes contained approximately 150 spores per cm^2. The Petri dishes were kept individually inside plastic bags to avoid dehydration. The dishes were placed in growth chambers under a 12 h photoperiod or in darkness (Lab-Line 844, Lab-Line Instruments, Inc., Melrose Park, IL, USA) at 15, 20, 25, 30 or 35 °C. For the light treatment, light was provided by white fluorescent (Sylvania, 20 W) and incandescent (Solar, 25 W) lamps. The photon flux density (PFD) in the chambers was 33 μmol m^{-2} s^{-1} (400–700 nm) as measured with a spectrophotometer (Li 185 B, LICOR Inc., Lincon, NE, USA). For the spores incubated in the dark, the plates were wrapped with a double layer of aluminium foil. Germination in the two light treatments was measured every 7 days for 4 weeks and germination percentage was calculated based on the total number of spores present per cm^2. After this time, the Petri dishes were transferred to 25 °C with light (12 h photoperiod). Spores were observed under a stereoscopic microscope (Stereo Star-Zoom American Optical, Scientific Instruments, USA). Germination was defined as the protrusion of the rhizoid and/or the first prothallial cell through the spore coat.

Table 1
Habitat reported by Mickel and Smith (2004) and Mendoza-Ruiz and Pérez-García (2009) and localities where the spores of the eight terrestrial fern species were collected.

Species	Habitat	Locality	Collection site	Voucher
Argyrochosma formosa (Liebm.) Windham	Dry, rocks, wooded slopes, limestone slopes, steep rocky, ravines and thorn scrub, pine forests, 1250–2700 m a.s.l.	Oaxaca, 2. 4 km after Santos Reyes, toward Juquila	In open areas of a pine-oak forest, 1752 m a.s.l.	A. Mendoza R. et al. −585
Blechnum appendiculatum Willd.	Along roadsides, moist slopes and stream banks, in montane rain forest, pine-oak forests, cloud forest and tropical rain forest, 400–2550 m a.s.l.	Puebla, 4 km after Patoltecoya, toward Tulancingo	In open areas and along roadsides in a pine-oak forest, 1320 m a.s.l.	B. Pérez-García et al. −1183
Dennstaedtia globulifera (Poir) Hieron	Wet montane forest, 600–2100 m a.s.l.	Puebla, 4 km after Patoltecoya, toward Tulancingo	Undisturbed pine-oak forest, 1752 m a.s.l.	B. Pérez-García et al. −1180
Hemionitis subcordata (D. C. Eaton *ex* Davenp.) Mickel	Shaded rocky banks in woods or by roads, moist rocky slopes, tropical deciduous, sub-deciduous forests, rarely in pine-oak forests, 200–1400 m a.s.l.	Jalisco, 1.3 km after Puente los Horcones, 11 km after Boca de Tomatlán Howard Tuito	Undisturbed pine-oak forest, 466 m a.s.l.	A. Mendoza R. et al. −765
Plagiogyria pectinata (Liebm.) Lellinger	Along rivers and streams, in moist and shady areas of pine forest, evergreen cloud forests, tropical rain forests, 1750–3250 m a.s.l.	Durango, 4 km after La Ermita, toward Concordia	Undisturbed pine-oak forest, 2568 m a.s.l.	A. Mendoza R. et al. −728
Polystichum mickelii A. R. Sm.	Wet forest, slopes and sides of the road in disturbed cloud forests, 450–2500 m a.s.l.	Veracruz, ~1 km after Santa Rita, toward Misantla	In a ravine in disturbed montane cloud forest, 1410 m a.s.l.	A. Mendoza R. et al. −715
Thelypteris glandulosa (Desv.) Proctor	Lowland rain forest, along stream rivers, 300–850 m a.s.l.	Nayarit, 7 km after the way toward Tepic	In open areas of a montane cloud forest, 830 m a.s.l.	A. Mendoza R. et al. −756
Thelypteris serrata (Cav.) Alston	Along rivers, streams swamps, and wet road side banks and swamps, sometimes in standing water, tropical rain forest and sub-deciduous tropical forest, 0–550 m a.s.l.	Nayarit, 10.4 km after Cuarenteño toward El Cora	In an open area of semi-evergreen seasonal forest, 222 m a.s.l.	A. Mendoza R. et al. −758

2.3. Statistical analyses

Intraspecific final spore germination was analysed using one way ANOVA and Tukey's test. For the statistical analysis applied to the spores that were initially incubated in the dark and after a month transferred to 25 °C and light, the percentage of germination for spores that were incubated continuously at 25 °C with light was used as the control.

