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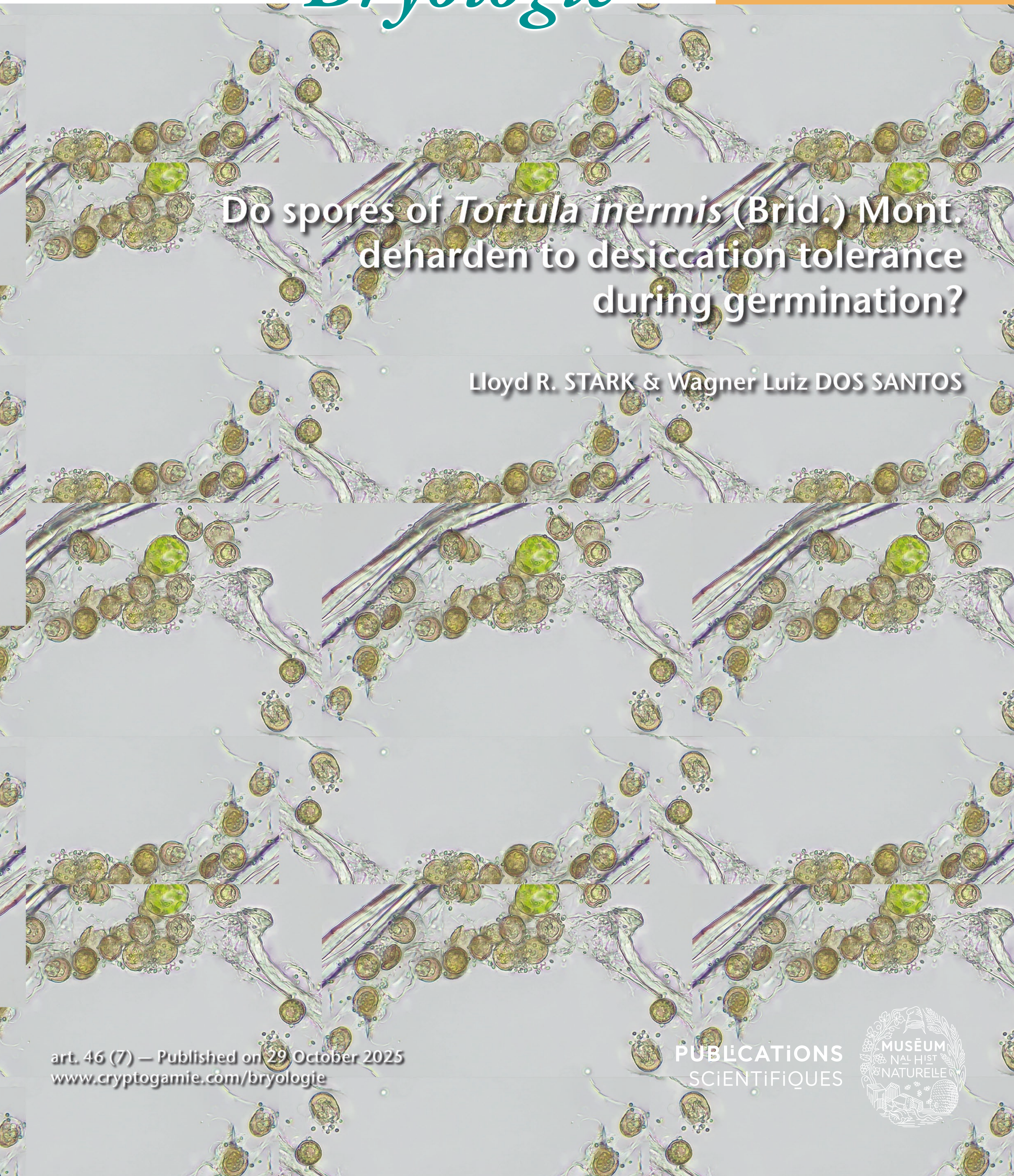

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Do spores of *Tortula inermis* (Brid.) Mont. deharden to desiccation tolerance during germination?

