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Effect of light and water availability on spore germination and protonemal growth of the Neotropical moss *Thamniopsis incurva* (Pilotrichaceae)

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Abstract – *Thamniopsis incurva* is a Neotropical moss whose populations are usually associated with streams in tropical rainforests. In this paper, the spore germination and longevity, as well as the protonemal growth under different irradiances and water potentials, were studied. Mature capsules from populations in Atlantic Forest of northeastern Brazil (state of Pernambuco) were sterilized and the spores distributed on Knop nutrient medium (liquid and solidified with 0.4% agar). The spores quickly lost viability over three months. The germination was observed in both light (photoperiod 12h) and continuous darkness conditions. Variations of irradiance (1.4 to 14.4 μmol m^{-2} s^{-1}) did not inhibit protonemal growth and appearance of buds. On the other hand, spore germination, protonemal growth and gametophyte establishment were constrained from –0.06 MPa water potential. It appears that *T. incurva* invests in high production of sporophytes and spores per capsule, whose ability to germinate is elevated in contrast to its longevity. Additionally, spores and protonemata are very sensitive to low water potentials, likely restricting the establishment of the species to very wet microhabitats.

Bryophyte / ecophysiology / germination / protonema / water availability / irradiance

INTRODUCTION

Long-term persistence of bryophyte species depends on whether successful immigration (dispersal, germination and establishment) can balance the losses of subpopulations by demographic stochasticity or losses of habitat patches

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(Söderström & During, 2005). Moreover, germination and establishment from spores may be successful only under a much more restricted set of conditions than that allowing adult plant survival (Miles & Longton, 1992; Sundberg & Rydin, 2002).

Characteristics of the diaspores, like size and storage products are directly connected to dispersal capacity and longevity (long-term viability), as well as indirectly associated to maintenance or establishment of populations (Lloyd & Klekowiski Jr., 1970; During, 2001; Glime, 2007). The presence of chlorophyllous spores is common in bryophytes (Mogensen, 1983). These green spores, typical of species that occur in moist habitats, have a shorter longevity due to their higher metabolic rate. They store starch, in contrast to longer-living yellow spores that typically store oils and are common in drier habitats (Schofield, 1985; Glime, 2007). Spore longevity interferes with germination rate, resulting in low germination values over time. In bryophytes, the longevity of spores can vary from several months (many liverworts) to many years (mosses such as *Sphagnum* – 3y, *Oedipodium* – 20y *Dicranella* – 50y) (Schofield, 1985; Sundberg & Rydin, 2002; Glime, 2007).

Germination, the most critical stage of the plant life cycle, in mosses starts from spore swelling followed by chlorophyll production, breaking of the exospore and protrusion of the protonema. Initial protonema includes a chlorophyllous filament called chloronema, and in many mosses, after some days of culture, it is possible to distinguish another filament with brown color and few chloroplasts called caulonema (Nishida, 1978; Nehira, 1983; Schofield, 1985; Hartman & Weber, 1990; Pressel *et al.*, 2008). The former have an assimilatory and the latter an adventitious role (Cove *et al.*, 2006). In general, from the caulonema the buds – structures with an apical three-face cell – will grow into new gametophores (Brandes, 1973; Nishida, 1978; Nehira, 1983).

Suitable conditions of temperature, light and water are prerequisites for spore germination and protonemal development. For tropical taxa, the optimum temperatures varying between 20-25^{o}C are adequate (Duckett *et al.*, 2004; Silva *et al.*, 2006). Light acts in two ways: it supplies the necessary energy for photosynthesis and acts as an external signal for developmental regulation, and constitutes probably the most crucial factor in plant ecology (Wada & Kadota, 1989; Hartman & Weber, 1990; Smith, 1995). Furthermore, water is fundamental for spore swelling and its limitation can reduce or inhibit germination (Schofield, 1985; Glime, 2007). The presence of water is responsible for the conversion of stored substances into glucose for the production of ATP and consequently protonemal growth (Glime, 2007).

Studies on spore establishment of bryophytes in the field have shown that the rate of establishment of new colonies from spores is very small (Miles & Longton, 1992) and that perennial and perhaps annual mosses reproduce almost entirely by other ways than spores (Anderson, 1963). However, Sundberg & Rydin (2002) observed spore establishment of perennial bryophytes in the field (like *Sphagnum* species, and probably *Pohlia nutans* (Hedw.) Lindb. and *Polytrichum strictum* Menzies ex Brid.). Conditions required for spore germination and protonemata growth usually differ from the optimal conditions for the growth of adult plants, which may explain the failure of spores to germinate in some field studies (Sundberg & Rydin, 2002). On the other hand, studying germination and establishment under controlled laboratory conditions makes it possible to isolate important abiotic factors and to analyze their effects accurately.

