

Response of spores and young gametophytes of *Cyathea delgadii* Sternb. (Cyatheaceae) and *Blechnum brasiliense* Desv. (Blechnaceae) to different light levels

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RESUMO – (Resposta de esporos e gametófitos jovens de *Cyathea delgadii* Sternb. (Cyatheaceae) e *Blechnum brasiliense* Desv. (Blechnaceae) aos diferentes níveis de luz). Luz é um dos fatores limitantes para o estabelecimento das pteridófitas, pois controla a germinação de esporos fotossensíveis. O objetivo deste trabalho foi estudar o efeito de níveis de luz na germinação de esporos de duas pteridófitas ornamentais nativas da Floresta Atlântica, sob condições ambientais. *Cyathea delgadii* é uma samambaia arbórea e *Blechnum brasiliense*, subarborescente. O efeito dos níveis de luz foi analisado em abril e em junho/2003, em Florianópolis, SC, Brasil. Esporos esterilizados foram inoculados em “erlenmeyers” contendo meio de cultura composto por macronutrientes, ferro e benomil a 0,01%. Os frascos foram mantidos em caixas de 50 cm³ cobertas por tela “sombrite” que forneceu 5, 22, 42 e 62% de luz natural. Irradiâncias e temperaturas foram medidas diariamente às 14 h no período de estudo. As maiores porcentagens de germinação foram observadas sob 5 e 22% de luz solar para ambas as espécies. A germinação de esporos de *Cyathea delgadii* sob 22% de luz atingiu 76% e o tempo médio de germinação foi de 19,7 dias; a 5% de luz a germinação atingiu 83,5% e o tempo médio de germinação foi de 20,16 dias. A germinação de *Blechnum brasiliense* sob 22% de luz atingiu 76% e o tempo médio de germinação foi de 9,06 dias; a 5% de luz a germinação atingiu 84% e o tempo médio de germinação foi de 13,18 dias. Os maiores níveis de luz inibiram parcialmente a germinação e os gametófitos morreram no decorrer do período de teste.

Palavras-chave: esporos, fotoinibição, germinação, níveis de luz, pteridófitas

ABSTRACT – (Response of spores and young gametophytes of *Cyathea delgadii* Sternb. (Cyatheaceae) and *Blechnum brasiliense* Desv. (Blechnaceae) to different light levels). Light is a limiting factor for fern establishment because it controls germination of light sensitive spores. The aim of this work was to study the effect of light levels on spore germination in two ornamental ferns native to the Atlantic forest, under natural conditions. *Cyathea delgadii* is a tree fern and *Blechnum brasiliense*, a subarborescent fern. The effect of light levels was analyzed in April and July/2003, in Florianópolis, Santa Catarina state, Brazil. Sterilized spores were sown in Erlenmeyer flasks containing mineral culture medium with macronutrients, iron, and benomyl 0.01%. The Erlenmeyer flasks were kept in 50 cm³ boxes covered with black shade netting, which gave 5, 22, 42, and 62% natural light. Irradiance and temperature were scored daily at 14:00 h during the study period. Higher percentages of germination were observed at 5 and 22% light for both species. Germination of *Cyathea delgadii* spores at 22% light reached 76% and mean germination time was 19.7 days; at 5% light, germination reached 83.5% and mean germination time was 20.16 days. Germination of *Blechnum brasiliense* at 22% light reached 76% and mean germination time was 9.06 days; at 5% light, germination reached 84% and mean germination time was 13.18 days. The highest light levels inhibited spore germination and the gametophytes died during the test period.

Key words: ferns, germination, light levels, photoinhibition, spores

Introduction

Ferns are an important plant group of the Brazilian flora. According to Tryon (1970; 1972) and Tryon & Tryon (1982), southeastern Brazil (from Minas Gerais to Rio Grande do Sul) contains about 600 species. Sehnem (1977) analyzed the geographic distribution, ecology, and fern migration routes in southern Brazil; he observed a broad fern distribution with some cosmopolitan species, which also occurred on several continents. According to the author, in natural conditions

ferns basically require shade and high humidity for their development.

