

Article

Differential Effects of Desiccation on Hornworts with Contrasting Life Histories in Tropical Montane Forests: A Functional Trait—Based Perspective

Gabriel F. Peñaloza-Bojacá ^{1,*} , Tiago Vilas-Boas ² , Juan C. Villarreal A ^{3,4}  and Adaíses S. Maciel-Silva ¹ 

¹ Laboratório de Sistemática Vegetal, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil

² Laboratório de Fisiologia Vegetal, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil

³ Département de Biologie, Université Laval, Québec, QC G1V 0A6, Canada

⁴ Smithsonian Tropical Research Institute, Panama City 0843-03092, Panama

* Correspondence: gpenaloza.bojaca@gmail.com

Abstract: Desiccation tolerance (DT) is the ability of an organism or structure to dry completely and subsequently survive in that air-dry state. Hornworts are excellent plant models to study desiccation effects as they have contrasting life histories which are likely associated with DT. We tested whether (1) epiphytic species had more efficient DT responses to drying and postrehydration than non-epiphytic species and whether (2) “green” spores were more sensitive than non-green spores to extreme drying. Hornwort species were collected from the Atlantic Forest of Rio de Janeiro, Brazil. We studied five species (gametophytes and spores: *Dendroceros crispus*, *D. crispatus*, *Nothoceros vincentianus*, *Phaeoceros carolinianus*; and only spores of *Anthoceros lamellatus*), using different relative humidity values, drying durations, and postrehydration conditions. All DT treatments affected the chlorophyll fluorescence (F_v/F_m) of gametophytes, with species-specific responses. *D. crispatus* and *D. crispus* (epiphytes) performed better than *P. carolinianus* and *N. vincentianus*, with fast recovery of F_v/F_m values postrehydration. The ability of non-green spores of *P. carolinianus* and *A. lamellatus* and green spores of *D. crispus* to support desiccation led us to reject our second hypothesis. The DT strategies of hornworts highlighted the trade-offs that are important in spore dispersal and plant establishment, such as fast colonization in *Dendroceros* spp. and potential spore soil banks in *Phaeoceros* and *Anthoceros* species.

Keywords: anthocerotophyta; Brazilian Atlantic forest; chlorophyll fluorescence; desiccation tolerance; green spores; life history traits; spore germination



Citation: Peñaloza-Bojacá, G.F.; Vilas-Boas, T.; Villarreal A, J.C.; Maciel-Silva, A.S. Differential Effects of Desiccation on Hornworts with Contrasting Life Histories in Tropical Montane Forests: A Functional Trait—Based Perspective. *Forests* **2023**, *14*, 255. <https://doi.org/10.3390/f14020255>

Academic Editors: Fernanda De Vasconcellos Barros and Caroline Signori-Müller

Received: 23 December 2022

Revised: 14 January 2023

Accepted: 26 January 2023

Published: 29 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Desiccation tolerance (DT) is the ability of an organism or structure to air-dry completely and subsequently survive in this state [1–4]. This trait occurs mainly in cyanobacteria, lichens, green algae, and bryophytes [5–7], and may have played an important role during the colonization of land by plants [2,8–10]. DT is a key factor in plant life cycles, influencing the responses of both their reproductive (e.g., spores and seeds) and vegetative tissues [2,8,11–13]. Desiccation tolerance has been reported in less than 0.15% of all vascular plant species in regards to their ability to tolerate water contents (WC) near 30%–40% at c. 94%–95% relative humidity (RH) [8,14,15]. Conversely, DT in bryophytes (liverworts, mosses, and hornworts) has been characterized by gametophytes surviving ~10% WC, which can result from a range of RH values less than 50%, and then recovering their metabolic activity even after long periods of time [2,8,16,17].

Multiple factors are used to accurately assess DT in bryophytes: (1) the rate of drying (RoD); (2) water content at equilibration (*equilibrating relative humidity*, $RHeq$); (3) the duration dry; and (4) the rate of rehydration (RoR ; for details see [2,18,19]). The desert moss

Syntrichia ruralis, for example, was kept in its dried state at ~30% RH for 20 years and when subsequently rehydrated for 28 days it showed regenerated shoots and a good parameter of chlorophyll fluorescence, at c. 0.35 [10]. Although the DT has been investigated in multiple taxa of bryophytes in recent decades ([2,8] and references therein), including the responses of their gametophytes and spores, there is long-standing uncertainty regarding whether hornworts are desiccation-tolerant species. For instance, Wood (2007) classified the hornwort *Dendroceros granulatus* as a desiccation-tolerant plant based on a personal communication from Michael C. F. Proctor, but there is no experimental data focusing on the ability of hornworts to withstand desiccation. Moreover, this knowledge gap makes our understanding of the hornwort ecology in tropical forests quite shallow, consequently hindering conservation proposals for rare and endangered species.

Hornworts are a small group of land plants, comprising c. 250 species [20] with a dominant gametophyte phase, and they display various features that are unique among land plants. All hornwort species have symbiotic relationships with fungi and/or endophytic nitrogen-fixing bacteria [21–23] and can present a pyrenoid-based biophysical carbon-concentrating mechanism [24,25]. Hornwort phylogeny has recently been resolved, with one of their key morphological traits being their spore color and ornamentation (Figure 1A; [25–28]). Most recent phylogenies propose hornworts as a sister group to mosses and liverworts [29], as part of the bryophyte clade [30,31].

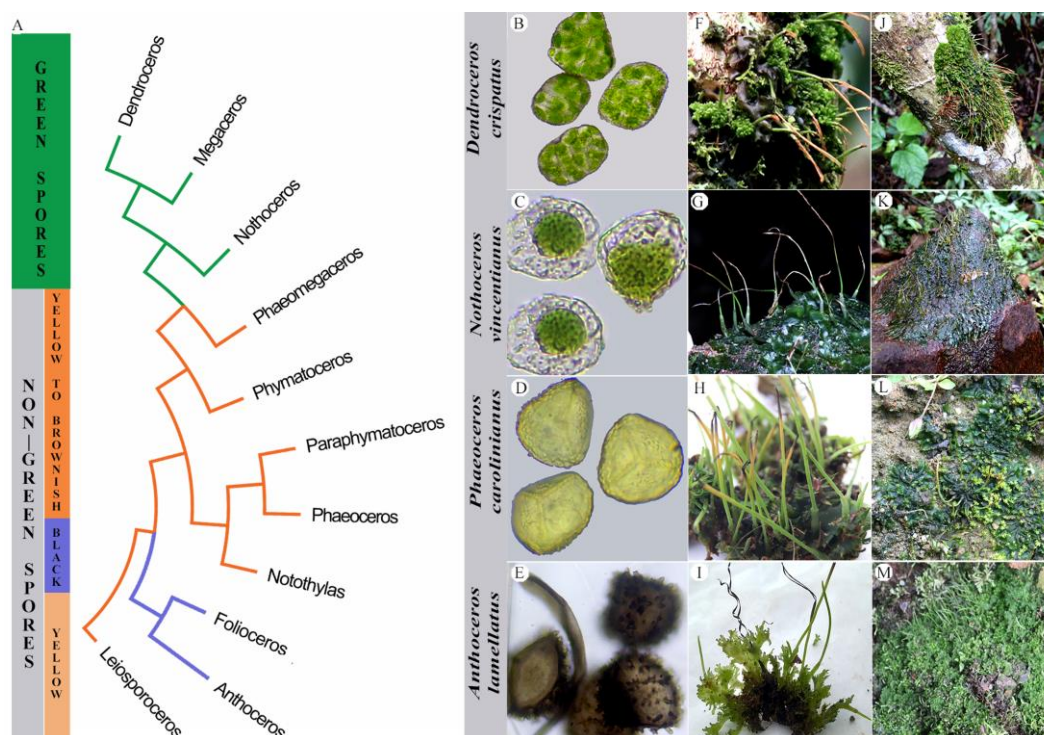


Figure 1. Diversity of spores and niches of hornwort species. (A) Phylogenetic diagram of the hornwort genera with green and non-green spores; (B,C) types of green spores—(B) multicellular spores; (C) unicellular spores; (D,E) unicellular non-green spores. (F–I) General view of hornwort gametophytes and sporophytes. (J–M) Different niches of hornwort species. (J) Epiphytic (on the bark of live trees and shrubs), *Dendroceros* genus. (K) Saxicolous (directly on rock surfaces), *Nothoceros vincentianus*. (L,M) Terricolous (on soils), (L) *Phaeoceros carolinianus* and (M) *Anthoceros lamellatus*.

Hornworts grow in many varied niches, such as on soils and rocks (e.g., *Anthoceros* and *Phaeoceros*) and on rotten logs near rivers and streams (most *Nothoceros* species), and they can also be found as epiphytes on live trunks, twigs, and leaves (the genus *Dendroceros*; [31–34]). Epiphytism is commonly related to desiccation tolerance, as most epiphytic plants have adaptations to deal with drying [35–37] and many bryophyte epiphytes tend to curl inwards

when in a dry state [38,39]. Following this idea, we expected that epiphyte hornworts would also display desiccation-tolerant traits.

Spore color and ornamentation have been suggested as features of protection against desiccation in hornworts. Yellow/dark spores with thick walls are supposedly more resistant than those with thin walls and a prominent plastid, thus appearing “green” [28,40–42]. Differential rates of metabolism, storage sources, and longevity have been recorded for the different spore types [28,36,43], with “green” spores assumed to germinate faster than darker spores, which are typically found in soil banks [44]. Although spores of *Phaeoceros* and *Anthoceros* are yellow, brown, or black (non-green spores), *Nothoceros* and *Dendroceros* have “green” spores [32,45], suggesting contrasting DT responses in these species. Hornwort spores also have different germination types, which are likely associated with DT strategies. For instance, unicellular spores (e.g., in *Nothoceros*, *Anthoceros*, and *Phaeoceros*; [28,46,47]) raise gametophytes directly in contact with the substrate, whereas multicellular spores develop sporelings located inside their walls and protected from desiccation (e.g., endospory in *Dendroceros* [28,36,48]).