3. Results

3.1. Germination in the light in a temperature gradient, followed by transfer to 25 °C

In all eight species there was a significant difference ($P < 0.0001$) in spore germination across the five temperature treatments in the light (Fig. 1). The optimal temperature for spore germination (where germination was significantly faster, $P < 0.05$) was 25 °C for *D. globulifera*, *P. mickelii*, *B. appendiculatum* and *T. glandulosa*; 20 °C for *P. pectinata*; and 25 and 30 °C for *A. formosa*, *H. subcordata* and *T. serrata*. When incubated in the dark, spores did not germinate for any of the species or at any of the temperatures tested. Times to reach maximal germination in each temperature are not shown.

A. formosa, *T. glandulosa* and *T. serrata* spores germinated between 15 and 30 °C (Fig. 1A–C). In the two first species germination was significantly less at 20 and 15 °C, while spore germination for *T. serrata* was only reduced significantly at 15 °C (Fig. 1C). In these three species, germination was completely inhibited at 35 °C, but this inhibition was alleviated when the spores were transferred to 25 °C. *P. pectinata* and *P. mickelii* spores germinated in a narrower interval of temperatures (15–25 °C, Fig. 1D, E). However, in *P. mickelii*, germination of most of the spores was inhibited at 15 °C. In both species, at