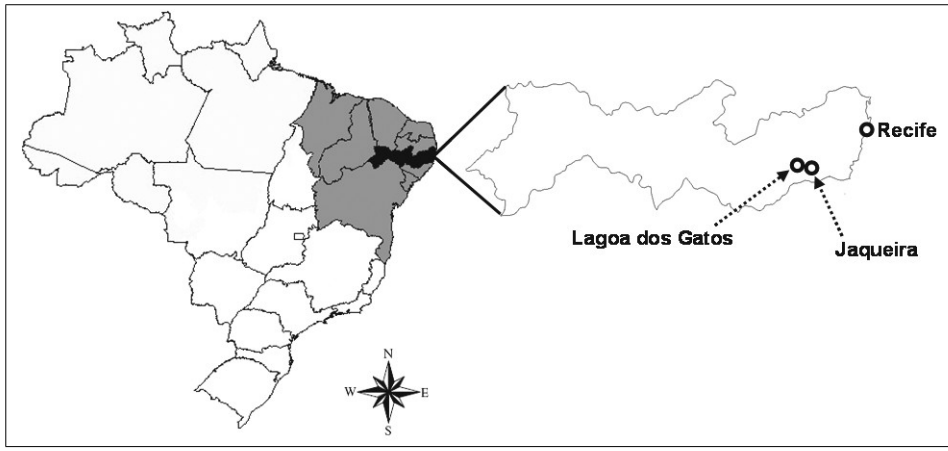


Fig. 1. Location of the field site between two close counties (arrows) in the State of Pernambuco (detail), northeastern region (grey) of Brazil.

We studied germination of spores and development of protonemata in the Neotropical moss *Thamniopsis incurva* (Hornsch.) W.R. Buck (Pilotrichaceae) under laboratory conditions. This species is autoicous and pleurocarpous, growing primarily in tropical rainforests, between 0–1400 m elevation (Buck, 1998; Vaz & Costa, 2006). It is common to find populations of this species between 0–1100 m on humid soil or rocks associated with streams (Buck, 1998; Yano & Peralta, 2007; Vaz & Costa, 2006). In Brazil, it is mainly present in the southeastern and southern regions of the country, with scarce records in the fragments of Atlantic Forest of the northeastern region (Fig. 1, in grey) above the São Francisco river, despite many floristic studies carried out in that region (Castro *et al*., 2002; Valdevino & Pôrto, 2002; Germano & Pôrto, 2005; Yano & Pôrto, 2006; Campelo & Pôrto, 2007).

Field observations revealed that subpopulations of *T. incurva* had a high production of sporophytes during consecutive months of the year, with many chlorophyllous spores per capsule. In addition, the subpopulations were restricted to a microhabitat where the water availability was high and the canopy cover was slightly dense. These observations lead us to ask the following questions: (1) are spores able to germinate rapidly and losing their viability over a period of a few months? (2) do spores show positively photoblastic response (i.e., light-germination) and is their germination inhibited under dark conditions? (3) are spore germination and protonemal growth negatively affected by low water availability and irradiances higher than those found next to subpopulations of *T. incurva* in the study site?

MATERIALS AND METHODS

Study site and species. Field expeditions were carried out at the Private Reserve of Natural Patrimony Frei Caneca (RPPN Frei Caneca), between Lagoa dos Gatos and Jaqueira counties, State of Pernambuco, Brazil (Fig. 1). The reserve is composed of Submontane Atlantic Forest fragments, from 500 to 750 m altitude,

22 to 26°C mean annual temperature and 1332 mm mean annual rainfall (RPPN Frei Caneca 2007). Species collection and field observations were done in 2005 and 2006.

Subpopulations of *T. incurva* were found in just one forest fragment in the reserve (08°42' S 35°50' W; 500 ha; 650 m altitude; mean month temperature 18 to 25°C and rainfall 13 to 570 mm measured during this study; Fig. 2), where it is possible to observe fog during the early and last hours of the day, especially during the rainy season and wetter days. *Thamniopsis incurva* was recorded colonizing rocks associated with water and humus accumulation. The greatest density of the subpopulations was found on crystalline rock, where there is a continuous water film during every month. These subpopulations occupied approximately 6 m^2 of rock surface and contained individuals in different phenophases, from protonemata and juvenile gametophores to mature gametophores with sporophytes and dehisced capsules. The area was shaded (1.20 to 4.75 µmol m^{-2} s^{-1} - sunny day) and received filtered sunlight under a canopy of sparse tall trees and tree ferns *Cyathea corcovadensis* (Raddi) Domin. Plant vouchers are deposited at the UFP Herbarium of the Universidade Federal de Pernambuco, Brazil (n° 44.828).

General procedures. Material was collected, placed in clear polyethylene bags and taken to the laboratory. Mature and closed capsules were used in the experiments. Sporophytes were separated from gametophytes, sterilized for two min in 1.5% sodium hypochlorite and washed in distilled water (Duckett *et al.*, 2004). Spores from several capsules were homogenized in a nutrient solution and spread on the medium (approximately 20 spores μL^{-1}).

Petri dishes (6 cm diameter) were used, containing 10 mL of sterilized Knop nutrient solution [$Ca(NO_3)_2$ H_2O: 0.5 g L^{-1}; $MgSO_4$ $2H_2O$: 0.17 g L^{-1}; KH_2PO_4: 0.175 g L^{-1}; KCl: 0.06 g L^{-1}; FeCl (3%): 1 mL L^{-1}] (Nehira, 1988) plus nistatin fungicide 100 U mL^{-1}. Liquid and solid (agar 0.4%) media were used (Silva *et al.*, 2006).

Dishes were sealed with PVC film and randomly positioned in a growth chamber at approximately 25±1°C and 14.4 µmol m^{-2} s^{-1} ("cool white" lamps). Glasses, nutrient solution and distilled water were autoclaved for 20 min at 120 ºC and 1 Kg cm^{-2} and the plastic materials were kept in ethanol solution 70% for 48h.