Our main goal was to test for DT in different hornwort species. As discussed above, hornworts present very contrasting life histories that may be associated with DT strategies, which prompts several questions. Are hornworts, in general, desiccation-tolerant? Does the species niche influence the DT of the plant? Do spore traits have differential effects on DT responses?

To that end, we investigated the gametophytes and spores of species of the genera *Dendroceros*, *Nothoceros*, *Phaeoceros*, and *Anthoceros* (only spores in the latter) and hypothesized that (1) Epiphytic species have a more efficient DT response to drying and *postrehydration* treatments than non-epiphytic species. We expected the gametophytes of the *Dendroceros* species, compared to the gametophytes of *Nothoceros* and *Phaeoceros*, to present higher or constant chlorophyll fluorescence during the different *RHeq*, *dry duration*, and *postrehydration* treatments. Conversely, we also hypothesized that (2) “green” spores would be more sensitive than non-green spores to extreme drying. We expected spores of *Anthoceros* and *Phaeoceros* to have higher survival and growth rates than those of *Dendroceros* and *Nothoceros* under the different desiccation treatments.

2. Materials and Methods

2.1. Species Descriptions

We examined the following five species of hornworts: (1) *Dendroceros crispatus* (Hook.) Nees: a gametophyte with galeate wings, a solid midrib, one chloroplast per cell, with pyrenoids, and rectangular cells in the capsule with nodular wall thickenings [27]. (2) *Dendroceros crispus* (Sw.) Nees: a flat undulate or crispate gametophyte with a solid midrib, one chloroplast per cell, with pyrenoids, and rectangular cells in the capsule with wall thickenings. Both of these epiphytic species produce multicellular green spores (Figure 1B) and are found in forests at 800–2000 m a.s.l. [27,32]; see Figure 1F,J. (3) *Nothoceros vincentianus* (Lehm.) J.C. Villarreal: a pinnately branched gametophyte with 1–2 chloroplasts per cell, the absence or the presence of pyrenoids, rectangular cells in the capsule, and unicellular spores that are green at maturity (Figure 1C). This species is terricolous or saxicolous in Neotropical forests above 400 m a.s.l., along streams [28,49]; see Figure 1G,K. (4) *Phaeoceros carolinianus* (Michx.) Prosk.: a gametophyte with a rosette shape with a smooth margin, one chloroplast per cell, with pyrenoids, rectangular cells in the capsule with stomata, and unicellular spores that are yellow at maturity (Figure 1D). This species has a worldwide distribution, growing on soils and exposed areas above 300 m a.s.l. [32,50,51]; see Figure 1H,L. (5) *Anthoceros lamellatus* Steph. A.: a gametophyte with a rosette shape, with crenulate margins, abundant dorsal lamellae, one chloroplast per cell, pyrenoids present, rectangular cells in the capsule with stomata, and unicellular spores that are dark brown to blackish at maturity (Figure 1E). It has a neotropical distribution, growing on soils and exposed areas above 1000 m a.s.l. [28,52]; see Figure 1I,M.

2.2. Field Sampling

We carried out field collections in January and February 2020 in the Atlantic Forest in the state of Rio de Janeiro, Brazil. We collected samples (c. 8 cm²) of the hornwort species (except *A. lamellatus*) in the forest located in the Serra da Bocaina National Park, 22°46′04″ S and 44°36′34″ W, 1237–1524 m a.s.l., 17 °C–23 °C, with a mean rainfall of 1700–3000 mm.year^{−1} [53]. *Anthoceros lamellatus* samples were collected along the roadside next to the forest on the way to the Três Picos State Park, 22°20′04″ S, 42°42′04″ W, 1250–1368 m a.s.l., 17 °C–21 °C, with a mean rainfall of 1000–2000 mm year [54]. All samples presented gametophytes with rhizoids and mature sporophytes. Plants were transported in hermetically sealed plastic pots to the laboratory, thus maintaining a high internal relative humidity (>98% RH) and preventing their acclimation (acclimation to desiccation occurs during a dry-down or partial drying event; [10]).

2.3. Deacclimation

To assess desiccation tolerance and avoid variations due to unknown field hardening effects, we deacclimated plants for 15 days (effective time in *Sphagnum* species; [10,55]) using a 12 h photoperiod, at 21 °C, 74 μmol of photons m^{−2} s^{−1}, and RH > 98% in a culture room. Deacclimation is a process in which plants collected in the field are subjected to uninterrupted hydration under non-stressful conditions, overcoming any physiological hardening to desiccation that may have been obtained in the field [10]. In the same way, a period of 15 days can help stimulate plant growth under non-stressful conditions, ensuring complete deacclimation (Figure 2). A shorter period of time may not ensure deacclimation, as in *Exormotheca Holstii* or *Crossidium*, in which signs of hardening still persisted after 7 days [10,56]. This process ensured that suprasaturation conditions were applied to gametophytes and spores before the experiments began. Twenty-four hours before the end of the deacclimation period, we selected adult gametophytes from each species (here defined as plants with (a) male and female gametangia, (b) cyanobacteria colonies, (c) lengths greater than 2 cm, and (d) mature sporophytes (with signs of apical dehiscence)). Those plants were cleaned while viewing them under a stereomicroscope with the help of needles and forceps, washing them several times with deionized water until all excess unwanted material was removed. Subsequently, they were kept in deionized water until the completion of the deacclimation period.

To control the rate of drying, we followed the methodology proposed by [2,10,13], with modifications, using cell culture well plates with two sheets of filter paper (18 mm², 3 μm porosity), 20 μL of sterilized water, a digital thermohygrometer (Htc-2A) to monitor the temperature and internal humidity of the desiccation chambers, and HR 85%. We standardized the *rate of drying* based on measurements conducted over 24 h, that is, from full turgor (blotted) to plant curling. The volume of water and the *rate of drying* period had been previously tested in the laboratory (unpublished data—for details *RoD* see Stark 2017 and references). Deacclimated gametophytes and sporophytes were gently passed through filter paper to remove excess surface water, before being placed into the well plates. We used a total of 27 gametophytes per species, with three replicates and one individual plant per well. We used a total of 81 sporophytes per species, with three replicates and three mature sporophytes per well (Supplementary File S1). Finally, we applied an equilibrium time period, in which the plates with gametophytes or sporophytes and covers were placed in the desiccation chambers for 24 h at different relative humidity treatments (*RHeq* [10]) and before the period referred to as the *duration dry* (Figure 2, see below). Once this period was complete, we removed the covers from the plates.

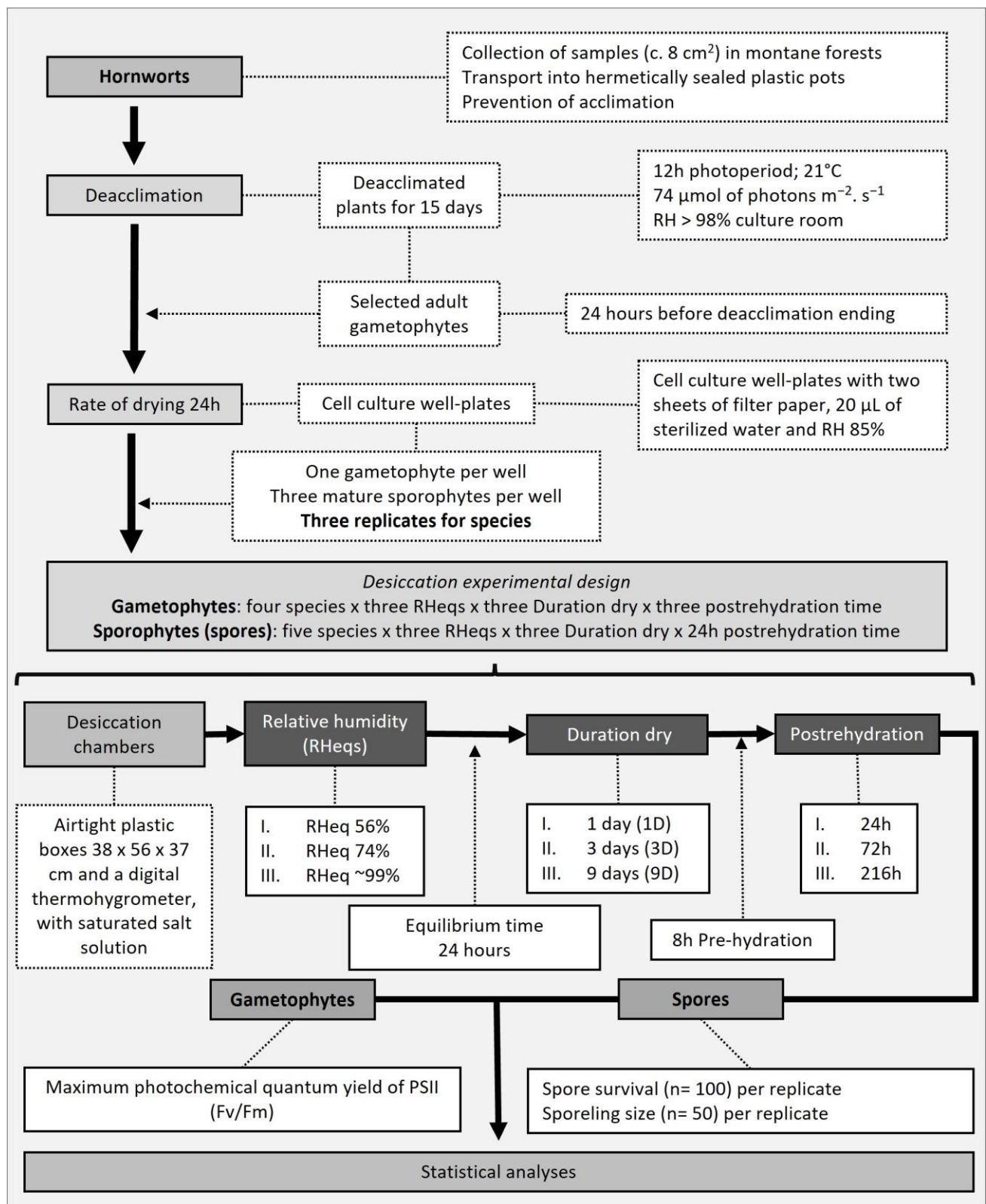


Figure 2. Summary of the desiccation experimental design for gametophytes and sporophytes of hornworts.