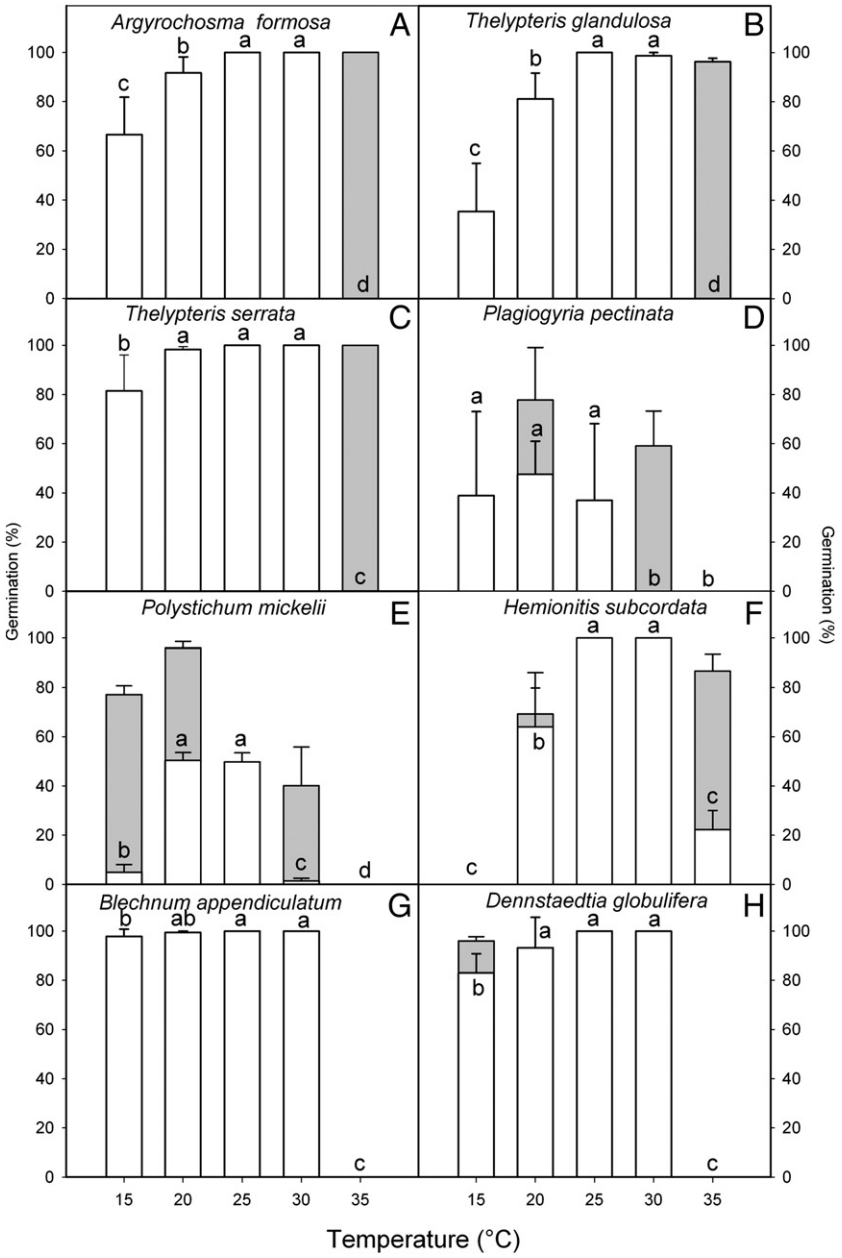

Fig. 1. Germination percentage of spores incubated for one month with light (12 h photoperiod) in a temperature gradient (white colour). The grey colour represents the proportion of spores that only germinated when transferred to 25 °C, with the same photoperiod. For each species, letters indicate significant differences in spore germination at $\alpha = 0.05$, before transfer to 25 °C. Comparisons were performed using ANOVA and the post hoc Tukey's test. SD is indicated.

20 °C, and at 30 °C in *P. mickelii*, inhibition occurred in a small proportion of the spores. Inhibition was removed at 25 °C, whereas spores incubated at 35 °C failed to germinate even when transferred to 25 °C. In both these species, the spores that were initially incubated at 20 °C and then transferred to 25 °C underwent induction of germination resulting in the percentage of germination being higher than with any other treatment (Fig. 1D, E). *H. subcordata* was a unique species that did not germinate at 15 °C (Fig. 1F). In contrast with *P. mickelii*, *H. subcordata* spores germinated between 20 and 35 °C. Spore germination at 35 °C was low, but germination inhibition was lost after transfer to 25 °C. A high percentage of *B. appendiculatum* and *D. globulifera* spores germinated between 15 and 30 °C. Similar to the three previously mentioned species, these two species did not germinate at 35 °C, even after transfer to 25 °C (Fig. 1G, H).

3.2. Germination in darkness in a temperature gradient, followed by transfer to 25 °C and light

When incubated in the dark, no spore germination was observed with any of the species tested at the different temperatures. After transfer to 25 °C (12 h photoperiod), with the exception of *T. glandulosa* ($P = 0.288$), the other seven species showed a significant difference ($P < 0.0001$) between treatments (Fig. 2). Germination of *A. formosa*, *T. glandulosa*, *T. serrata* and *B. appendiculatum* spores was high for all of the temperatures tested (15, 20, 25, 30 and 35 °C) (Fig. 2A–C, G). *P. pectinata* and *D. globulifera* and spores did not germinate at 15 and 35 °C, and 35 °C, respectively (Fig. 2D, H). *P. pectinata* spores incubated at 20, 25 or 30 °C (Fig. 2D) had a higher percentage of germination than spores that were incubated and germinated at the light at the same