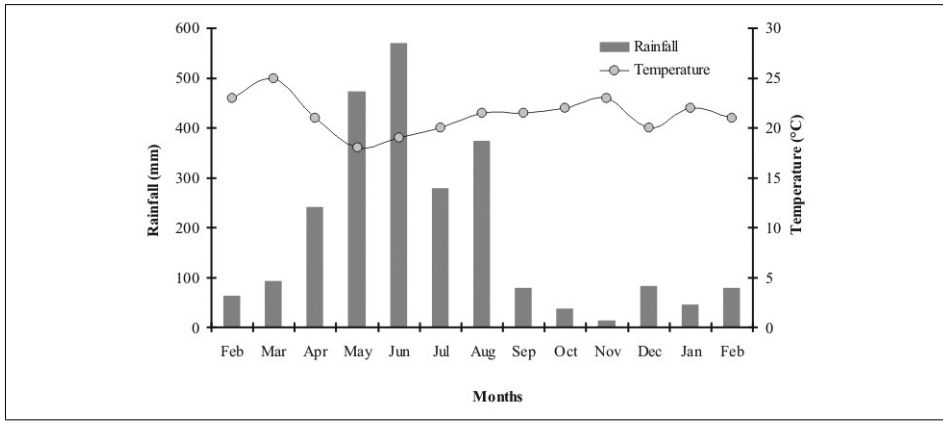


Fig. 2. Mean monthly rainfall (mm) and temperature (°C) measured in the field site from February 2005 to February 2006.

Each experiment consisted of five replicates. The percentage of germinated spores was assessed with a light microscope at 1-day intervals. A subsample of 100 spores in each replicate was examined and classified as germinated if the spore wall was broken by the germ tube, with protonemal protrusion. Daily evaluations were done to stabilize the data.

Germination.

a) *light effect* – treatments with light/dark periods of 12/12h (light treatment) and continuous darkness (dark treatment) were used, the latter was carried out by using Petri dishes in three black plastic bags (Cox *et al.*, 2003).
b) *spore longevity* – spore longevity under dry conditions was investigated by placing spores on a layer of aluminum paper kept in Petri dishes with dry filter paper and sealed with PVC film. Five replicates were placed in a growth chamber under the same conditions as for the previous germination experiments (Wiklund & Rydin, 2004). Recently collected spores and those kept dry for 30 and 60 days in the chamber (30-60% relative air humidity) were added to the nutrient solution. Longevity of spores was defined as the time when 1% of originally viable spores were predicted as still being alive (Sundberg & Rydin, 2000). Spores were quantified similarly to the general procedures above cited.
Protonemal growth. Experimental procedures were similar to those described for the germination experiments, with light/dark periods of 12/12h, 25±1°C temperature and 14.4 μmol m^{-2} s^{-1} irradiance. Three replicates (Petri dishes 9 cm diameter) were used per each day of treatment evaluation. A subsample of 30 protonemata per replicate was examined every 15 days during 90 days of culture, under a light microscope. The number of cells was chosen as the protonemal growth parameter, and the protonemal growth rate was calculated.
a) *irradiance effect* – the influence of the irradiance on protonemal growth and morphogenesis was analyzed under the treatments of 14.4; 10.08; 7.20; 4.32 and 1.44 μmol m^{-2} s^{-1}, respectively, based on measurements from the field site (1.20 and 4.75 μmol m^{-2} s^{-1}). These treatments were carried out using plastic shade nets with different percentages of shade in relation to full light (approx. 14.4 μmol m^{-2} s^{-1}), involving the Petri dishes.
b) *water availability* – as previously mentioned, it is known that *T. incurva* individuals in the field generally occur associated with high water availability (for instance, the subpopulations analyzed in this study). Based on this observation, the limitation of water on protonemal growth and morphogenesis was previously investigated under the water potentials –0.2, –0.4, –0.6, –0.8 and –1.0 MPa with no germination success, although spores of certain mosses are known to germinate at very low potential, e.g. –2.0 MPa (Wiklund & Rydin, 2004). Hence, higher water potentials (–0.02, –0.04, –0.06, –0.08, –0.1 MPa) were used. Water potentials were adjusted with PEG 6000 (polyethylene glycol) in the nutrient solution (Villela *et al.*, 1991). Polyethylene glycol is often used to maintain culture media at predetermined water potentials, as it is an inert, non-ionic, organic polymer that alters the matric potential of the solution (Michel & Kaufman, 1973; Steuter *et al.*, 1981). Water potential 0 corresponds to treatment with no addition of PEG, although it is slightly negative due to nutrient addition (Wiklund & Rydin, 2004).
Statistical analysis. Germination values were expressed as percentages in the text and transformed into ArcSin $\sqrt{(\%/100)}$ for statistical analysis. Student-t test ($p < 0.05$) was utilized for average comparison among germination treatments, and when pre-requisites such as variance homogeneity and residue normality

(Kolmogorov-Smirnov test) were not achieved, the data were analyzed with non-parametric test of Mann-Whitney (Bioestat 4.0, Ayres *et al.*, 2003). Cumulative germination curves for each replicate were modeled using the Gompertz growth model (Moreau-Valancogne *et al.*, 2007; Wiklund & Rydin, 2004): g(t) = gmax exp(–b0 exp(–b1 * t), where *g(t)* is germination at a given time, *t* is time in days from the start of cultivation, and *gmax*, *b0* e *b1* are parameters estimated by non-linear regression (Marquardt iterative method; Systat 11). Median germination time (*t50*, time for 50% germination) was calculated from the parameters *b0* e *b1*. *Gmax* is the final cumulative germination. The time for 50% of germination *t*50 was calculated because the germination rate was faster in the first days (Labouriau, 1983, Ranal & Santana, 2006). Protonemal growth values were expressed in $\log_{10}$ number of protonematal cells and the growth rate values from calculation as: growth rate = (number of cells at time 2 – number of cells at time 1) (time 2 – time 1)$^{-1}$, were square root transformed. The period with linear growth rate (15–30 days in the different experiments) was used (Wiklund & Rydin, 2004). Repeated measures ANOVA for protonemal growth analysis between 15 and 30 days, and one–way ANOVA for growth rates; with a posteriori Tukey $p< 0.05$) were used for comparison among growth treatments. (Statistica 6.0)