Some Brazilian ferns are used as ornamental plants and members of the Cyatheaceae and Dicksoniaceae have been indiscriminately exploited by commercialization of pots and substrate used in the production of ornamental plants (Fernandes 2000; Windisch 2002). For this reason, understanding fern ecophysiology is necessary for the development of methods that may assist in their conservation and management.

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Light has signaling functions for any given plant's development in the forest and is the energy source for CO₂ assimilation (Lüttge 1997). According to Chazdon & Fetcher (1984), 0.5 to 4% sunlight reaches the forest floor. The effect of light on the induction of fern spore germination has been extensively studied for many years. It has been known for a long time that light promotes spore germination through a low fluency response (LFR) mediated by the phytochrome system (Sugai & Furuya 1967; 1968; Miller 1968; Furuya 1983; 1985; Raghavan 1993). In spite of this, the international literature has very little information concerning the effects of light levels on spore germination. Inhibition of spore germination at high light levels was observed for spores of *Dicksonia sellowiana* Hook. (Dicksoniaceae) and *Rumohra adiantiformis* (G. Forst.) Ching (Dryopteridaceae) (Filippini *et al.* 1999; Brum & Randi 2002; Renner & Randi 2004).

This paper investigated the effect of light levels on germination and gametophyte survival of two ornamental fern species from the Atlantic Forest, under natural conditions. *Cyathea delgadii* Sternb. (Cyatheaceae) is a tree fern of gallery, montane, cloud, and rain forests (Tryon & Tryon 1982). *Blechnum brasiliense* Desv. (Blechnaceae) is a subarborescent fern, which also occurs in a wide variety of environments, including cloud forests, lowland rain forests, gallery forests, swamps and savannahs (Tryon & Tryon 1982).

Material and methods

Sporophylls of several *Cyathea delgadii* Sternb. and *Blechnum brasiliense* Desv. plants were harvested in February/2002 along a path in the São Francisco de Assis Natural City Park, situated in Blumenau, Santa Catarina state (26° 55' 15"S; 49° 05' 30"W). The study site is a 22.29-hectare natural Atlantic forest fragment. Sporophylls were air-dried in an oven at 30 °C for three days on filter paper in order to induce dehiscence. The spores were removed and separated from debris by filtering through lens paper, and stored in glass jars under refrigeration at 7 ± 1 °C.

Spores were surface-sterilized using a 10% (v/v) solution of commercial bleach (2% active chlorine) for a period of 20 min. after which they were filtered through sterile filter paper and washed several times with sterile distilled water. Spores were sown in Erlenmeyer flasks containing 20 mL of mineral solution as proposed by Mohr (1956), modified by Dyer (1979)

and supplemented with benomyl 0.01%. The flasks were plugged with two layers of autoclaved, transparent, commercial polypropylene film (7 x 7 cm) fixed with a rubber band. All procedures were carried out in a laminar hood.

For the study of the effects of light levels, four Erlenmeyer flasks per treatment (four replications) were inoculated with surface sterilized spores (10 mg) and kept in 50 cm³ boxes covered with black shade netting that provided 62, 42, 22, and 5% natural light. A maximum and minimum thermometer was placed inside the boxes. Minimum and maximum temperatures were recorded daily at 14:00 h. Irradiance inside the boxes was also recorded daily at 14:00 h. Irradiance levels were measured with a quantameter LICOR 250, using a PAR sensor (400 to 700 nm). The boxes were kept in an open area. The tests were carried out from 1-30 April 2003 for *Cyathea delgadii* and from 13 June to 1 July 2003 for *Blechnum brasiliense*, in Florianópolis, Santa Catarina state, Brazil. During the test period, light levels were measured in the São Francisco de Assis Natural City Park.