2.4. Experimental Design and Desiccation

Deacclimated gametophytes and sporophytes were used in all our assays (Figure 2). During the DT experiments, the plants were maintained under constant temperature and

light conditions ($21\text{ }^{\circ}\text{C}$, $74\text{ }\mu\text{mol}$ of photons $\text{m}^{-2}\text{ s}^{-1}$) in a culture room with a 12 h photoperiod. To evaluate the effects of desiccation times and intensities for each species (as described above), we prepared desiccation chambers (airtight plastic boxes of $38 \times 56 \times 37\text{ cm}$ and, internally, an Htc-2A digital thermohygrometer), with saturated salt solution in Petri dishes to provide constant internal RHs. Equilibrating relative humidity treatments (*RHeqs*) were achieved using the following saturated saline solutions: *RHeq* 56% (Ca $[\text{NO}_3]_2$), *RHeq* 74% (NaCl), and *RHeq* ~99% with deionized water.

Our experimental design for the measurement of DT in gametophytes was as follows: four species with three replicates each $\times$ three *RHeqs* $\times$ three *dry durations* $\times$ three *postrehydration times*. For sporophytes: five species with three replicates each $\times$ three *RHeqs* $\times$ three *duration dry* $\times$ one *postrehydration time*. The *duration dry* periods were 1 day (1D), 3 days (3D), and 9 days (9D). *Postrehydration* was applied to all replicates, with three different times: 24 h for gametophytes and sporophytes and 72 h and 216 h for gametophytes. These *Postrehydration times* were observed in the same number of replicates for gametophytes. One period of 8 h of pre-hydration (in which desiccated gametophytes and sporophytes were submitted to a relative humidity of ~99% *RHeq*) was applied in all *postrehydration* treatments before adding liquid water [10,57]. The plants and sporophytes were subsequently rehydrated by adding 2 mL of deionized water directly onto the filter paper, keeping them immersed until the completion of the different *postrehydration times* [10,13].

To assess gametophyte responses to DT, we measured the maximum photochemical quantum yield of PSII (F_v/F_m), using the equation: $F_v/F_m = (F_m - F_o)/F_m$, where F_m is the maximum fluorescence and F_o is the fluorescence in the absence of actinic light [58,59]. F_v/F_m measurements were taken in the morning, before the start of the light period, so that the plants had been under 12 h of dark adaptation. The latter is related to PSII reaction centers, which are open after dark adaptation and which decrease under dehydration stress; thus, this can be used as a measure of survival under drought conditions in bryophytes [17,60,61]. Chlorophyll fluorescence emissions were assessed using a pulse-amplitude modulation fluorometer (model MINI-PAM, WALZ, Effeltrich, Germany) with a light saturation pulse of $\sim 5000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$.

To assess DT in spores, we collected spores from the capsule apex (sporogenesis is asynchronous in these hornworts and the capsule apex contains mature spores) from the sporophytes under different treatments. Spores from three capsules were mixed and cleaned with deionized and sterilized water and plated for 30 days in Petri dishes on solid culture medium (Knop II with 0.5% agar, pH 5.6; [62], using a 12 h photoperiod at $21\text{ }^{\circ}\text{C}$ and $74\text{ }\mu\text{mol}$ of photons $\text{m}^{-2}\text{ s}^{-1}$. We measured spore survival ($n = 100$) and sporeling size (50) per replicate. Survival was measured as viable spores or germinated spores when they showed signs of germination after 30 days (i.e., the development of a rhizoid or germ tube or several green cells inside the spore wall), or, to the contrary, non-viable spores after 30 days (no signs of development, being hyaline or dark brown, with no green chloroplasts and no rhizoid or germ tube). Sporeling size was analyzed using BEL Capture Application software ($400\times$ magnification), assessing their lengths (in mm). Additionally, after deacclimation and before beginning our experiments, we determined F_v/F_m reference values for the gametophytes, as well survival and growth values for spores of the study species at *RHeq* 56%, 74%, and *RHeq* ~99% (Supplementary Figure S1).

2.5. Statistical Analyses

We generated generalized linear models (GLMs) to test our hypotheses and verify our predictions. The effects of “*species*”, “*RHeq*”, “*dry duration*”, and “*postrehydration*” treatments on the “ F_v/F_m ” values were tested with the GLM (Gaussian distribution and identity link function; model details provided in Supplementary File S2). The effects of “*species*”, “*RHeq*”, and “*dry duration*” on “*spore survival*” and “*sporeling size*” were analyzed with a model equivalent to the one above. Since the response variable “*sporeling size*” had spores as a random factor nested in each replicate, we attempted to include that factor in our previous model. Because of singularity effects, however, we preferred to exclude it and

to use the simplest and most powerful (Akaike's information criteria, AIC) model for our analyses. Additionally, analyses of deviance for GLMs and *post hoc* comparisons (Tukey test) were applied. The latter test was used to evaluate all pairwise differences at $\alpha = 0.05$. All analyses and graphs were implemented in R [63] using RStudio V.3.6.2 software [64], with the ggplot2 [65] and MASS packages [66].

3. Results

3.1. Desiccation Effects on Hornwort Gametophytes

All DT treatments (*RHeq*, *dry duration*, *postrehydration*) had significant effects on the F_v/F_m of gametophytes of the study species; significant interactions of these treatments with “*species*” indicated that the F_v/F_m in hornwort species exhibited very distinct behaviors under different desiccation treatments (Table 1; Figure 3). In general, the species with the highest F_v/F_m values were *D. crispatus* (mean $\pm$ se: 0.515 ± 0.012) and *D. crispus* (0.443 ± 0.018), as compared to *P. carolinianus* (0.188 ± 0.025) and *N. vincentianus* (0.144 ± 0.020 ; Supplementary Figure S2, File S3). *RHeq* treatments of 56% and 74% had similar negative effects on the gametophytes, compared to 99% *RHeq*, generating damage mostly in the gametophytes of *P. carolinianus* and *N. vincentianus*. We observed signs of regeneration in the gametophytes of *P. carolinianus* at 99% *RHeq* after three days of desiccation (Figure 4, red arrows). Although several plants recovered their photosynthetic capacities, the chlorophyll fluorescence of the gametophytes in our experiment did not reach the reference values of recently deacclimated plants.

Table 1. Generalized linear model of the effects of desiccation tolerance in gametophytes and spores of hornworts species (GLM with Gaussian distribution and identity link function; bold indicates statistical significance).

Source	Degree of Freedom (d.f.)	Deviance	Resid. Df	Resid.Dev	F	Pr(>F)
Chlorophyll fluorescence parameters for hornwort gametophytes						
Null model			323	184.989		
Species	3	82.696	320	102.293	2.130.616	<0.001
<i>RHeq</i>	2	21.851	318	80.443	844.457	<0.001
<i>Duration dry</i>	2	12.203	316	68.240	471.616	<0.001
<i>Postrehydration</i>	2	12.289	314	55.951	474.911	<0.001
Species: <i>RHeq</i>	6	12.782	308	43.169	164.665	<0.001
Species: <i>duration dry</i>	6	0.2100	302	41.069	27.054	0.014
Species: <i>postrehydration</i>	6	0.2773	296	38.296	35.723	<0.001
Model: AIC: −460.44; Residual deviance: 3.8296; 296 degrees of freedom (df)						
Survival spores						
Null model			134	55441		
Species	4	19447.3	130	35993	190.645	<0.001
<i>RHeq</i>	2	967.2	128	35026	18.963	0.154
<i>Duration dry</i>	2	9.7	126	35016	0.0189	0.981
Species: <i>RHeq</i>	8	4295.7	118	30721	21.056	0.041
Species: <i>dry duration</i>	8	2668.6	110	28052	13.080	0.246
Model: AIC: 1155.5; Residual deviance: 28052; 110 degrees of freedom (df)						
Sporeling Size						
Null model			6749	580.75		
Species	4	198.547	6745	382.21	1.132.689	<0.001
<i>RHeq</i>	2	17.994	6743	364.21	205.306	<0.001
<i>Duration dry</i>	2	22.908	6741	341.30	261.377	<0.001
Species: <i>RHeq</i>	8	26.078	6733	315.23	74.386	<0.001
Species: <i>duration dry</i>	8	20.523	6725	294.70	58.540	<0.001
Model: AIC: −1928.8; residual deviance: 294.70; 6725 degrees of freedom (df)						

In terms of the *dry duration*, the F_v/F_m values of hornwort gametophytes decreased from 1D (0.360 ± 0.022) to 3D (0.371 ± 0.023) and to 9D (0.023 ± 0.020) (Supplementary Figure S2). The significant interactions we observed between “*dry duration*” and “*species*” and “*RHeq*” were mostly the result of the gametophytes of *D. crispus* and *D. crispatus* being

less affected by drying than those of *P. carolinianus* and *N. vincentianus*. These latter species had the lowest F_v/F_m values among the different treatments (Figure 3).