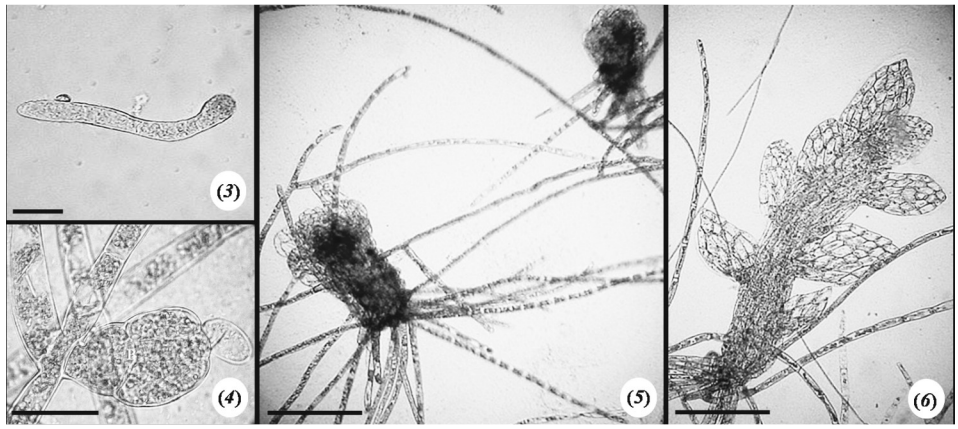
RESULTS

Subpopulations of *Thamniopsis incurva* produced numerous sporophytes (from one to three per plant) throughout the year, with a slight reduction of the number of mature sporophytes during the drier season (i.e., between December and March; Fig. 2). Each sporophyte is estimated to hold approximately 120,000 to 200,000 spores.

Germination. Under light conditions, spores germinated one day after being sown on the medium, producing chloronemata of long cylindrical cells. Germination is exosporic, bipolar and buds were observed up to 60d of culture, not necessarily associated with caulonema (Figs 3-6; for more details about protonemata morphogenesis, see Silva et al. 2006). Spore germination was significantly higher under light conditions (97%) than under darkness (6%) ($Z = 2.88$, $p = 0.0039$). Germination under darkness was characterized by cellular protrusion and elongation without division of the initial protonemal cell. On the other hand, under light the chloronema branched off quickly into side branches.

Differences in the physical nature of the nutrient medium did not influence the cumulative germination percentage of spores, that is, the spores germinated at similar rates in the liquid medium and on solid medium (97 and 98%; n.s. $p>0.05$; Fig. 7). The median germination time also did not differ between the media (0.254 and 0.242 days, n.s. $p>0.05$). Under these two conditions, the protonemata developed similarly, producing simultaneously primary chloronema and side branches. Based on these results, subsequent experiments used either liquid or solid media, according to which was the more adequate medium to access samples of spores or protonemata.

Viability of spores was quickly lost over 60 days, reducing to only 7% after the first 30 days after spore collection and maintenance under laboratory conditions. Cumulative germination percentage was reduced and the time to germinate increased significantly after 30 days of storage in the laboratory (97 and 4%, $t = 34.90$, $p<0.0001$; Fig. 8) and after 60 days all spores were achlorophyllous and collapsed, probably due to water loss.



Figs 3-6. Germinated spore, protonemata and gametophores of the moss *Thamniopsis incurva* cultured in Knop nutrient medium with light/dark periods of 12/12 h and at 25°C. **3.** Primary protonema from spore cultured for seven days. **4.** Bud (**B**) proceeding from protonemal filaments after 60 days of culture. **5-6.** Gametophores cultured for 75 days. Scale Bars, (*a, b*) = 20 µm, (*c*, *d*) = 100 µm.