All tests were done in a completely randomized design. The percentage of germination was scored every three days for *Cyathea delgadii* and daily for *Blechnum brasiliense*. Each observation was based on one slide analyzed from each flask per treatment, and 100 spores or young gametophytes counted on each slide. Spores were considered germinated when a rhizoidal cell protrusion was present. Gametophytes were considered alive when green protallial cells were present and were considered dead when the protallial cells were discolored or light-brown. Mean germination time was calculated for each replication per treatment according to the equation: $\bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$, where t_i is the time in days from sowing to the last observation and n_i is the number of spores completing the germination process on day t_i (Labouriau 1983; Santana & Ranal 2004). The Kolmogorov-Smirnov test showed absence of normality for the germinability data, which were submitted to arcsine transformation. The Kolmogorov-Smirnov and Bartlett's tests (= 0.05) were also applied to the transformed data, before the analysis of variance. To compare the effect of light levels on mean germination time and on maximum germinability, pairwise comparisons were done using Tukey test at 0.05 significance (Santana & Ranal 2004). To compare the effects of light levels between germinability and survivor gametophytes of *Cyathea delgadii* and *Blechnum brasiliense* the Student *t* test was applied at the end of the test period.

Results

The data obtained for *Blechnum brasiliense* spore germination under different light levels showed that during the test period, which occurred from 13 June to 1 July (1 to 20 days of cultivation), minimum temperatures in Florianópolis ranged from 14 to 19 °C and maximum temperatures from 21 to 32 °C (Fig. 1A). Data obtained in the São Francisco de Assis Natural City Park showed that 0.9% natural sunlight reaches the floor of this Atlantic forest fragment. During the test period, irradiance at 14:00 h varied from 6 to 49 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (5% light); from 28 to 215 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (22% light); from 53 to 411 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (42% light) and finally, from 78 to 606 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (62% light) (Fig. 1B). Lower irradiance values were observed on the first and after the seventh day of *Blechnum brasiliense* spore cultivation, which were rainy days. Lower percentages of *Blechnum brasiliense* germination were observed at 62 and 42% and higher at 22 and 5% light (Tab. 1, Fig. 1C). The lowest mean germination time was found at 62% light (Tab. 1). Gametophyte survival began to decrease after 11 days of cultivation at 62% light during the test period (Fig. 2A).

Data for spore germination of *Cyathea delgadii* under different light levels were obtained in April/2003. During the test period, minimum daily temperatures in Florianópolis ranged from 9 to 23 °C, maximum daily temperatures ranged from 28 to 36 °C (Fig. 3A) and irradiance values at 14:00 h varied from 12 to 64 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (5% light); from 55 to 284 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (22% light); from 163 to 542 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (42% light); and from 241 to 800 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (62% light) (Fig. 3B). The third day of *Cyathea delgadii* cultivation was very cloudy and rainy, and had the lowest irradiance of the test period. Lower percentages of *Cyathea delgadii* germination were observed at 62 and 42% light and higher were observed at 22 and 5% light, which did not differ statistically (Tab. 1, Fig. 3C). The lowest mean germination time was found at 62% light (Tab. 1). After a few days of cultivation, the percentage of *Cyathea delgadii* gametophyte survival decreased at 62 and 42% light (Fig. 2B).

Discussion

The first observation of this study was germinability photoinhibition at higher light levels. There was wide daily alternation between minimum and maximum temperatures over the test period; some days were

very warm and some nights very cold. On some days, the difference between daytime and nocturnal temperatures was over 15 °C. On windy days, higher temperatures were observed inside the 5% light box and lower temperatures inside the 62% light box. This was probably a consequence of air circulation inside the boxes, considering that the box which received 62% sunlight was more ventilated due to the larger holes in the black shade netting. According to Haupt (1990; 1991; 1992) phytochrome-mediated spore germination in *Dryopteris filix-mas* L. and *D. paleacea* (Sw) C. Chr. (Dryopteridaceae) was inhibited by raising the temperature from 22 to 27 or 32 °C; the elevated