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ABSTRACT
The potential for spores of a moss to deharden to desiccation tolerance during the 48 hours prior to germination is investigated for perhaps the first time in a moss. Spores from capsules of *Tortula inermis* (Brid.) Mont. stored for 13 years desiccated in darkness were rehydrated for 3, 6, 12, 24, 36, or 48 hours and then rapidly dried to equilibration with *c.* 34% relative humidity at *c.* 22°C. The spores were then rehydrated and germination observed on days 3, 7, and 14. Control spores that were not subject to desiccation began to germinate on day 3, and reached 50% germination by day 7 postrehydration. Spores dried rapidly 3-24 hours postrehydration exhibited control levels of germination (50-60%) by day 14 postrehydration, indicating that spores did not deharden during the first 24 hours. However, the germination percentage of spores dried rapidly at 36 and 48 hours postrehydration declined to *c.* 20%, indicating a vulnerability to desiccation just prior to germination. During this latter period, the percentage of abortive spores is relatively high, and appeared to have aborted in a 12-hour period (24-36 h). Nevertheless, 20% of the spores survived a rapid drying event just prior to germination. This strategy of water stress tolerance ensures survival capabilities even during interruptions of the germination process by sudden, harsh, desiccation events where the hydrated spore desiccates in under an hour. Given known dehardening times of ≥7 days in bryophyte species studied, dehardening dynamics of spores is of the shortest duration known thus far in bryophytes (1.5 days).

KEY WORDS
Pregermination, protonema, abortive spores, water stress, sporeling, germ tube, rehydration.

RÉSUMÉ
Les spores de Tortula inermis *(Brid.) Mont. deviennent-elles résistantes à la dessiccation pendant la germination?*
La possibilité pour les spores d'une mousse de se dessécher jusqu'à la tolérance à la dessiccation pendant les 48 heures précédant la germination est étudiée, peut-être pour la première fois, chez une mousse. Des spores de capsules de *Tortula inermis* (Brid.) Mont. conservées pendant 13 ans et desséchées dans l'obscurité ont été réhydratées pendant 3, 6, 12, 24, 36 ou 48 heures, puis séchées

rapidement jusqu'à l'équilibre avec une humidité relative de *c.* 34% à *c.* 22°C. Les spores ont ensuite été réhydratées et la germination a été observée aux jours 3, 7 et 14. Les spores témoins qui n'ont pas été soumises à la dessiccation ont commencé à germer le troisième jour et ont atteint 50 % de germination le septième jour après la réhydratation. Les spores séchées rapidement 3-24 heures après la réhydratation présentaient des niveaux de contrôle de la germination (50-60%) au 14ème jour après la réhydratation, ce qui indique que les spores ne se sont pas déshydratées au cours des premières 24 heures. Cependant, le pourcentage de germination des spores séchées rapidement à 36 et 48 heures après la réhydratation a diminué à *c.* 20%, indiquant une vulnérabilité à la dessiccation juste avant la germination. Au cours de cette dernière période, le pourcentage de spores avortées est relativement élevé et semble avoir avorté sur une période de 12 heures (24-36 heures). Néanmoins, 20 % des spores ont survécu à une dessiccation rapide juste avant la germination. Cette stratégie de tolérance au stress hydrique garantit des capacités de survie même en cas d'interruption du processus de germination par des événements de dessiccation soudains et brutaux, où la spore hydratée se dessèche en moins d'une heure. Compte tenu des temps de dessiccation connus de ≥7 jours chez les espèces de bryophytes étudiées, la dynamique de dessiccation des spores est de la durée la plus courte connue jusqu'à présent chez les bryophytes (1,5 jour).

MOTS CLÉS
Pré-germination, protonéma, spores avortées, stress hydrique, spore, tube germinatif, réhydratation.