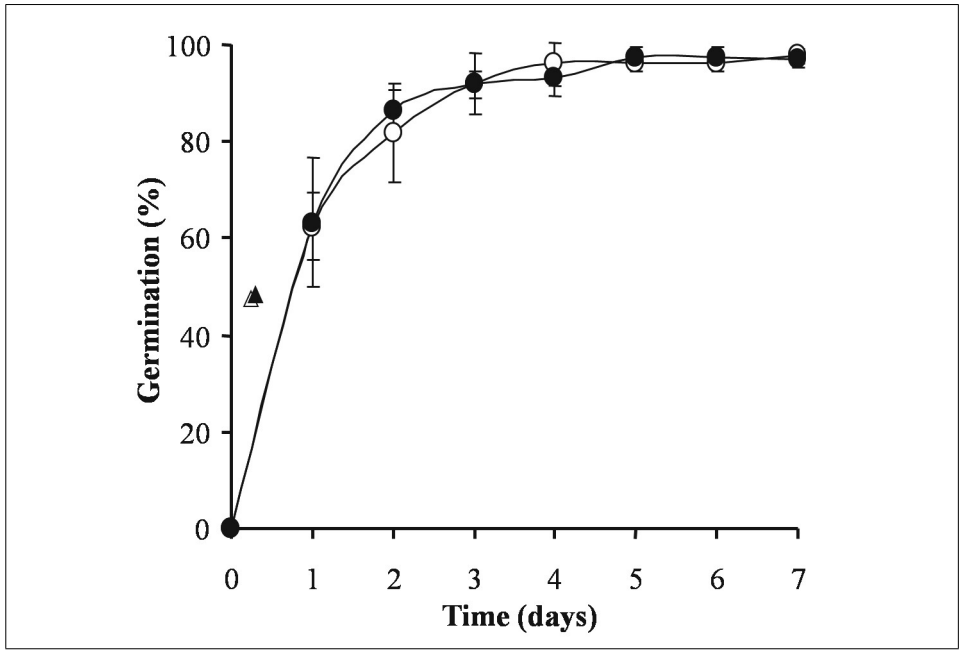


Fig. 7. Daily germination percentage of *Thamniopsis incurva* spores, cultured in liquid and solid Knop nutrient medium with light/dark 12/12h periods at 25°C. Empty circles = solid medium; filled circles = liquid medium; triangles = median germination time. Values represent means ±1 standard deviation.

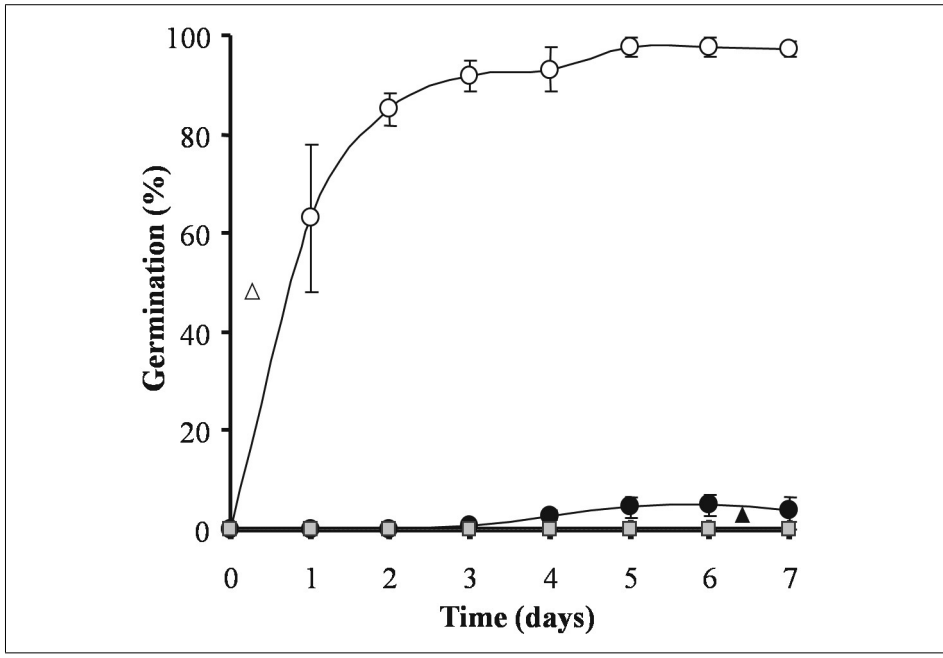


Fig. 8. Daily germination percentage of *Thamniopsis incurva* spores, recently collected and after 30 and 60 days of storage at 25°C, in laboratory. Empty circles = spores recently collected; filled circles = spores after 30 days; squares = spores after 60 days; triangles = median germination time. Values represent means ±1 standard deviation.

Protonemal growth

Irradiance – *s*pore germination was observed in all applied light conditions. No treatment inhibited morphogenesis, including the production of buds and gametophores. The number of protonematal cells differed significantly both among treatments and evaluation days in each treatment (15 and 30 days). ANOVA showed that all the responses to the main effects (treatments: F= 926.70, d.f.= 4; days: F= 1350.09, d.f.=1) and the treatment X time interactions (F= 5.05, d.f.= 4) were significant at $p<0.001$

The number of protonematal cells decreased with the decline of irradiance, and consequently the protonemal growth rate significantly slowed with lower irradiances (F= 197.10, $p< 0.001$, d.f.= 4), Figs. 9 and 10). For instance, higher protonemal growth rates occurred under irradiances of 14.4 to 7.20 than at 4.32 and 1.44 μmol m^{-2} s^{-1} (Fig. 10).

Water availability – Spore germination was not detected at water potentials from –0.2 to –1.0 MPa. However, the spores germinated when cultured between 0 and –0.1 MPa. The protonemata morphogenesis was inhibited from –0.06 to –0.1 MPa, and under potentials –0.08 and –0.1 MPa the protonemata were not able to branch or to produce buds and gametophores.