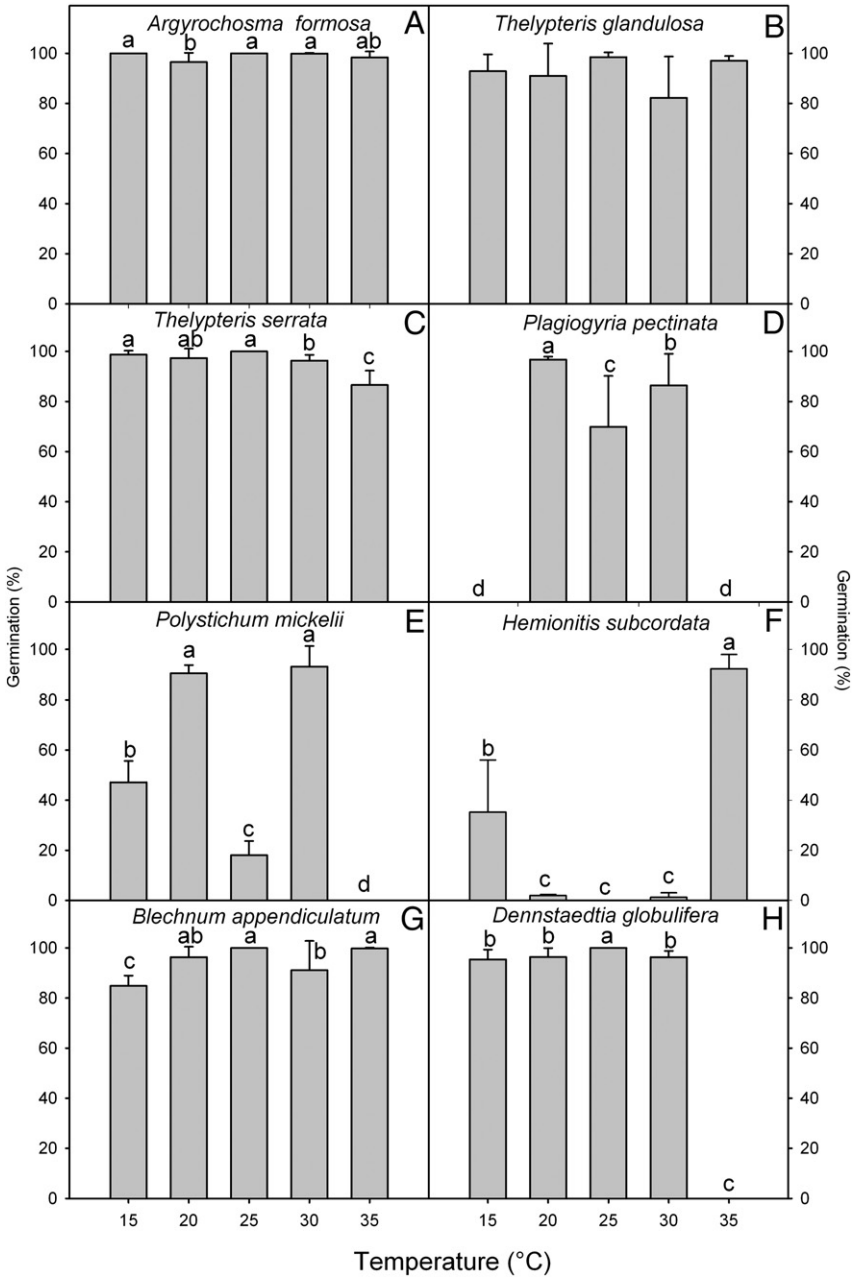

Fig. 2. Germination percentage of spores incubated for one month in darkness in a temperature gradient and then transferred to 25 °C. Spores did not germinate in the dark, thus, only germination with light is shown. Letters indicate significant differences in spore germination at $\alpha = 0.05$, after transfer to 25 °C. For each species, the control treatment was the germination of spores incubated with light at 25 °C. Comparisons were performed using ANOVA and the post hoc Tukey's test. SD is indicated.

temperatures (Fig. 1D). The spores of *P. mickelii* and *H. subcordata* died when they were incubated in darkness at 25 °C, and at 20–30 °C, respectively (Fig. 2E, F). At these temperatures spores were covered by fungi and bacteria and showed plasmolysis. The spores of *H. subcordata* incubated initially in darkness at 15 °C and 35 °C (Fig. 2F) showed similar germination percentages to those observed when the spores were incubated at these temperatures with light and then transferred to 25 °C with light (Fig. 1F). In *P. mickelii*, spores incubated in darkness at 20 °C and 30 °C showed high levels of germination when transferred to 25 °C with light (Fig. 2E). Spores initially incubated in the dark at 30 °C germinated better than spores incubated with light at this temperature (Fig. 1E). No spores of this species incubated at 35 °C germinated.