INTRODUCTION

Bryophyte plants and structures are mostly poikilohydric, subject to fluctuations in water content depending on prevailing moisture conditions in the microenvironment. As a result, gametophores, rhizoids, and propagules in the field may be variably acclimated (hardened) to drying. In general, juvenile phenophases (protonemata, juvenile shoots, embryos) and rapidly growing tissues exhibit an inducibly desiccation-tolerant (IDT) ecological strategy of desiccation tolerance (DT), whereas adult shoots of mosses may be either IDT or constitutively DT (CDT; Werner *et al.* 1991; Pressel & Duckett 2010; Stark *et al.* 2012, 2016; Greenwood *et al.* 2019; Coe *et al.* 2021). The DT strategy of bryophyte propagules (spores, gemmae, tubers) is largely unknown, aside from propagules being generally DT (Duckett & Ligrone 1994). Diaspores of several species are DT at maturity (e.g. Duckett *et al.* 1993; Goode *et al.* 1994). However, the strategy of gemma DT is seldom explored, with protonemal gemmae (IDT) and leaf gemmae (CDT) exhibiting differing ecological strategies in the genus *Syntrichia* Brid. (Stark *et al.* 2016; Coe *et al.* 2021). As pointed out by Michael Proctor in Proctor *et al.* (2007), "For all but species of the most humid habitats, spores must be DT if they are to be effective agents of dispersal." Within cylindrical capsules of many mosses, spores are expected to experience multiple wet/dry cycles, and when drying out the exothecial cells of the capsule wall constrict and force the spores distally (Vitt 1981), ensuring a desiccated (and desiccation-tolerant) spore at dispersal. Exceptions include species with hydrocastique peristomes and some annual bryophytes, which release spores under wet conditions. Unlike gametophores and gemmae, sporophytes of many mosses may be homoiohydric, with capsules restricting water loss during spore maturation; mature spores in this case have experienced a protracted slow-drying period (or cycles of hydration and then slow drying) culminating in a typically hardened condition (Duckett & Pressel 2022). This hardened condition allows spores to tolerate, during dispersal, severe atmospheric conditions of long- and short-term desiccation, freezing, and UV exposure (Van Zanten & Pócs 1981; Glime 2017a).

Variation in spore viability and the ability to germinate can be affected by differences in: 1) nutrition and moisture availability within the capsule where position within the capsule could place some spore regions at an advantage; 2) gametophore to gametophore variability in energy available to transfer to the sporophyte; 3) spore size and carbohydrate reserves of the spore; and 4) proximity to columella (Glime & Knoop 1986; Mogensen 1978; Glime 2017b). Few studies have addressed bryophyte spore tolerance of wet/dry cycles (Fan *et al.* 2023), and few/none investigated potential dehardening dynamics in germinating spores. Newton (1972) came closest to testing for the presence of spore dehardening. However, the species tested (*Plagiomnium undulatum* (Hedw.) T.J.Kop.) had spores that began germination (distended spores and germ tubes) on day 2 postrehydration, resulting in desiccation of a combination of spores and sporelings. In addition, 45% of spores were abortive at dispersal in the populations studied, and the rate of drying and equilibrating relative humidity (RH) was not given but was probably a slow drying period over *c.* 2 days. Nevertheless, at 20°C, germination was 15% lower (85 vs 100%) in spores that were desiccated and subsequently rehydrated, and at 10°C, germination was significantly delayed (day 11 vs day 3) and germination was 40% below controls. Her data suggest that a slow drying period during pregermination may have a negative effect on germination levels in *P. undulatum*.