Number of protonematal cells declined significantly with water potential reduction, but enhanced over the culture days except under –0.08 and –0.1 MPa (F= 21.975, $p<0.001$, d.f.= 5, Fig. 11). Protonemata cultured at 0 MPa

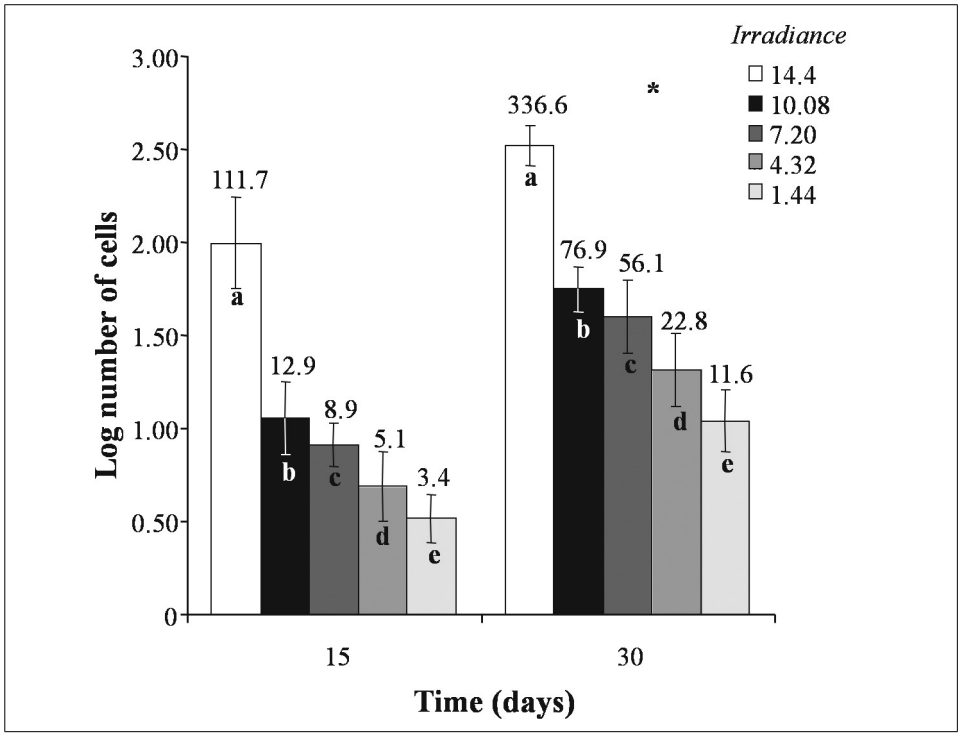


Fig. 9. Log number of protonematal cells of *Thamniopsis incurva* cultured at different irradiances (μmol m^{-2} s^{-1}) at 25°C during a 30-day period. Significant differences (Tukey test; $\alpha = 0.05$) between treatments are indicated by different letters and between days by asterisks. Values represent means ±1 standard deviation.

demonstrated the maximum number of cells, followed by the treatments –0.02, –0.06 and –0.04 MPa. Protonemal growth rates were higher at the potentials from 0 to –0.06 MPa, with clear reduction from –0.08 MPa ($F=103.20$, $p<0.001$, d.f.= 5, *Fig. 12*).

DISCUSSION

Spores of *Thamniopsis incurva* germinated quickly and in great number, but rapidly lost the ability to germinate over three months. Spores were positively photoblastic, with little germination in darkness. Spore germination and protonemal growth were negatively affected by low water availability and the protonemal growth decreased with reduction of irradiance.

Green spores of *T. incurva* could be associated with high and fast germination and protonemal growth rates, in addition to a rapid reduction of spore viability over time. These types of spore are typical of species that occur in moist habitats and have a shorter viability due to their higher metabolic rate and water content, in contrast to non-green spores that contain much higher levels of

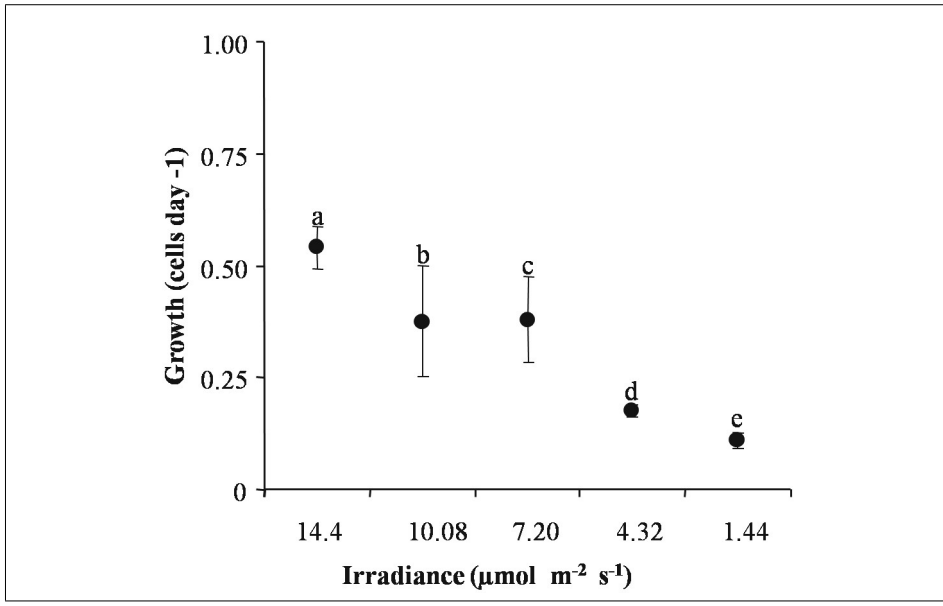


Fig. 10. Protonemal growth rate (cells · day $^{-1}$) of *Thamniopsis incurva* cultured at different irradiances at 25°C during a 30-day period (data transformed in square root). Significant differences (Tukey test; $\alpha = 0.05$) between treatments are indicated with different letters. Values represent means ±1 standard deviation.

lipids, fat and protein (Schofield, 1985; Glime, 2007). It is known in pteridophytes that chlorophyll-bearing spores have a higher metabolism and generally germinate in less than three days and have longevity of one year or less (Lloyd & Klekowiski, 1970). Chlorophyllous spores are common to wet-mesophytic habitats, which are in general unfavorable to spore dispersal due to forest canopy and very wet environment (Lloyd & Klekowiski, 1970). In field conditions it is possible that spores of *T. incurva* germinate quickly after dispersal and under conditions suitable for germination, but protonemata must be able to develop and establish new plants.