4. Discussion

All of the studied species were positively photoblastic, as are most fern species (Raghavan, 1989), and germination in darkness was not stimulated by supraoptimal temperatures as occurs in some other fern species (Miller, 1968; Towill, 1978). Thus, germination of the studied species would be limited to the seeds found in the first few mm of soil. Germination inhibition and the possible induction of secondary dormancy occurred at both suboptimal and supraoptimal temperatures. Spores of most of the species included in this study did not germinate at 35 °C in the light, except for *H. subcordata* (21%) and only two species (*P. pectinata and P. mickelii*) did not germinate at 30 °C with light. Thus, these species may be considered to be thermophilous (Orozco-Segovia et al., 1996). Lack of germination in several species at high and low temperatures was caused by thermoinhibition or possible thermodormancy (secondary dormancy). The assumption of the development of secondary dormancy in the spores of several species, including *B. appendiculatum* at 35 °C and *H. subcordata* and *P. pectinata* at 15 °C, was based on the observation that no germination was more related to the initial light condition during incubation (light or darkness) than to the effect of initial incubation temperature by itself. Additionally, the spores that might acquire secondary dormancy, unlike dead spores, maintained its morphology and structure along all the treatments and the development of fungi in the dishes was scant. Germination does not occur at high temperatures (>30 °C) in the five Cyatheaceae species studied by Pérez-García and Riba (1982) and in *P. lepidopteris* (Langsd. & Fisch.) Kunze (Polypodiaceae; Viviani and Randi, 2008). However, no germination, in these species, at some of the temperatures included in these studies just was reported, but this fact was not related to any physiological process (thermoinhibition, thermodormancy or spore death). In contrast, eight species studied by Ranal (1999) including five Polypodiaceae and two Adiantaceae, and *R. adiantiformis* (Forst.) Ching (Dryopteridaceae) (Brum and Randi, 2002) are thermophilous (these have high germination at 29 and 29.8 °C).

Under a 12 h photoperiod, at the temperatures tested in this study, most of the species exhibited thermoinhibition, except for *B. appendiculatum* spores, which showed only apparent thermodormancy at 35 °C. In *A. formosa*, *T. glandulosa* and *T. serrata*, full thermoinhibition was observed at 35 °C, close to the temperature where germination of *O. sensibilis* is fully thermoinhibited (i.e. 36 °C, Towill, 1978). The small proportion of the *H. subcordata* spores that germinate at 35 °C may increase the probability of colonisation in harsh environments, such as rocky slopes and roads where this species is also established (Mickel and Smith, 2004; Mendoza-Ruiz and Pérez-García, 2009).

In *P. pectinata* and *P. mickelii*, full thermoinhibition occurred at 30 °C, whereas at lower temperatures, there was partial germination inhibition (20 °C and 15/20 °C, respectively). Germination of *O. sensibilis* (growing in a temperate area) was not studied at 15 °C, but 30 °C is the optimal temperature for germination and at 20 °C, spore germination is partially inhibited. *P. pectinata* and *P. mickelii* commonly grow at high elevations in moist and shaded environments, such as the montane cloud forest (Mickel and Smith, 2004; Mendoza-Ruiz and Pérez-García, 2009). However, other species included in this study grow at the same elevations and habitats but germinate at higher or lower temperatures. Low germination at 15 °C has been observed in other species, such as *Trichipteris scabriuscula* and *Nephelea mexicana* (Cyatheaceae), although the occurrence of thermoinhibition was not tested in these species (Pérez-García and Riba, 1982).

Thermoinhibition at supraoptimal or suboptimal temperatures might prevent spore germination under adverse environmental conditions, as occurs in seeds. However, in most species, sensitivity to temperature was modified after spores were incubated in darkness, which probably may occur in the soil spore bank. Prolonged exposure to darkness may modify the ability of spores to germinate in diverse habitats and microhabitats. Changes in sensitivity to temperature by incubation in the dark have been previously reported for *O. sensibilis* (Towill, 1978).