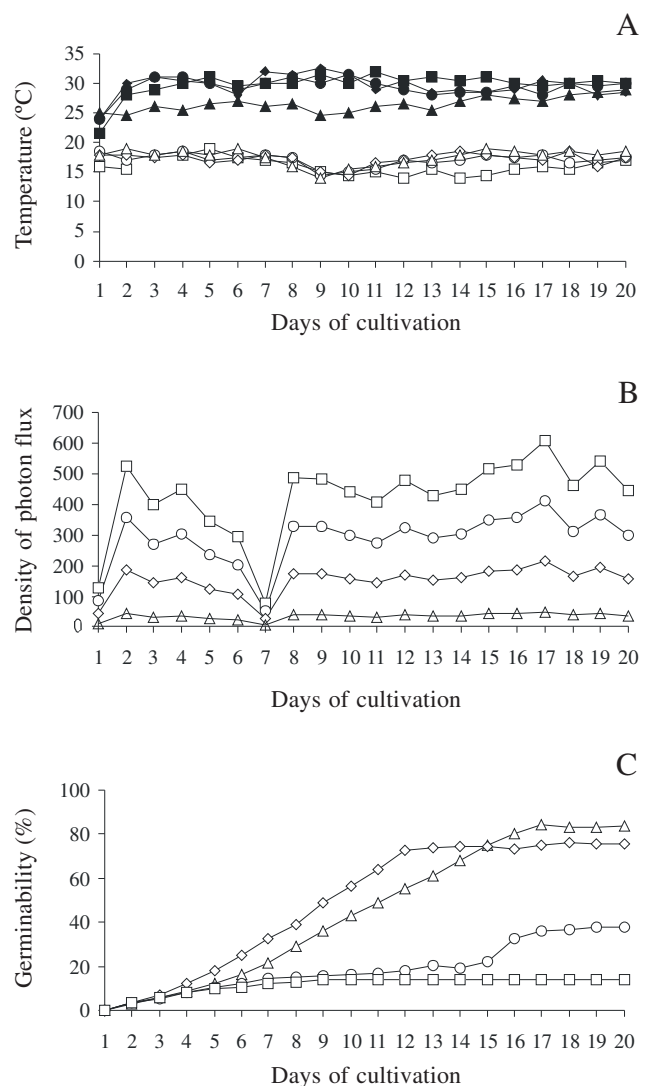


Figure 1. Minimum and maximum temperature (A) and irradiance ($\mu\text{mol m}^{-2}\text{s}^{-1}$) (B) measured at 14:00 h, inside boxes of black shade netting where spores were kept to provide different light levels from 13 of June to 01 of July (B); germinability of *Blechnum brasiliense* Desv. (C). Open symbols are minimum temperatures and dark symbols are maximum temperatures (\square = 62% light; \circ = 42% light; \diamond = 22% light; \triangle 5% light).

Table 1. Days to reach maximum germination, mean germination time, and final percentage of germination of *Blechnum brasiliense* and *Cyathea delgadii* Sternb. spores under different light levels. DMG = days to reach maximum germination; MGT = mean germination time; MG = maximum percentage of germination. Mean \pm standard deviation. χ^2 = statistic of Bartlett's test for variances; D_{max} = statistic of the Kolmogorov-Smirnov test for normality ($n = 16$, $\alpha = 0.05$, $\delta = 0.5$). Lines with different letters denote statistical differences (Tukey test). *After angular transformation.

Characteristics and statistical assumptions	Light level (%)			
	62	42	22	5
	<i>Blechnum brasiliense</i>			
DMG	9	20	18	17
MGT (days)	6.9 \pm 0.211a	13.92 \pm 0.30c	9.06 \pm 0.112b	13.18 \pm 0.05c
$\chi^2 = 6.512$				
$D_{max} = 0.165$				
MG (%)	13.5 \pm 0.96c	37.75 \pm 2.22b	76.00 \pm 4.24a	84.00 \pm 0.82a
$\chi^2 = 4.903$				
$*D_{max} = 0.156$				
	<i>Cyathea delgadii</i>			
DMG	12	21	27	30
MGT (days)	8.36 \pm 0.47a	14.68 \pm 0.41b	19.74 \pm 0.14c	20.16 \pm 0.23c
$\chi^2 = 3.960$				
$D_{max} = 0.171$				
MG (%)	23.0 \pm 6.68c	40.25 \pm 4.03b	76.00 \pm 3.46 a	83.50 \pm 3.87a
$\chi^2 = 1.701$				
$*D_{max} = 0.158$				

temperature was effective during the “coupling phase” when the far-red phytochrome (Pfr) initiates the processes leading to germination. The data presented here suggest that photoinhibition of spore germination observed at 62 and 42% light is a consequence of high light levels. Apparently, the results were not influenced by daily temperature oscillation since they were observed at all light levels analyzed in this study.