Spores of *T. incurva* lost viability quickly under laboratory conditions. In the field this would constrain germination and establishment to days immediately following dispersal. Spore viability is directly associated with the storage substance (During, 2001). Duckett & Renzaglia (1993) found that bryophyte spores and asexual propagules have protein and lipids as storage substances, whereas shorter longevity diaspores usually contain starch. In bryophytes, spore viability may also be linked to resistance against dryness, coldness and UV radiation during long-distance dispersal. Zanten & Pócs (1981) and Zanten & Gradstein (1988), conducting spore viability experiments, perceived a conspicuous correlation between the spore tolerance and the geographic distribution of species. Spores from species endemic to New Zealand were shown to be less tolerant to the treatments than the spores of wide-ranging species. Spore characteristics of *T. incurva*, such as abundant chlorophyll, apparently few storage products and high water content (traits that increase the possibilities of desiccation and reduction of viability of spores) in addition to the environmental

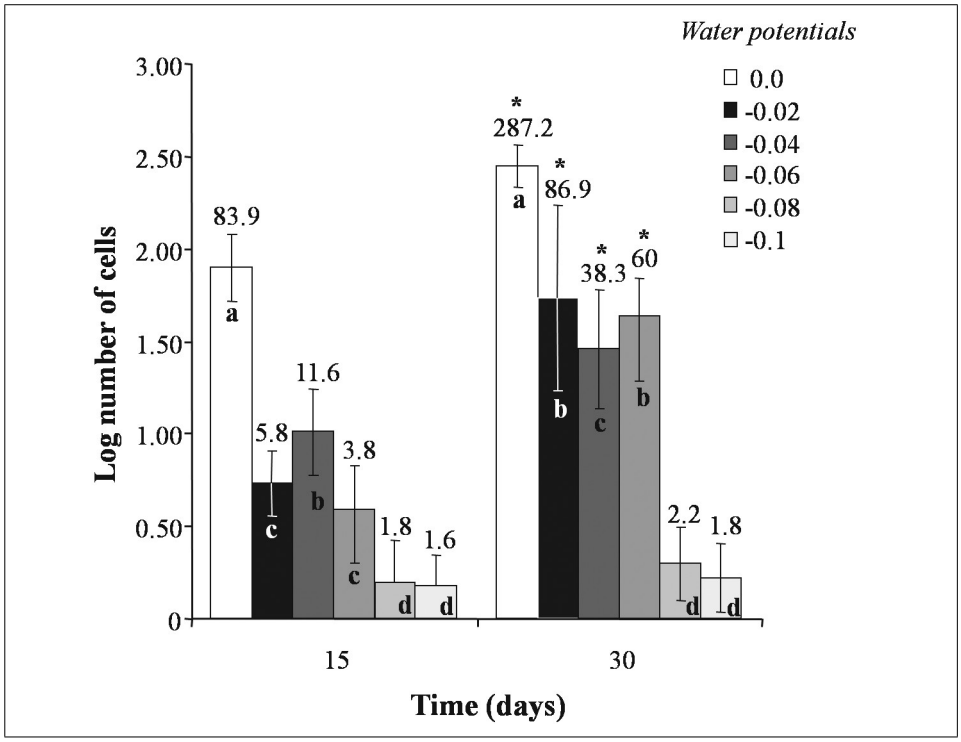


Fig. 11. Log number of protonematal cells of *Thamniopsis incurva* cultured at different water potentials (MPa) at 25°C during a 30-day period. Significant differences (Tukey test; $\alpha = 0.05$) between treatments are indicated by different letters and between days by asterisks. Values represent means ±1 standard deviation.

conditions in tropical forests, probably interfere with the success of spore dispersal for more distant forest fragments.

According to Glime (2007) there are trade-offs between spore size and the number of spores produced per capsule. Bryophytes species that produce small spores invest in spore number per capsule and increase the probabilities in the long distance dispersal. In *T. incurva* a capsule may produce many spores (>120,000) with sizes varying around 12 μm (Buck, 1998), considered a small spore among bryophytes (Söderström & During, 2005). Another trade-off is a negative correlation between spore size and the presence of asexual propagules in bryophytes. According to Longton & Schuster (1983) species with small spores and few storage substances would also invest in asexual propagules. There are no records of specialized asexual propagules for *T. incurva* in nature (Buck, 1998), although protonemata cultured *in vitro* may produce protonemal gemmae (pers. obs.).