In contrast to germination inhibition at supraoptimal and suboptimal temperatures, secondary dormancy is not overcome after spores or seeds are transferred to a suitable temperature for germination (Hills and van Staden, 2003; Murdoch and Ellis, 2000). Secondary dormancy is acquired during prolonged incubation under adverse conditions, indicating that a deeper rest state is more difficult to overcome than spores just inhibited for germination (Gabriel y Galán and Prada, 2010). In secondary dormancy, low and high temperatures (as in dormancy cycling) can induce this state and release seeds from it (Vleeshouwers et al., 1995). This sensitivity to temperature has been related to the season of species seed germination; thus, winter or summer temperatures can break dormancy, allowing germination to occur in spring or autumn (Baskin and Baskin, 1985). The soil ethylene/CO_2 ratio, which is affected by different processes, such as litter decomposition and respiration of soil organisms, might also be able to release spores from dormancy (Edwards, 1977; Hargurdeep et al., 1986; Hilhorst, 2007). In seeds, these gases present in the soil atmosphere and other chemical stimuli present in the soil, such as nitrite, nitrate and azide, which may have cyclic variations during the year, can also break secondary dormancy (Murdoch and Ellis, 2000). In this study, secondary dormancy was probably induced in several species after incubation at suboptimal and supraoptimal temperatures. In *B. appendiculatum*, *D. globulifera*, *P. pectinata* and *P. mickelii* secondary dormancy was possibly induced by high temperatures (35 °C), although further studies would need to be carried out to confirm this. Species of these taxa are able to grow in shaded areas of the tropical rain forest and the montane cloud forest (Mickel and Smith, 2004). In contrast, in *H. subcordata*, this dormancy was induced at a low temperature (15 °C), confirming the preference of this species for warm habitats, such as undisturbed areas in semi-deciduous and deciduous forests and rarely in pine-oak forests (Mickel and Smith, 2004). Thus, the temperatures that might induce secondary dormancy in these species may be more related to the season of spore germination, and the safe site for germination and gametophyte development, than to their habitat itself. In soil spore bank samples, *Athyrium pycnocarpon* and *Athyrium thelypterioides* (Woodsiaceae) were found to undergo secondary dormancy (Hamilton, 1988). The spores of the bryophyte *Sphaerocarpos texanus* (Sphaerocarpaceae) experience a dormancy/non-dormancy spore cycle, which is similar to the dormancy cycle in winter annuals (McLetchie, 1999). In *B. appendiculatum*, *P. pectinata* and *H. subcordata*, induction of secondary dormancy by the combination of light and temperature was confirmed by the germination of spores that were initially incubated in darkness at 35 °C (for *B. appendiculatum*) and 15 °C for *H. subcordata* and *P. pectinata* (when this last species was incubated always with light) after transferring the dishes to 25 °C with light. In *D. globulifera* and *P. mickelii*, the apparent dormant state that developed at 35 °C was concluded by the spore health after two months of incubation. Secondary dormancy was not present in the other three species. Interestingly, *H. subcordata* spores died when incubated in darkness at 20, 25 or 30 °C. This was apparent from the spore plasmolysis and abundant fungi development on the spores. This species had a high

percentage of germination when incubated with light at these same temperatures. Spore death also occurred in *P. mickelii* incubated in darkness at 25 °C.

In some species, spore transfer from darkness at different temperatures, to 25 °C with light resulted in a higher percentage of germination than that found after transferring the spores incubated at different temperatures for a 12 h photoperiod to 25 °C. As described previously by Hills and van Staden (2003) in seeds and Towill (1978) in fern spores, thermoinduction of germination occurred in *P. mickelii* spores upon transfer from 30 °C (darkness) to 25 °C (12 h photoperiod).