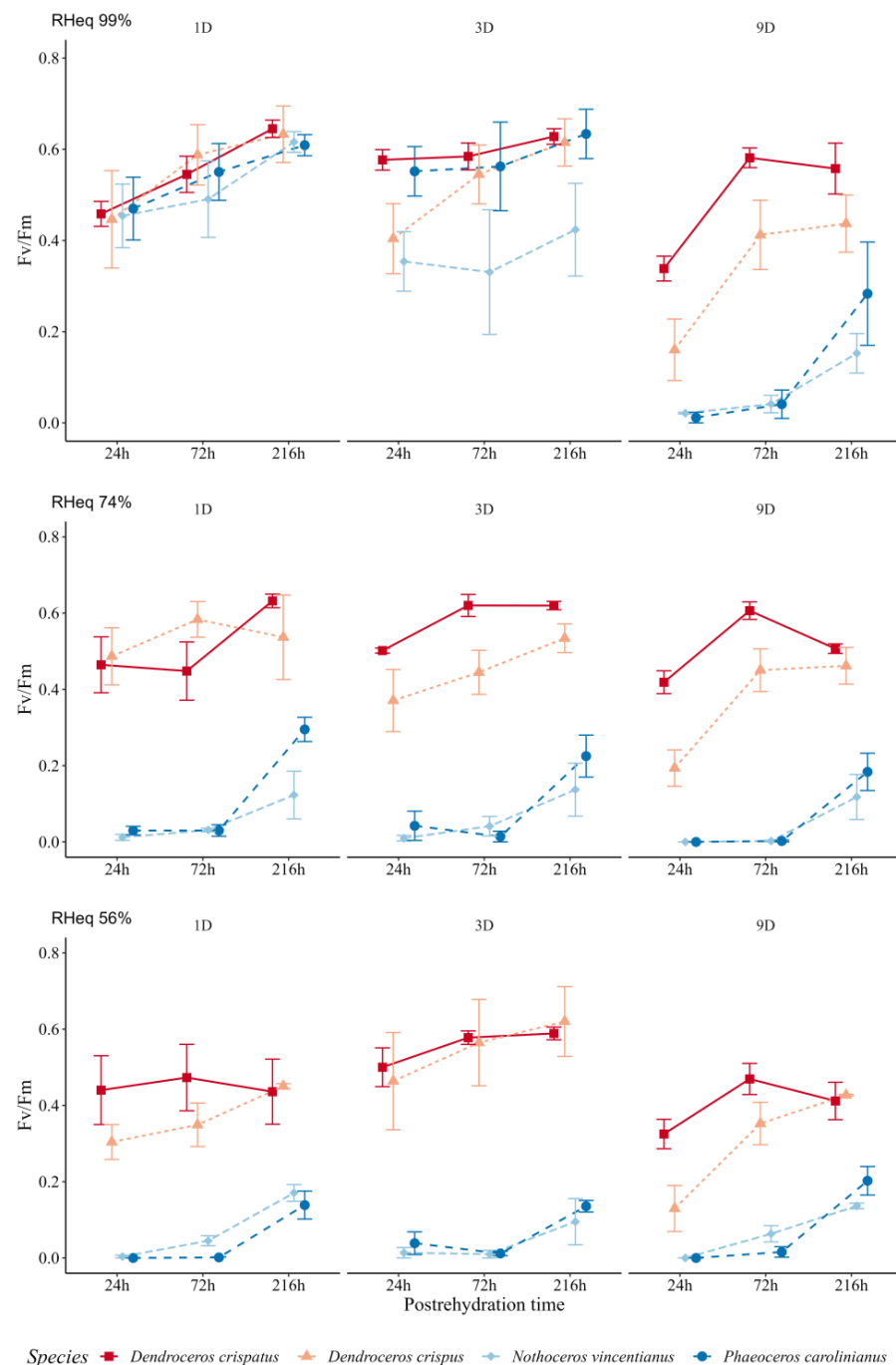


Figure 3. Means of the F_v/F_m chlorophyll fluorescence parameters of hornwort gametophytes with equilibrating relative humidity treatments (RHeq) of 99%, 74%, and 56%. Experiments were conducted with the different species placed under drying treatments with differing durations in terms of days (1D, 3D, and 9D) and postrehydration times, in terms of hours (24 h, 74 h, and 216 h); error bars represent ± 1 SE.

We observed a tendency of the gametophytes to recover their F_v/F_m values after all the tested *postrehydration* times, with better recovery rates recorded after the *postrehydration* times of 24 h (0.25 ± 0.021), 72 h (0.32 ± 0.024), and 216 h (0.40 ± 0.020) (Supplementary Figure S2). The F_v/F_m values in *D. crispatus* and *D. crispus* increased similarly after 24 h and

72 h of *postrehydration* for all *RHeqs* values, and after *dry durations* of 1D and 3D. At 216 h, the F_v/F_m of both species increased for all *RHeqs* values only under 1D of drying (except for *D. crispus* at 74% *RHeq* and for *D. crispus* at 56% *RHeq*), however, indicating an effect of *duration dry* on species' responses after *postrehydration*. Chlorophyll fluorescence in *N. vincentianus* and *P. carolinianus* showed an inverse pattern, with low values at 24 h and 72 h that increased after 216 h for all *dry durations* and *RHeqs* treatments (Figure 3).

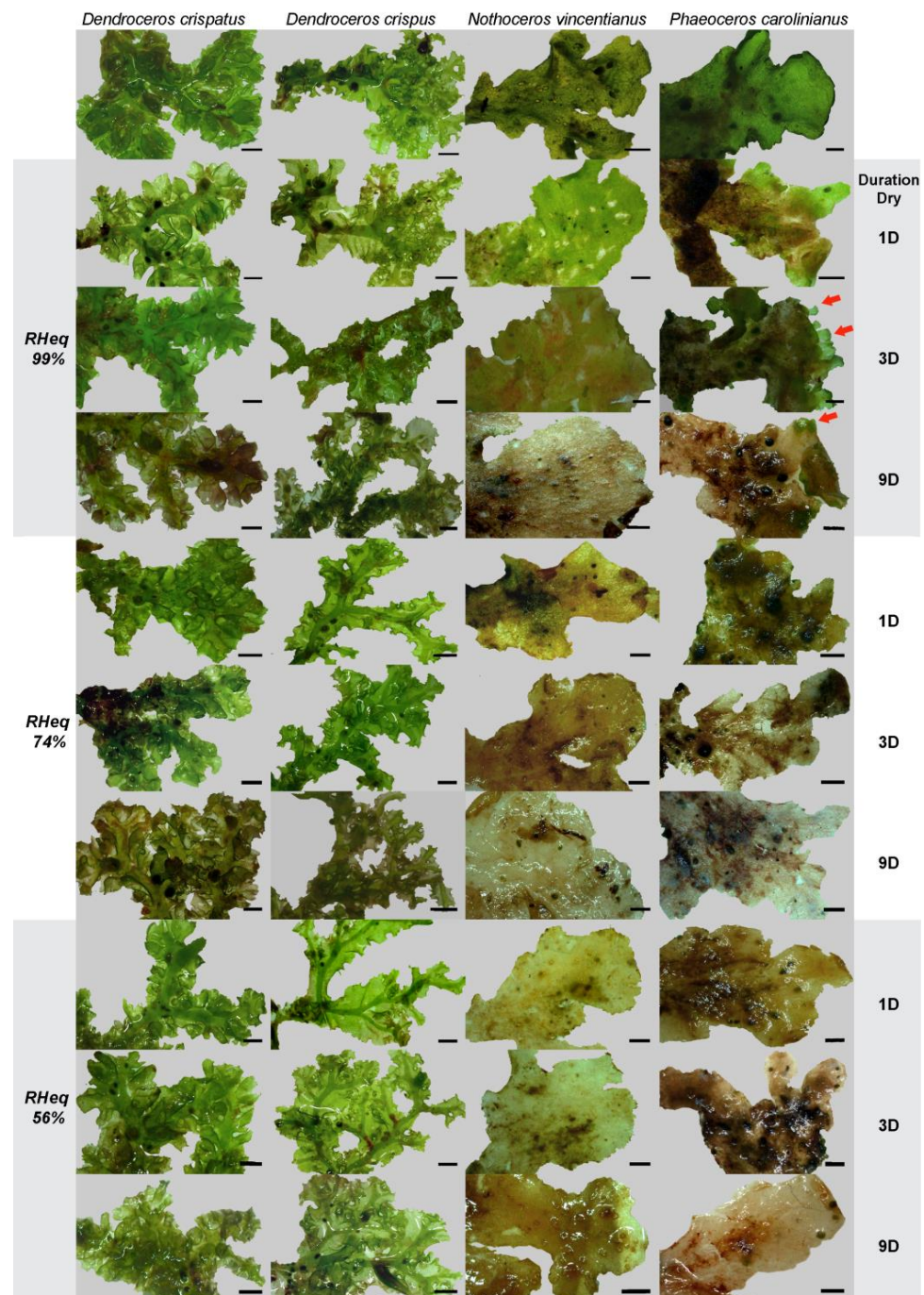


Figure 4. Effects of drying on hornwort gametophytes in the three treatments consisting of differing *RHeq* values (Left) and *dry durations* (right), with a *postrehydration* time of 216 h. The first line displays gametophytes with deacclimation for 15 days and without drying effects. Scale-bar: 1 mm. Red arrows: gametophyte regeneration.

3.2. Desiccation Effects on Hornwort Spores

Although the hornwort species differed significantly in terms of their spore survival rates, no main effect of *RHeq* values and *dry durations* was observed on the spore survival of the hornworts (Table 1 and Figure 5A). In general, spore survival differed among hornwort species, as follows: *Phaeoceros carolinianus* ($84.5\% \pm 1.56\%$), followed by *D. crispus* ($82.0\% \pm 1.02\%$), *A. lamellatus* ($69.4\% \pm 3.67\%$), *N. vincentianus* ($61.3\% \pm 4.55\%$), and *D. crispatus* ($53.0\% \pm 3.68\%$). We observed significant interactions of “species” with “*RHeq*” (Table 1) in *A. lamellatus* and *P. carolinianus*, with better spore survival in lower *RHeqs* treatments. In contrast, *D. crispus*, *D. crispatus*, and *N. vincentianus* exhibited their best spore survival at higher *RHeqs* (Figure 5A). *P. carolinianus* and *A. lamellatus* spores exhibited similar survival rates after desiccation treatments as compared to recently collected plants.