Spore germination of *T. incurva* under dark conditions, although in small numbers, can be sufficiently important for the establishment dynamic from spores of this species in tropical rainforests. It is possible that spores germinate quickly in the field after dispersal and finding suitable conditions (e.g., high water availability), even though they are very shadowed or covered by leaf litter or thin soil layer. Consequently, these sites where adults are not easily found reduce

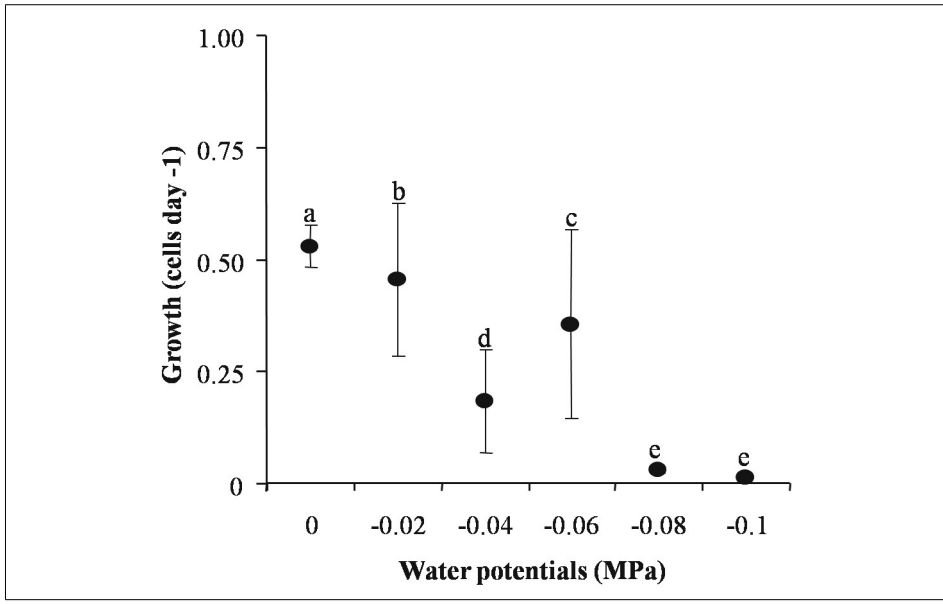


Fig. 12. Protonemal growth rate (cells · day $^{-1}$) of *Thamniopsis incurva* cultured at different water potentials during a 30-day period (data transformed in square root). Significant differences (Tukey test; α=0.05) between treatments are indicated with different letters. Values represent means ±1 standard deviation.

future competition of protonemata with adults. On the other hand, fluctuations in the microclimate associated to light can promote the protonemal development from spores that germinated earlier than did not others.

Irradiances higher than those measured next to the subpopulations of *T. incurva* in the field promoted both spore germination and protonemal growth. In the same way, Egunyomi (1978) found that spores of *O. albidum* germinated and produced protonemata under all irradiances used, with 100% germination at 16.5 µmol m^{-2} s^{-1} and 5.1% at 0.01 µmol m^{-2} s^{-1} (data transformed from lux to µmol m^{-2} s^{-1}, Larcher, 2004). On the other hand, the author also verified that the protonemata at 16.5 µmol m^{-2} s^{-1} were damaged at the end of the experiment, attributing its cause to a possible effect of high luminescence. High irradiance can promote protonemal growth (Monroe, 1968; Brandes, 1973) and secondary caulonemata instead of bud formation (Glime, 2007). This can be important by extending the plant to a darker location or merely as a way of occupying all the available illuminated space for successful gametophores (Glime, 2007). The results in the laboratory from spore germination and protonemal growth of *T. incurva* at irradiances from 1.44 to 14.4 µmol m^{-2} s^{-1} can indicate that the species in the field would demonstrate larger amplitude to colonize environments with lower and higher irradiances than those detected next to subpopulations at the study site.

Negative influence on spore germination and protonemal growth of *T. incurva* from water potentials lower than –0.04 MPa explains the habitat preferences of this species, such as soil and rocks close to streams in tropical rainforests where water availability is abundant. In contrast, spores of

Octoblepharum albidum germinated from 0 to –1.46 MPa (water potential where many seeds were not able to germinate), and the protonemal growth delayed with the reduction of water potentials, but there was not cell deformation or color alteration (Silva et al. unpubl. data). The mosses *Neckera pennata* Hedw. and *Buxbaumia viridis* (DC) Moug. et Nestl. possess distinct habits and responses. While spores of *Neckera* (epiphytic) had higher tolerance to very low water potentials like –2 MPa, spores of *Buxbaumia* (epixylic) germinated up to –1.5 MPa. Similarly, the protonemal growth of these mosses was also negatively influenced by the water potentials used (Wiklund & Rydin, 2004). Spores and protonemata of *T. incurva* are not tolerant to low water potentials, and this response is still more conspicuous when compared to the tolerance verified among the above-cited species.

At least for the subpopulations of *Thamniopsis incurva* studied here, the life history is situated between perennial shuttle (species that require stable environments, where end of habitat is predictable – e.g. dead wood) and perennial stayers (where end of habitat is unpredictable – e.g. rocks) (During, 1979), with high to moderate sexual reproductive effort throughout the year, elevated number of spores per capsule, small size of spores and absence of specialized asexual propagules recorded in nature. The high constraint on spore germination and protonemal growth by reduced water potentials could be associated with spore features like lots of chlorophyll and elevated metabolism, and physiological optimum for species establishment in nature. Low spore longevity together with the sensitivity of these diaspores to water potentials lower than –1.0 MPa (as well as probable sensitivity to desiccation during air travels) can negatively affect the establishment of new plants. Furthermore, it is interesting to verify the effect of the interaction between abiotic (e.g. light intensity and quality, pH, water, nutrients) and biotic (competition) factors on spore germination and protonemal growth, to determinate accurately what are the factors that control and restrict a species to many or few sites.

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