Dehardening is the process of dissipating the hardened condition to DT, becoming vulnerable to a rapid drying event. Bryophyte gametophores and embryos are known to deharden in at least five species, with reported dehardening times of ≥7 days (Hellwege *et al.* 1994; Beckett 1999; Hájek & Vicherová 2014; Stark *et al.* 2014; Brinda *et al.* 2016). The presence of dehardening indicates an IDT ecological strategy. In plants, desiccation constrains, or trades-off, with growth, the productivity-tradeoff hypothesis (Alpert 2006). This applies not only to clades, but to individual plant structures,

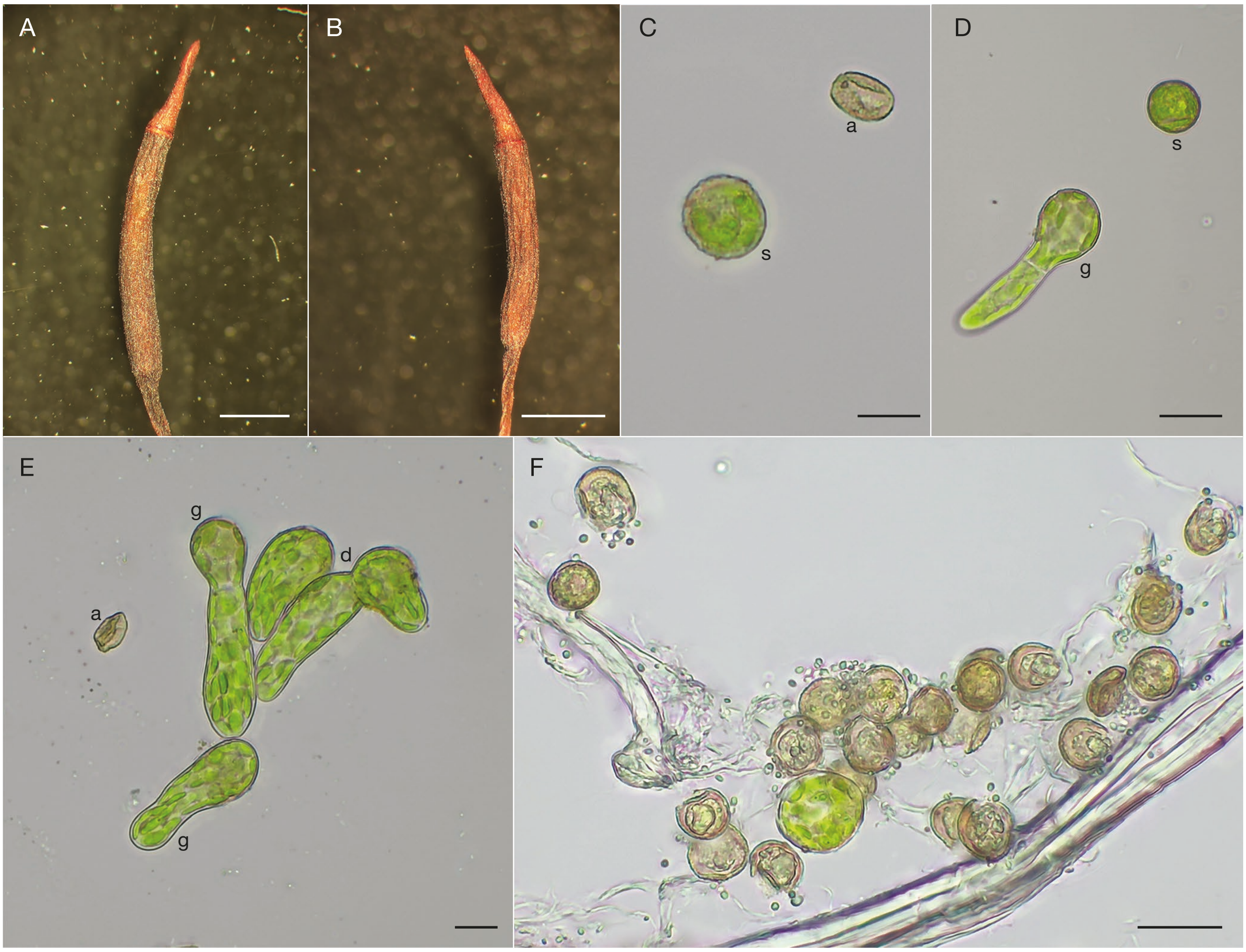

Fig. 1. — *Tortula inermis* (Brid.) Mont.: **A**, **B**, 13-year-old typical experimental capsules; **C**-**F**, spores at various phases of germination: **a**, abortive; **d**, distended; **g**, with germ tube; **s**, swelling; **C**, control day 2; **D**, control day 6; **E**, control day 12; **F**, 48 h dehardened day 14, showing mostly abortive spores under microbial attack and one swollen spore. Scale bars: A, B, 1 mm; C-E, 10 µm; F, 20 µm.

consistent with rapidly growing juvenile phenophases often exhibiting a completely dehardened condition (references above). Spores of many bryophytes germinate in culture in a few days (Valanne 1966; Glime 2017b). We ask the question, do spores of *Tortula inermis* (Brid.) Mont. deharden to desiccation tolerance in the 48 hours leading up to visibly germinating spores (called the *pregermination* period here)? The possibility of spores dehardening to DT was acknowleged by Mogensen (1978) in *Cinclidium* Sw., where