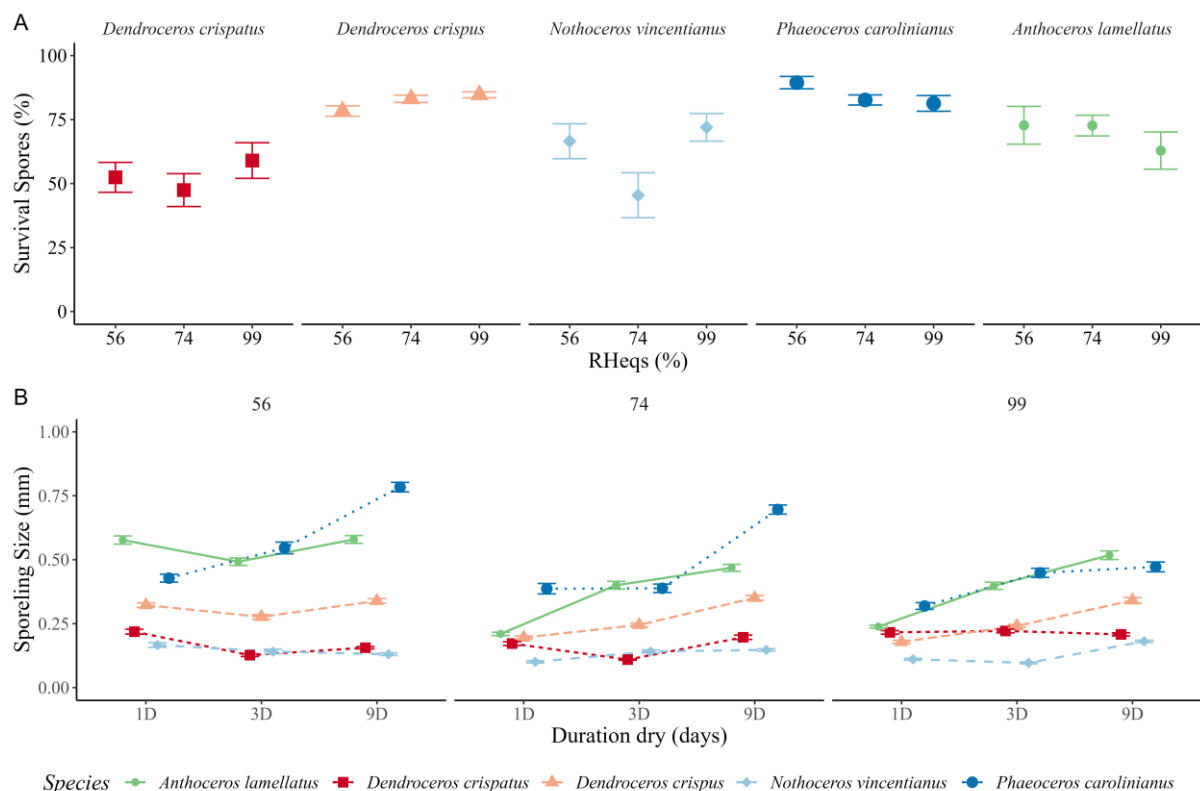


Figure 5. Spore survival percentages and sporeling sizes of hornworts cultured for 30 days. (A,B) after *RHeq* and 24 h *postrehydration* before cultivation—(A) spore survival with *RHeq* treatment; (B) sporeling size with *RHeq* and *duration dry* treatments. Error bars represent ± 1 SE.

Sporeling sizes varied in response to *RHeq* treatments, with species exhibiting decreasing sizes from 56% *RHeq* ($0.40 \text{ mm} \pm 0.007$), followed by 74% ($0.296 \text{ mm} \pm 0.005$) and 99% ($0.298 \text{ mm} \pm 0.005$; Figure 5B). *Phaeoceros carolinianus*, *A. lamellatus*, and *D. crispus* had smaller sporelings with 74% and 99% *RHeqs* treatments. *Dendroceros crispatus* and *N. vincentianus* produced small sporelings in all treatments. Significant interactions were observed between “dry duration” and “species” (Table 1), with the longer the dry duration, the larger the sporelings of *P. carolinianus*, *A. lamellatus* and *D. crispus*; whereas *D. crispatus* and *N. vincentianus* had the smallest sporelings at 3D as compared to 1D and 9D. The sporelings of *P. carolinianus*, *A. lamellatus*, and *D. crispus* reached the reference values of sporeling size after the 9D desiccation treatment with 56% and 74% *RHeqs* (Supplementary Figure S1).

4. Discussion

We corroborated our first hypothesis: the gametophytes of epiphytic species (*D. crispatus* and *D. crispus*) were more tolerant of desiccation than the non-epiphytic species, and

Phaeoceros carolinianus and *N. vincentianus* gametophytes suffered greater physiological and morphological damage. Our second hypothesis was rejected, as *P. carolinianus* and *A. lamellatus* (non-green spores) and *D. crispus* (green spores) showed better spore survival and sporeling sizes in desiccation treatments, distinct from *D. crispatus* and *N. vincentianus* (green spores), of which the same parameters were the lowest.

4.1. Desiccation Tolerance of Hornwort Gametophytes

The gametophytes of the epiphytic species studied here exhibited less morphological injury and damage to photosystem II (Figures 2 and 3), as was suggested earlier for *Dendroceros* species [2,36]. Epiphytism, unique plastid traits, and a star-shaped chloroplast with a complex pyrenoid are assumed to provide protection for the photosynthetic apparatus of *Dendroceros* during drying periods [2,36,67]. A relationship between DT and epiphytism has been widely observed in other tropical plants, including the mosses *Prionodon densus* and *Dicranoloma fragiliforme*, the liverwort *Frullania peruviana*, and the fern *Microgramma reptans*, of which the gametophytes do not show morphological or physiological damage after drying periods with *RHeqs* values lower than 50% [2,35,37,68].

Additionally, the failure of *N. vincentianus* and *P. carolinianus* gametophytes to restore normal morphological and physiological parameters after prolonged drying regimes at *RHeqs* <99% expressed their low DT capacities (Figures 2 and 3). The niches of both species may be linked to their DT responses, as *N. vincentianus* is commonly found in very humid and shady areas (e.g., along rivers and streams or inside forests) and *P. carolinianus* usually grows on moist soil in exposed sites (e.g., roadsides and ravines). These niches contrast greatly, with the latter usually being colonized by bryophytes that are resistant to occasional dry periods [8,69]. We assume, therefore, that the lower DTs of *N. vincentianus* and *P. carolinianus* gametophytes, even if they are only for short periods of time, are related to protective mechanisms, such as their sugar contents or constitutive cellular protection by abscisic acid (ABA; [2,8,10,70]).

4.2. Postrehydration Effects

Epiphytic species responded more effectively to short *postrehydration* periods of 24 h and 72 h (with a pre-hydration period of 8 h for all of the treatments used here) than the non-epiphytic species did, which can be explained by the rate at which bryophytes rehydrate—as the highest performance plants commonly have high surface areas and low weights [10,71]. *Phaeoceros* and *Nothoceros* plants are thick, with several layers of cells in their thalli, whereas *Dendroceros* species are thinner and have uniseriate wings. We observed different responses among the gametophytes of *Dendroceros* species after a *postrehydration* period of 216 h; *Dendroceros crispus* responded better than *D. crispatus* with longer rehydration (Figure 3). Although *D. crispatus* is a galeate gametophyte with many macro-perforations and pores in its wings, *D. crispus* is a crispate gametophyte (with a higher surface area) with few and irregular pores [27].

The responses of the *N. vincentianus* and *P. carolinianus* gametophytes, which showed better recovery after 216 h of *postrehydration*, suggest the need for longer rehydration times. The desiccated cell membrane in those species may require greater exposure to a saturated atmosphere and free water to recover and mitigate the injuries caused by drying, compared to *Dendroceros* species [10,72]. Another important and unprecedented factor in hornworts is the strategy used by *P. carolinianus* at an *RHeq* of 99% with a *postrehydration* time of 216 h for all drying treatments (mainly 1D and 3D) for gametophyte regeneration (Figure 4). This strategy has been reported for other groups of bryophytes (e.g., *Octoblepharum*, *Orthodontium*, and *Dicranoweisia*), with asexual propagule or vegetative regeneration being associated with habitat drying and directly influencing dispersal and establishment processes in these species [8,73–75].

4.3. Desiccation Tolerance of Hornwort Spores

Phaeoceros carolinianus and *A. lamellatus* exhibited higher spore survival and larger sporeling sizes under the different desiccation treatments, as was expected. Both species have thicker spore walls than those found in green spores, which apparently provides protection against desiccation [28,76]. Additionally, the inner spore walls in *Phaeoceros* and *Anthoceros* contain callose [77], an important polymer for the construction and maintenance of wall integrity [78]. Spores with callose may deal well with desiccation as that polymer provides a protective matrix [77,79,80], although that trait alone is not enough to explain all of the differences observed in our study (e.g., *Nothoceros* vs. *Dendroceros*).

Phaeoceros and *Anthoceros* spores also contain oils and starch as storage compounds, which can help provide greater longevity and drought resistance [28,36]. However, unlike green spores, they require longer times to mobilize their storage compounds and to develop protonemata (e.g., early gametophytes [28,41,43,81]). *Phaeoceros* and *Anthoceros*, as well as other hornworts with non-green spores [82], produce gametophytes with short lifespans that are able to resist dry seasons as spores in soil banks [44].