For *Rumohra adiantiformis* (G. Forst.) Ching (Dryopteridaceae), the highest germinability was achieved at 9% light in April and August/2000, at Florianópolis, Santa Catarina state, Brazil; at 54 and 72% light in March/2000, germination was completely inhibited and partially inhibited at 72% light in April 2000 (Brum & Randi 2002). On the other hand, high germinability of *Cheilanthes feei* Moore was achieved under a continuous light regime of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but this species can germinate under a wide variety of conditions including constant darkness (Nondorf *et al.* 2003). The authors applied a similar photon flux density to *C. feei* spores in relation to the values registered for *Blechnum brasiliense*, on the first day of cultivation under 62% light, when the day was cloudy. The canopy phenomenon which is responsible for the low Pfr/P_{total} under the canopy must be taken into account on forest soils. It results from the fact that green leaves absorb

red light because of their high chlorophyll content but are relatively transparent to far-red light (Taiz & Zeiger 1998). According to Bewley & Black (1994) photoinhibition of seed germination is a manifestation of the high-irradiance reaction (HIR) as a mechanism for discouraging seed germination under high field irradiance when seedlings would be subjected to harsh drying conditions. Shinomura *et al.* (1996) explain that seed germination of light-sensitive species is a low fluence response (LFR) controlled by the detection of changes in the red/far-red ratio (R/FR) through phytochrome B (Phy B). Similar photoinhibition behavior could be proposed for spores of *Cyathea delgadii* and *B. brasiliense*

For *Dicksonia sellowiana*, lower mean germination time was observed for spores cultivated at 5 and 20% light in relation to 36 and 50% light (Renner & Randi 2004). A different response was observed in the present paper for *Blechnum brasiliense* and *Cyathea delgadii* spores, which presented lower mean germination time at high light levels.

The second observation in this paper was gametophyte death for both species at 62 and 42% light, after a few days of cultivation. This effect was probably a consequence of chlorophyll oxidation at higher light levels, which reduces photosynthetic

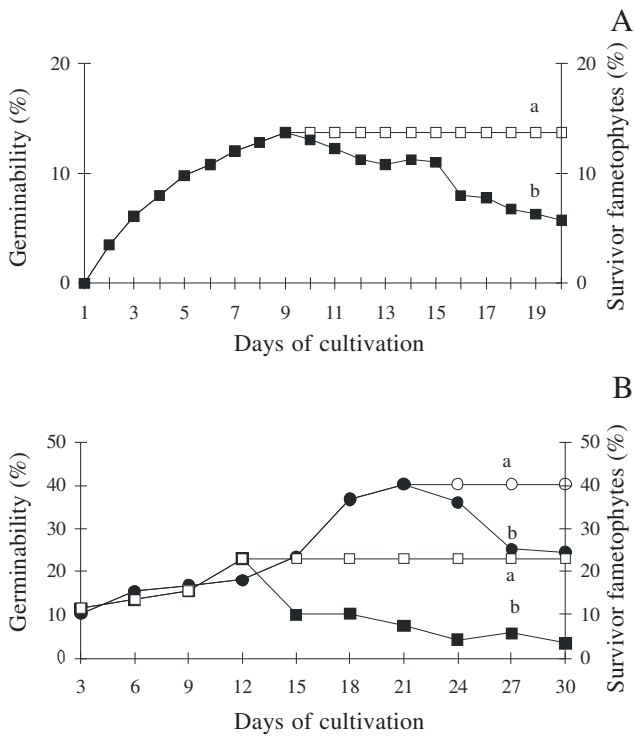


Figure 2. Survivor gametophytes of *Blechnum brasiliense* Desv. (A) and *Cyathea delgadii* Sternb. (B). Letters denote statistical differences between germinability and survivor gametophytes (Student *t* test). Open symbols are germinability. Closed symbols are survivor gametophytes (\square = 62% light; \circ = 42% light).

efficiency, limiting plant growth and eventually causing the death of plants as pointed out by Demming-Adans & Adams (1992), Sonoike (1996) and Kitao *et al.* (2000).