spores in the swollen stage (within the capsule in this genus) tended to abort if desiccated prior to dispersal. Juvenile structures in bryophytes deharden when experiencing relatively long periods of hydration, and the spore can be considered a juvenile haploid structure. We therefore predict that spores deharden to desiccation tolerance during the pregermination process, and adopt an IDT strategy of DT in the hours prior to visible distention. The expected tradeoff between rapid growth (germination) and stress tolerance predicts a diminution of constitutive tolerance during the pregermination period, as the spore commits its resources to germination and away from a tolerance to rapid drying, switching to the less costly inducible strategy (although not explicitly tested here). An alternative (and null) hypothesis is that the spore, constitutively protected at dispersal in *T. inermis*, retains the CDT condition in the days leading up to germination. The presence or absence of dehardening in spores should greatly influence the fitness of newly dispersed spores.

MATERIAL AND METHODS

Study species

Tortula inermis (Pottiaceae) is one of the dominant bryophyte species of the Southwestern United States deserts (Flowers 1973), and can occupy very xeric sites in the Mojave Desert of California and Nevada (Stark 1997; Malcolm *et al.* 2009). The species occurs in aridlands of Northern America, Mediterranean Europe, Southern Asia, and Northern Africa (Mishler 1994; Zander & Eckel 2007). *Tortula inermis* is characterized by ligulate leaves that often have a mucronate apex; leaf