The DT responses of green and multicellular spores (e.g., endosporic germination) of *Dendroceros* species are likely associated with endospory. This trait is present in other bryophytes and assumed to be an adaptation to environments that experience periodic desiccation [36,83,84]. The differential responses of the spores of the two *Dendroceros* species could explain why *D. crispus* is widely distributed compared to *D. crispatus* [85]. The spores of *N. vincentianus* demonstrated the lowest performance due to their exosporic germination, which exposes the delicate protonema to drought conditions. The green spores of *Dendroceros crispus*, *D. crispatus*, and *N. vincentianus* also lack storage space for large amounts of oil [28,77,86]. This is unlike the unicellular green spores of some ferns, such as *Osmunda regalis*, which present lipids with the ability to protect the integrity of the membrane during desiccation [87].

4.4. Ecological Strategies of DT in Hornworts

We observed complex trade-offs of DT responses among the different life phases of hornworts, with contrasting life strategies linked to niche requirements, including (1) gametophytes that were sensitive and spores that were strongly tolerant of drying (*P. carolinianus* and *A. lamellatus*), (2) gametophytes and spores that were tolerant of dry periods (*D. crispus*), (3) tolerant gametophytes and sensitive spores (*D. crispatus*), and (4) both gametophytes and spores that were sensitive to drying (*N. vincentianus*). The above responses are similar to those described in the *DT continuum hypothesis* proposed for bryophytes [1], which states that plant life history phases (e.g., protonemata, juvenile shoots, adult shoots, spores, and others) can have different DT strategies, even within the same species and along an inducible or constitutive tolerance gradient [1,88].

This is the first study to highlight these trade-offs in hornworts, in line with similar differential DT strategies reported in both vascular and non-vascular plants. Sporophytes of the desert moss *Tortula inermis*, for example, were found to be more sensitive to rapid drying than their gametophytes [89]. The protonemata vs. juvenile shoots in *Bryum argenteum* [13] and the asexual propagules vs. shoots of *Syntrichia pagorum* also exhibited different DT responses. Equally, the gametophytes of some fern species (e.g., *Asplenium auritum* and *Polystichum retroso-paleaceum*) are more tolerant than their sporophytes [68,88,90]. The differential life strategies of hornworts may be important evolutionary triggers for spore dispersal and the maintenance of gametophytes in the field.

Finally, we have highlighted the importance of hornworts in tropical montane cloud forests, especially *Dendroceros* species as potential indicators of climate change. This genus presents unique ecological characteristics, such as its altitudinal distribution range (700–1000 m in Brazilian forests and up to 2300 in Colombian forests), its epiphytic niche, the height of the sporophyte (20–200 cm), and the degree of DT (gametophytes vs. multicellular spores). The aforementioned characteristics make this plant group particularly sensitive to climate change, as reported for vascular epiphytic species [91–93]. In addition, these

unique characteristics of *Dendroceros* can be related to the seasonality and conservation status of cloud forests and indicate possible alterations in their ecosystems [94]. Climate change may directly affect the climatic conditions of the forest, together with the diversity, distribution, establishment, and spread of *Dendroceros* and other hornwort species [95–97].

5. Conclusions

Our study highlights the existence of a DT continuum among hornwort species, with different life strategies associated with life phases (spores vs. gametophytes), niches, genera, and species. We corroborated the observed DT behaviors of *Dendroceros* gametophytes, which were linked to their epiphytic niche in montane forests. The endospory of *Dendroceros* spores conferred a moderate DT response, especially in *D. crispus*, although the high performance of the non-green spores of *Phaeoceros* and *Anthoceros* indicated their excellent potential to form soil banks in open habitats. The high desiccation sensitivities of both the spores and gametophytes of *Nothoceros vicentianus* are likely associated with the very humid niches in which that species lives. Differential DT strategies may increase the chances of spore dispersal and fast colonization (e.g., *Dendroceros*), or even spore bank formation, by *Phaeoceros* and *Anthoceros* species. Finally, rehydrating cycles suggest that long-term studies will provide evidence for a more complex DT continuum across many species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f14020255/s1>, File S1—Descriptions of the materials used in the desiccation tolerance tests of five hornwort species; File S2—Summary of the generalized linear models of the desiccation tolerances of the gametophytes and spores of the hornwort species tested; File S3—Summary of the post hoc Tukey honest significant difference (HSD) test of the gametophytes and spores of the hornwort species; Figure S1—Reference values for DT experiments with hornworts in this study. (A) Means of the F_v/F_m chlorophyll fluorescence parameters of hornwort gametophytes. (B,C) Hornworts cultured for 30 days. (B) Percentage spore survival. (C) Sporeling reference sizes. Error bars represent ± 1 SE; Figure S2—Means of the F_v/F_m chlorophyll fluorescence parameters of hornwort gametophytes. (A) Balanced relative humidity treatments; (B) *Dry durations*; (C) *postrehydration* periods. Error bars represent ± 1 SE.

Author Contributions: Conceptualization and methodology, G.F.P.-B. and A.S.M.-S.; collected data, T.V.-B. and G.F.P.-B.; data curation, validation, and statistical analysis, G.F.P.-B., J.C.V.A. and A.S.M.-S.; writing—original draft preparation, G.F.P.-B., J.C.V.A. and A.S.M.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded and supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES- Finance Code 001); Emerging Leaders in the Americas Program—ELAP (called 2018–2019); International Association for Plant Taxonomy (IAPT—Research Grant 2019); and the Rufford Foundation (Small Grants 2018–2019).

Data Availability Statement: All data for this study are available in Supplemental Materials.

Acknowledgments: This research was carried out with permission from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO, license number 58259-8). The first author thanks the park rangers and Diniz, V.S, Magalhaes, M.D, and Tomasi, M.O. for their help in the field, as well as Oliveira, M.F., for his invaluable help with the experiments and recording the information.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Stark, L.; Brinda, J. Developing sporophytes transition from an inducible to a constitutive ecological strategy of desiccation tolerance in the moss *Aloina ambigua*: Effects of desiccation on fitness. *Ann. Bot.* **2015**, *115*, 593–603. [CrossRef] [PubMed]
2. Wood, A.J. The nature and distribution of vegetative desiccation-tolerance in hornworts, liverworts and mosses. *Bryologist* **2007**, *110*, 163–177. [CrossRef]
3. Koster, K.; Balsamo, R.; Espinoza, C.; Oliver, M. Desiccation sensitivity and tolerance in the moss *Physcomitrella patens*: Assessing limits and damage. *Plant Growth Regul.* **2010**, *62*, 293–302. [CrossRef]
4. Alpert, P. The discovery, scope, and puzzle of desiccation tolerance in plants. *Plant Ecol.* **2000**, *151*, 5–17. [CrossRef]