Several authors also verified the effects of light levels on the growth of young sporophytes. Suzuki *et al.* (2005) showed that young *Dicksonia sellowiana* sporophytes did not survive at 100, 75, and 50% light at Florianópolis, Santa Catarina state, Brazil. They observed the highest development and fresh mass/dry mass ratio, which reflects the level of water, in plants of *D. sellowiana* growing at 10% light. Concerning the effects of light levels on sporophyte development of *Alsophila firma* (Baker) D.S. Conant and *Sphaeropteris horrida* (Liebm.) Tryon, members of the Cyatheaceae, Bernabe *et al.* (1999) concluded that the forest edge was an appropriate habitat for their establishment, where PAR was nine times higher ($160 \mu\text{mol m}^{-2} \text{s}^{-1}$) than in the forest interior ($18 \mu\text{mol m}^{-2} \text{s}^{-1}$) on the observation date. Therefore, appropriate light levels and light quality seem to be of fundamental importance for fern establishment, together with water, temperature and nutrient supplies; they must guarantee germination

and photosynthetic activity for sporophyte development.

The present data suggest that fern spore photoinhibition is a mechanism that can guarantee spore germination and gametophyte establishment under appropriate environmental conditions. If spores are dispersed in open areas (gaps) they can germinate on cloudy or rainy days, but gametophytes probably will not survive on sunny days. Additional information on the effect of light levels on gametophyte and sporophyte growth will improve the understanding of fern physiology and establishment under natural conditions.

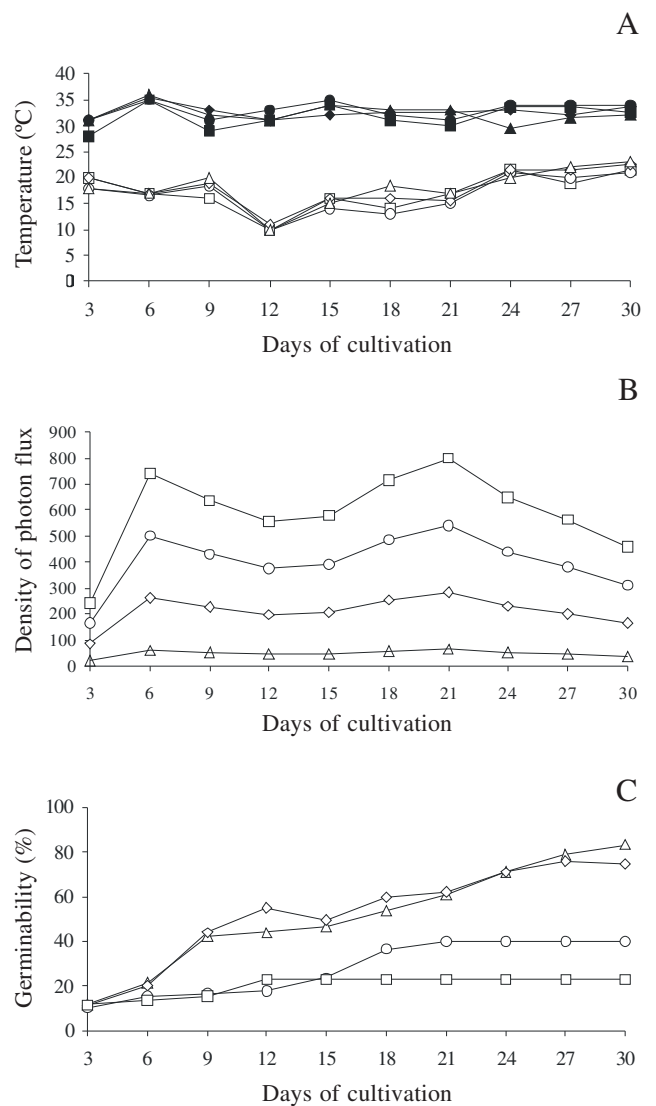


Figure 3. Minimum and maximum temperature (A) and irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (B) measured at 14:00 h, inside boxes of black shade netting where spores were kept to provide different light levels from 1 to 30 April; germinability of *Cyathea delgadii* Sternb. (C). Open symbols are minimum temperatures and dark symbols are maximum temperatures (\square = 62% light; \circ = 42% light; \diamond = 22% light; \triangle 5% light).

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