Although thermoinhibition and thermoinduction usually occur at supraoptimal temperatures (Vidaver and Hsiao, 1975), inhibition and probably dormancy at suboptimal temperatures are functionally similar and were identified in the studied species. In seeds, at the cellular, biochemical and molecular levels, inhibition of germination and seed dormancy can be caused by different factors and biochemical pathways, as seen in primary and secondary dormancy (Bewley and Black, 1994; Vleeshouwers et al., 1995; Murdoch and Ellis, 2000; Corbineau et al., 2007). Thus, from an ecological point of view, secondary dormancy and germination inhibition at suboptimal and supraoptimal temperatures have similar consequences in seeds and spores. The dormancy and inhibition of germination can ensure spore germination in suitable period of the year and microenvironment, thereby spreading germination in time and space. Similarly, darkness can improve seed germination and plantlet establishment when adequate conditions are present. Studying spore germination in a temperature gradient can be useful for understanding species distribution. Dormancy induction can be related to dormancy cycling in the soil spore bank, which regulates seasonal germination in the fern habitats. It is necessary to further explore the induction of secondary dormancy by supraoptimal and suboptimal temperatures to understand spore germination and colonisation in the field.

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References

Baker, K.S., Steadman, K.J., Plummer, J.A., Merritt, D.J., Dixon, K.W., 2005. The changing window of conditions that promotes germination of two fire ephemerals, *Actinotus leucocephalus* (Apiaceae) and *Tersonia cyathiflora* (Gyrostemonaceae). Annals of Botany 96, 1225–1236.

Baskin, J.M., Baskin, C.C., 1985. The annual dormancy cycle in buried weed seeds: a continuum. BioScience 35, 492–498.

Baskin, C.C., Baskin, J.M., 2001. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA, USA.

Bewley, J.D., Black, M., 1994. Seeds: Physiology of Development and Germination. Plenum Press, New York, USA.

Brum, F.R., Randi, M.A., 2002. High irradiance and temperature inhibit the germination of spores of the fern *Rumohra adiantiformis* (Forst.) Ching (Dryopteridaceae). Revista Brasileira de Botânica 25, 391–396.

Corbineau, F., Vinel De Leymarie, J., Côme, D., 2007. Thermoinhibition nucleotide triphosphate synthesis and energy metabolism in primary dormant and thermodormant oat seeds. In: Adkins, S.W., Ashmore, S., Navie, S.C. (Eds.), Seeds: Biology, Development and Ecology. CABI International, Wallingford, Oxon, UK, pp. 254–261.

Edwards, M.E., 1977. Carbon dioxide and ethylene control of spore germination in *Onoclea sensibilis* L. Plant Physiology 59, 756–758.

Gabriel y Galán, J.M., Prada, C., 2010. Pteridophyte spores viability. In: Kumar, A., Hernández, H. (Eds.), Working with Ferns: Issues and Applications. Springer, New York, USA, pp. 193–206.

Hamilton, R.G., 1988. The significance of spore banks in natural populations of *Athyrium pycnocarpon* and *A. thelypterioides*. American Fern Journal 78, 96–104.

Hargurdeep, S.S., Consolacion, E.D., Bassi, P.K., Spencer, M.S., 1986. Requirement for ethylene synthesis and action during relief of thermoinhibition of lettuce seed germination by combinations of gibberellic acid, kinetin, and carbon dioxide. Plant Physiology 81, 950–953.

Hilhorst, H.W.M., 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. Seed Science Research 8, 77–90.

Hilhorst, H.W.M., 2007. Definitions and hypothesis of seed dormancy. In: Bradford, K., Nonogaki, H. (Eds.), Seed Development, Dormancy and Germination. Blackwell Publishing, Oregon, Corvallis, USA, pp. 50–71.

Hills, P.N., van Staden, J., 2003. Thermoinhibition of seed germination. South African Journal of Botany 69, 455–561.

Karssen, C.M., 1980–1981. Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds. Israel Journal of Botany 29, 45–64.

Leymarie, J., Robayo-Romero, M.E., Gendreau, E., Benech-Arnold, R.L., Corbineau, F., 2008. Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) Seeds. Plant & Cell Physiology 49, 1830–1838.