5. Holzinger, A.; Karsten, U. Desiccation stress and tolerance in green algae: Consequences for ultrastructure, physiological and molecular mechanisms. *Front. Plant Sci.* **2013**, *4*, 327. [\[CrossRef\]](#)
6. Kranner, I.; Beckett, R.; Hochman, A.; Nash, T. Desiccation-tolerance in Lichens: A review. *Bryologist* **2008**, *111*, 576–593. [\[CrossRef\]](#)
7. Potts, M.; Slaughter, S.M.; Hunneke, F.U.; Garst, J.F.; Helm, R.F. Desiccation tolerance of prokaryotes: Application of principles to human cells. *Integr. Comp. Biol.* **2005**, *45*, 800–809. [\[CrossRef\]](#)
8. Proctor, M.; Oliver, M.; Wood, A.; Alpert, P.; Stark, L.; Cleavitt, N.; Mishler, B. Desiccation-tolerance in bryophytes: A review. *Bryologist* **2007**, *110*, 595–621. [\[CrossRef\]](#)
9. Oliver, M. Desiccation tolerance in bryophytes: A reflection of the primitive strategy for plant survival in dehydrating habitats? *Integr. Comp. Biol.* **2005**, *45*, 788–799. [\[CrossRef\]](#)
10. Stark, L.R. Ecology of desiccation tolerance in bryophytes: A conceptual framework and methodology. *Bryologist* **2017**, *120*, 130–165. [\[CrossRef\]](#)
11. Oliver, M.; Farrant, J.; Hilhorst, H.W.M.; Mundree, S.; Williams, B.; Bewley, J.D. Desiccation tolerance: Avoiding cellular damage during drying and rehydration. *Annu. Rev. Plant Biol.* **2020**, *71*, 435–460. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Lughadha, E.N.; Govaerts, R.; Belyaeva, I.; Black, N.; Lindon, H.; Allkin, R.; Magill, R.E.; Nicolson, N. Counting counts: Revised estimates of numbers of accepted species of flowering plants, seed plants, vascular plants and land plants with a review of other recent estimates. *Phytotaxa* **2016**, *272*, 82–88. [\[CrossRef\]](#)
13. Greenwood, J.L.; Stark, L.; Chiquoine, L. Effects of rate of drying, life history phase, and ecotype on the ability of the moss *Bryum Argenteum* to survive desiccation events and the influence on conservation and selection of material for restoration. *Front. Ecol. Evol.* **2019**, *7*, 388. [\[CrossRef\]](#)
14. Porembski, S.; Barthlott, W. Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation tolerant vascular plants. *Plant Ecol.* **2000**, *151*, 19–28. [\[CrossRef\]](#)
15. Rascio, N.; Rocca, N. La Resurrection plants: The puzzle of surviving extreme vegetative desiccation. *CRC Crit. Rev. Plant Sci.* **2005**, *24*, 209–225. [\[CrossRef\]](#)
16. Bewley, J. Physiological aspects of desiccation tolerance. *Annu. Rev. Plant Physiol.* **1979**, *30*, 195–238. [\[CrossRef\]](#)
17. Carvalho, R.C.; Branquinho, C.; da Silva, J.M. Physiological consequences of desiccation in the aquatic bryophyte *Fontinalis antipyretica*. *Planta* **2011**, *234*, 195–205. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Green, T.G.A.; Sancho, L.G.; Pintado, A. Ecophysiology of Desiccation/Rehydration Cycles in Mosses and Lichens. In *Plant Desiccation Tolerance. Ecological Studies (Analysis and Synthesis)*; Lüttge, U., Beck, E., Bartels, D., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; Volume 215, pp. 89–120. ISBN 978-3-642-19106-0.
19. Brinda, J.; Stark, L.; Clark, T.; Greenwood, J. Embryos of a moss can be hardened to desiccation tolerance: Effects of rate of drying on the timeline of recovery and dehardening in *Aloina ambigua* (Pottiaceae). *Ann. Bot.* **2016**, *117*, 153–163. [\[CrossRef\]](#)
20. Söderström, L.; Hagborg, A.; von Konrat, M.; Bartholomew-Began, S.; Bell, D.; Briscoe, L.; Brown, E.; Cargill, D.C.; da Costa, D.P.; Crandall-Stotler, B.J.; et al. World checklist of hornworts and liverworts. *PhytoKeys* **2016**, *59*, 1–828. [\[CrossRef\]](#)
21. Desirò, A.; Duckett, J.; Pressel, S.; Villarreal, J.C.; Bidartondo, M. Fungal symbioses in hornworts: A chequered history. *Proc. R. Soc. B Biol. Sci.* **2013**, *280*, 20130207. [\[CrossRef\]](#)
22. Villarreal, J.C.; Renzaglia, K. Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): A novel symbiosis in land plants. *Am. J. Bot.* **2006**, *93*, 693–705. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Nelson, J.; Hauser, D.; Li, F.-W. Symbiotic cyanobacteria communities in hornworts across time, space, and host species. *bioRxiv* **2020**. [\[CrossRef\]](#)
24. Villarreal, J.C.; Renner, S. Hornwort pyrenoids, carbon-concentrating structures, evolved and were lost at least five times during the last 100 million years. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18873–18878. [\[CrossRef\]](#)
25. Li, F.-W.; Villarreal, J.C.; Szövényi, P. Hornworts: An overlooked window into carbon-concentrating mechanisms. *Trends Plant Sci.* **2017**, *22*, 275–277. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Villarreal, J.C.; Renzaglia, K. Sporophyte structure in the neotropical hornwort *Phaeomegaceros fimbriatus*: Implications for phylogeny, taxonomy, and character evolution. *Int. J. Plant Sci.* **2006**, *167*, 413–427. [\[CrossRef\]](#)
27. Peñaloza-Bojacá, G.; Villarreal, J.C.; Maciel-Silva, A. Phylogenetic and morphological infrageneric classification of the genus *Dendroceros* (Dendrocerotaceae; Anthocerotophyta), with the addition of two new subgenera. *Syst. Biodivers.* **2019**, *17*, 712–727. [\[CrossRef\]](#)
28. Renzaglia, K.; Villarreal, J.C.; Duff, R.J.; Goffinet, B. New Insights into Morphology, Anatomy, and Systematics of Hornworts. In *Bryophyte Biology*; Shaw, A.J., Ed.; Cambridge University Press: Cambridge, UK, 2009; pp. 139–172.
29. Renzaglia, K.; Villarreal, J.C.; Garbary, D. Morphology supports the setaphyte hypothesis: Mosses plus liverworts form a natural group. *Bryophyt. Divers. Evol.* **2018**, *40*, 11–17. [\[CrossRef\]](#)
30. Leebens-Mack, J.; Barker, M.; Carpenter, E.; Deyholos, M.; Gitzendanner, M.; Graham, S.; Grosse, I.; Li, Z.; Melkonian, M.; Mirarab, S.; et al. One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **2019**, *574*, 679–685. [\[CrossRef\]](#)
31. Wickett, N.J.; Mirarab, S.; Nguyen, N.; Warnow, T.; Carpenter, E.; Matasci, N.; Ayyampalayam, S.; Barker, M.S.; Burleigh, J.G.; Gitzendanner, M.A.; et al. Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E4859–E4868. [\[CrossRef\]](#)
32. Duff, J.; Villarreal, J.C.; Cargill, C.; Renzaglia, K. Progress and challenges toward developing a phylogeny and classification of the hornworts. *Bryologist* **2007**, *110*, 214–243. [\[CrossRef\]](#)

33. Gradstein, S.R.; Churchill, S.P.; Salazar-Allen, N. *Guide to the Bryophytes of Tropical America*; The New York Botanical Garden Press: New York, NY, USA, 2001; Volume 86, ISBN 0-89327-435-6.
34. Villarreal, J.C.; Renzaglia, K. The hornworts: Important advancements in early land plant evolution. *J. Bryol.* **2015**, *37*, 157–170. [\[CrossRef\]](#)
35. Hosokawa, T.; Kubota, H. On the osmotic pressure and resistance to desiccation of epiphytic mosses from a beech forest, south-west Japan. *J. Ecol.* **1957**, *45*, 579. [\[CrossRef\]](#)
36. Schuette, S.; Renzaglia, K. Development of multicellular spores in the hornwort genus *Dendroceros* (Dendrocerotaceae, Anthocerotophyta) and the occurrence of endospory in Bryophytes. *Nov. Hedwigia* **2010**, *91*, 301–316. [\[CrossRef\]](#)
37. Leon-Vargas, Y.; Engwald, S.; Proctor, M. Microclimate, light adaptation and desiccation tolerance of epiphytic bryophytes in two Venezuelan cloud forests. *J. Biogeogr.* **2006**, *33*, 901–913. [\[CrossRef\]](#)
38. Stark, L.; Greenwood, J.; Brinda, J.; Oliver, M. The desert moss *Pterygoneurum lamellatum* (Pottiaceae) exhibits an inducible ecological strategy of desiccation tolerance: Effects of rate of drying on shoot damage and regeneration. *Am. J. Bot.* **2013**, *100*, 1522–1531. [\[CrossRef\]](#) [\[PubMed\]](#)
39. McLetchie, N.; Stark, L. Rate of drying influences tolerance of low water contents in the moss *Funaria hygrometrica* (Funariaceae). *Bryologist* **2019**, *122*, 271. [\[CrossRef\]](#)
40. Lloyd, R.M.; Klekowski, E.J. Spore germination and viability in Pteridophyta: Evolutionary significance of chlorophyllous spores. *Biotropica* **1970**, *2*, 129. [\[CrossRef\]](#)
41. Maciel-Silva, A.; Da Silva, F.; Válio, I. All green, but equal? Morphological traits and ecological implications on spores of three species of mosses in the Brazilian Atlantic Forest. *An. Acad. Bras. Cienc.* **2014**, *86*, 1249–1262. [\[CrossRef\]](#)
42. Pence, V. Survival of *Chlorophyllous* and *Nonchlorophyllous* fern spores through exposure to liquid nitrogen. *Am. Fern J.* **2000**, *90*, 119–126. [\[CrossRef\]](#)
43. Sundue, M.; Vasco, A.; Moran, R. Cryptochlorophyllous spores in ferns: Nongreen spores that contain chlorophyll. *Int. J. Plant Sci.* **2011**, *172*, 1110–1119. [\[CrossRef\]](#)
44. Bisang, I. Quantitative analysis of the diaspore banks of bryophytes and ferns in cultivated fields in Switzerland. *Lindbergia* **1996**, *21*, 9–20.
45. Villarreal, J.C.; Cargill, D.; Hagborg, A.; Soderstrom, L.; Renzaglia, K. A synthesis of hornwort diversity: Patterns, causes and future work. *Phytotaxa* **2014**, *9*, 150. [\[CrossRef\]](#)
46. Renzaglia, K.; Vaughn, K. Anatomy, development and classification of hornworts. In *Bryophyte Biology*; Shaw, A.J., Goffinet, B., Eds.; Cambridge University Press: Cambridge, UK, 2000; pp. 1–35.
47. Renzaglia, K. A comparative morphology and developmental anatomy of the Anthocerotophyta. *J. Hattori Bot. Lab.* **1978**, *44*, 31–90.
48. Villarreal, J.C.; Campos, L.V.; Uribe-M., J.; Goffinet, B. Parallel evolution of endospory within hornworts: *Nothoceros renzagliensis* (Dendrocerotaceae), sp. nov. *Syst. Bot.* **2012**, *37*, 31–37. [\[CrossRef\]](#)
49. Villarreal, J.C.; Renner, S. A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a taxonomically cleaned-up genus *Nothoceros*. *Mol. Phylogenet. Evol.* **2014**, *78*, 25–35. [\[CrossRef\]](#)
50. Penjor, P.; Chantanaorrapint, S.; Meesawat, U. Morphological and anatomical features of cosmopolitan hornwort: *Phaeoceros carolinianus* (Michx.) Prosk. *Walailak J. Sci. Technol.* **2016**, *13*, 769–779.
51. Proskauer, J. Studies on Anthocerotales V. *Phytomorphology* **1957**, *7*, 113–135.
52. Ibarra-Morales, A.; Muñiz, M.E.; Valencia, S. The genus *Anthoceros* (Anthocerotaceae, Anthocerotophyta) in central Mexico. *Phytotaxa* **2015**, *205*, 215–228. [\[CrossRef\]](#)
53. IBAMA Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Available online: <https://uc.socioambiental.org/> (accessed on 16 March 2020).
54. INEA Plano de Manejo—Parque Estadual Dos Três Picos (PETP). Available online: <http://www.inea.rj.gov.br/> (accessed on 23 December 2022).
55. Hájek, T.; Vicherová, E. Desiccation tolerance of *Sphagnum* revisited: A puzzle resolved. *Plant Biol.* **2014**, *16*, 765–773. [\[CrossRef\]](#)
56. Hellwege, E.M.; Dietz, K.-J.; Volk, O.H.; Hartung, W. Absciscic acid and the induction of desiccation tolerance in the extremely xerophilic liverwort *Exormothes holstii*. *Planta* **1994**, *194*, 525–531. [\[CrossRef\]](#)
57. Beckett, R. Partial dehydration and ABA induce tolerance to desiccation-induced ion leakage in the moss *Atrichum androgynum*. *S. Afr. J. Bot.* **1999**, *65*, 212–217. [\[CrossRef\]](#)
58. Butler, W.L.; Kitajima, M. Fluorescence quenching in Photosystem II of chloroplasts. *Biochim. Biophys. Acta Bioenerg.* **1975**, *376*, 116–125. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Genty, B.; Briantais, J.-M.; Baker, N. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta Gen. Subj.* **1989**, *990*, 87–92. [\[CrossRef\]](#)
60. Cruz de Carvalho, R.; Maurício, A.; Pereira, M.F.; Marques da Silva, J.; Branquinho, C. All for one: The role of colony morphology in Bryophyte desiccation tolerance. *Front. Plant Sci.* **2019**, *10*, 1360. [\[CrossRef\]](#)
61. Marks, R.A.; Burton, J.F.; McLetchie, D.N. Sex differences and plasticity in dehydration tolerance: Insight from a tropical liverwort. *Ann. Bot.* **2016**, *118*, 347–356. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Nakazato, T.; Kadota, A.; Wada, M. Photoinduction of spore germination in *Marchantia polymorpha* L. is mediated by photosynthesis. *Plant Cell Physiol.* **1999**, *40*, 1014–1020. [\[CrossRef\]](#)

63. R Core Team. R: A Language and Environment for Statistical Computing. Available online: <https://www.r-project.org/> (accessed on 15 October 2018).
64. RStudio Team. RStudio: Integrated Development Environment for R. Available online: <http://www.rstudio.com/> (accessed on 20 February 2019).
65. Wickham, H.; Chang, W. Ggplot2: An Implementation of the Grammar of Graphics. R Package Version 0.8.3. Available online: <http://CRAN.R-project.org/package=ggplot2>, 3 (accessed on 4 December 2019).
66. Kemp, F. Modern Applied Statistics with S. *J. R. Stat. Soc. Ser. D Stat.* **2003**, *52*, 704–705. [\[CrossRef\]](#)
67. Renzaglia, K.; Schuette, S.; Duff, J.; Ligrone, R.; Shaw, A.J.; Mishler, B.D.; Duckett, J.G. Bryophyte phylogeny: Advancing the molecular and morphological frontiers. *Bryologist* **2007**, *110*, 179–213. [\[CrossRef\]](#)
68. Watkins, J.; Mack, M.; Sinclair, T.; Mulkey, S. Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. *N. Phytol.* **2007**, *176*, 708–717. [\[CrossRef\]](#)
69. Abel, W. Die Austrocknungsresistenz der Laubmoose. *Sitzungsberichte der Akad. der Wissenschaften Math. Klasse* **1956**, *165*, 619–707.
70. Lakatos, M. Lichens and Bryophytes: Habitats and Species. In *Plant Desiccation Tolerance. Ecological Studies (Analysis and Synthesis)*; Lüttge, U., Beck, E., Bartels, D., Eds.; Ecological Studies; Springer: Berlin/Heidelberg, Germany, 2011; Volume 215, pp. 65–87.
71. Larson, D.W. Differential wetting in some lichens and mosses: The role of morphology. *Bryologist* **1981**, *84*, 1. [\[CrossRef\]](#)
72. Hoekstra, F.; Golovina, E.; Buitink, J. Mechanisms of plant desiccation tolerance. *Trends Plant Sci.* **2001**, *6*, 431–438. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Zhang, L.; Ma, P.; Chu, L.; But, P.P. Three modes of asexual reproduction in the moss *Octoblepharum albidum*. *J. Bryol.* **2003**, *25*, 175–179. [\[CrossRef\]](#)
74. Duckett, J.; Ligrone, R. A survey of diaspore liberation mechanisms and germination patterns in mosses. *J. Bryol.* **1992**, *17*, 335–354. [\[CrossRef\]](#)
75. Duckett, J.; Goode, J.; Matcham, H. Studies of protonemal morphogenesis in mosses. VIII. The gemmiferous protonemata of *Orthodontium* and *Dicranoweisia*. *J. Bryol.* **2001**, *23*, 181–193. [\[CrossRef\]](#)
76. Mogensen, G. The biological significance of morphological characters in bryophytes: The Spore. *Bryologist* **1981**, *84*, 187. [\[CrossRef\]](#)
77. Renzaglia, K.; Lopez, R.; Welsh, R.; Owen, H.; Merced, A. Callose in sporogenesis: Novel composition of the inner spore wall in hornworts. *Plant Syst. Evol.* **2020**, *306*, 16. [\[CrossRef\]](#)
78. Ellinger, D.; Voigt, C.A. Callose biosynthesis in arabidopsis with a focus on pathogen response: What we have learned within the last decade. *Ann. Bot.* **2014**, *114*, 1349–1358. [\[CrossRef\]](#)
79. Yim, K.; Bradford, K.J. Callose deposition is responsible for apoplastic semipermeability of the endosperm envelope of muskmelon seeds. *Plant Physiol.* **1998**, *118*, 83–90. [\[CrossRef\]](#)
80. Johri, B.M. (Ed.) *Embryology of Angiosperms*; Springer: Berlin/Heidelberg, Germany, 1984; ISBN 978-3-642-69304-5.
81. Mogensen, G. The Spore. *New Man. Bryol.* **1983**, 325–342.
82. Oliveira, B.A.; Pereira, A.F.d.N.; Pôrto, K.C.; Maciel-Silva, A.S. Spore germination and young gametophyte development of the endemic Brazilian hornwort *Notothylas vitalii* Udar & Singh (Notothyladaceae—Anthocerotophyta), with insights into sporeling evolution. *Acta Bot. Bras.* **2017**, *31*, 313. [\[CrossRef\]](#)
83. López-Pozo, M.; Fernández-Marín, B.; García-Plazaola, J.I.; Ballesteros, D. Desiccation tolerance in ferns: From the unicellular spore to the multi-tissular sporophyte. In *Current Advances in Fern Research*; Fernández, H., Ed.; Springer International Publishing: Cham, Switzerland, 2018; pp. 401–426. ISBN 978-3-319-75103-0.
84. Maciel-Silva, A.; Pôrto, K. Reproduction in Bryophytes. In *Reproductive Biology of Plants*; Ramawat, K., Mérillon, J.-M., Shivanna, R., Eds.; CRC Press: Boca Raton, FL, USA, 2014; pp. 57–84. ISBN 9781482201321.
85. Peñaloza-Bojacá, G.; Oliveira, B.; Araujo, C.A.T.; Villarreal, J.C.; Fantecelle, L.B.; Maciel-Silva, A. Dendrocerotaceae in Flora Do Brasil 2020. Available online: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB97157> (accessed on 19 March 2021).
86. Ligrone, R.; Renzaglia, K. The sporophyte–gametophyte junction in the hornwort, *Dendroceros tubercularis* Hatt (Anthocerotophyta). *New Phytol.* **1990**, *114*, 497–505. [\[CrossRef\]](#) [\[PubMed\]](#)
87. López-Pozo, M.; Gasulla, F.; García-Plazaola, J.I.; Fernández-Marín, B. Unraveling metabolic mechanisms behind chloroplast desiccation tolerance: Chlorophyllous fern spore as a new promising unicellular model. *Plant Sci.* **2019**, *281*, 251–260. [\[CrossRef\]](#)
88. Testo, W.; Watkins, J. Influence of plant size on the ecophysiology of the epiphytic fern *Asplenium auritum* (Aspleniaceae) from Costa Rica. *Am. J. Bot.* **2012**, *99*, 1840–1846. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Stark, L.; Oliver, M.; Mishler, B.; McLetchie, N. Generational differences in response to desiccation stress in the desert moss *Tortula inermis*. *Ann. Bot.* **2007**, *99*, 53–60. [\[CrossRef\]](#)
90. Sato, T.; Sakai, A. Freezing resistance of gametophytes of the temperate fern, *Polystichum retroso-paleaceum*. *Can. J. Bot.* **1980**, *58*, 1144–1148. [\[CrossRef\]](#)
91. Mendieta-Leiva, G.; Ramos, F.N.; Elias, J.P.C.; Zotz, G.; Acuña-Tarazona, M.; Alvim, F.S.; Barbosa, D.E.F.; Basilio, G.A.; Batke, S.P.; Benavides, A.M.; et al. EpIG-DB: A database of vascular epiphyte assemblages in the Neotropics. *J. Veg. Sci.* **2020**, *31*, 518–528. [\[CrossRef\]](#)
92. Laube, S.; Zotz, G. Neither host-specific nor random: Vascular epiphytes on three tree species in a panamanian lowland forest. *Ann. Bot.* **2006**, *97*, 1103–1114. [\[CrossRef\]](#)
93. Zotz, G.; Bogusch, W.; Hietz, P.; Ketteler, N. Growth of epiphytic bromeliads in a changing world: The effects of CO₂, water and nutrient supply. *Acta Oecologica* **2010**, *36*, 659–665. [\[CrossRef\]](#)

94. Benzing, D.H. Vulnerabilities of tropical forests to climate change: The significance of resident epiphytes. *Clim. Chang.* **1998**, *39*, 519–540. [[CrossRef](#)]
95. Pardow, A.; Lakatos, M. Desiccation tolerance and global change: Implications for tropical bryophytes in lowland forests. *Biotropica* **2013**, *45*, 27–36. [[CrossRef](#)]
96. Walsworth, T.E.; Schindler, D.E.; Colton, M.A.; Webster, M.S.; Palumbi, S.R.; Mumby, P.J.; Essington, T.E.; Pinsky, M.L. Management for network diversity speeds evolutionary adaptation to climate change. *Nat. Clim. Chang.* **2019**, *9*, 632–636. [[CrossRef](#)]
97. Jump, A.S.; Penuelas, J. Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecol. Lett.* **2005**, *8*, 1010–1020. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.