McLetchie, N.D., 1999. Dormancy/nondormancy cycles in spores of the liverwort. *Sphaerocarpos texanus*. The Bryologist 102, 15–21.

Mendoza-Ruiz, A., Pérez-García, B., 2009. Helechos y licopodios de México, vol. I. México Comisión Nacional Para el Conocimiento y Uso de la Biodiversidad y Universidad Autónoma Metropolitana, México, D. F.

Mickel, J.T., Smith, A.R., 2004. The pteridophytes of Mexico. Memoirs of the New York Botanical Garden 88, 1–1054.

Miller, J.H., 1968. Fern gametophytes as experimental material. The Botanical Review 34, 361–440.

Murdoch, A.J., Ellis, R.H., 2000. Dormancy viability and longevity. In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities. CAB International, Wallingford, Oxon, UK, pp. 183–214.

Orozco-Segovia, A., González-Zertuche, L., Mendoza, A., Orozco, S., 1996. A mathematical model that uses Gaussian distribution to analyze the germination of *Manfreda brachystachya* (Agavaceae) in a thermogradient. Physiologia Plantarum 98, 431–438.

Pérez-García, B., Riba, R., 1982. Germinación de esporas de Cyatheaceae bajo diversas temperaturas. Biotropica 14, 281–287.

Pérez-García, B., Orozco-Segovia, A., Riba, R., 1994. The effects of white fluorescent light, far-red light, darkness, and moisture on spore germination of *Lygodium heterodoxum* (Schizaeaceae). American Journal of Botany 81, 1367–1369.

Pérez-García, B., Mendoza-Ruiz, A., Sánchez-Coronado, M.E., Orozco-Segovia, A., 2007. Effects of light and temperature on spore germination of spores of four tropical ferns species. Acta Oecologica 32, 172–179.

Probert, R.J., 2000. The role of temperature in the regulation of seed dormancy and germination. In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities. CAB International, Wallingford, Oxon, UK, pp. 261–292.

Raghavan, V., 1989. Physiology of spore germination. In: Barlow, P.W., Bray, D., Green, P.B., Slack, J.M.W. (Eds.), Development and Cell Biology Series. University Press, Cambridge, MA, USA, pp. 27–52.

Ranal, M.A., 1999. Effects of temperature on spore germination in some ferns species from semideciduous mesophytic forest. American Fern Journal 8, 149–158.

Thompson, P.A., 1970a. Characterization of the germination to temperature of species and ecotypes. Nature 225, 827–831.

Thompson, P.A., 1970b. Germination of species of Caryophyllaceae in relation to their geographical distribution in Europe. Annals of Botany 34, 427–449.

Thompson, P.A., 1973. Geographical adaptation of seeds. In: Heydecker, W. (Ed.), Seed Ecology. Butterworths, London, UK, pp. 31–58.

Thompson, P.A., 1975. Characterization of the germination responses of *Silene dioica* (L.) Clairv. Populations from Europe. Annals of Botany 41, 1363–1365.

Towill, L.R., 1978. Temperature and photocontrol of *Onoclea* spore germination. Plant Physiology 6, 116–119.

Vázquez-Yanes, C., Orozco-Segovia, A., 1994. Signals for seeds to sense and respond to gaps. In: Caldwell, M., Pearcy, R. (Eds.), Exploitation of Environmental Heterogeneity by Plants: Ecophysiological Processes Above and Below Ground. Academic Press, New York, pp. 209–236.

Vidaver, W.A., Hsiao, I., 1975. Secondary dormancy in light-sensitive lettuce seeds incubated anaerobically or at elevated temperature. Canadian Journal of Botany 53, 2557–2560.

Viviani, D., Randi, M.A., 2008. Effects of pH, temperature and light intensity on spore germination and growth analysis of young sporophytes of *Polypodium lepidopteris* (Pteridophyta, Polypodiaceae). Rodriguésia 59, 751–760.

Vleeshouwers, L.M., Bouwmeester, H.J., Karssen, C.M., 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. Journal of Ecology 83, 1031–1037.