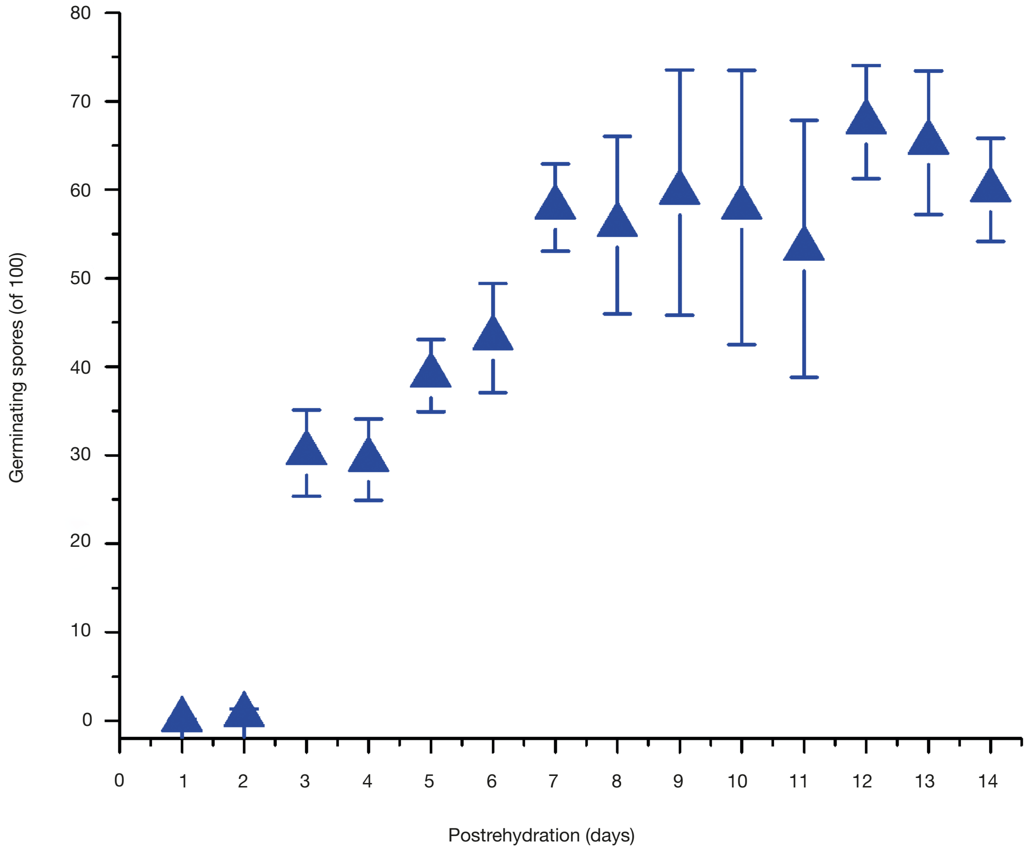

FIG. 2. — Spore germination from 13-year old capsules of *Tortula inermis* (Brid.) Mont. in the control (undried) treatment. Means ± one S.E. (standard error). N = 9 for postrehydration days 3, 7, and 14, N = 3 for other days, with each N value corresponding to a count of 100 spores from a single capsule.

margins recurved from base to apex; a gonioautoicous sexual condition; and when dry the leaves twist spirally around the stem in a characteristic manner (Zander & Eckel 2007). Patches frequently produce sporophytes, which take at least 18 months to mature, with spore dispersal occurring gradually over a three-year period (Stark 2001). Operculate capsules were collected in 2011 from southern Nevada, United States, and stored dry in the dark until the onset of the present experiment: United States, Nevada, Clark County, Newberry Mts, Grapevine Canyon, on north facing soiled slope, *c.* 850 m alt., *Llo Stark 8 May 2011* (voucher specimen at *UNLV*). The upright and relatively long (*c.* 2-4 mm) cylindrical capsules of *T. inermis* (Fig. 1A) lose their opercula during dry periods in the Mojave Desert, and spores are liberated gradually, with some spores remaining inside the capsule for up to 3 years without germinating precociously (Stark 2001).

EXPERIMENTAL DESIGN

Spores of *Tortula inermis* were allowed to rehydrate for six different periods (3, 6, 12, 24, 36, 48 h) under conditions given below for spore rehydration (24-90 µmol m^{-2} s^{-1} Photosynthetic Active Radiation (PAR), 12-h photoperiod, 18-24 °C), and after each dehardening time were subjected to a rapid-dry (RD) treatment (<1 h from full turgor to equilibration with 34% RH). Control spores were allowed to germinate normally (without interruption). The dehardening periods were selected after control germination trials demonstrated that distended spores and spores with germ tubes first appeared on day 3 postrehydration (Fig. 2), and we did not want to mix spores with sporelings. Thus, dehardening dynamics were assessed during the period leading up to the first visible signs of spore distention: the 48-h pregermination period.

SPORE REHYDRATION AND GERMINATION

Prior to the germination tests, various media or substrates for spore germination were assessed, including water, nutrient solution, agar, sand, and filter paper. Ultimately, the best results for the fastest germination with high germination percentages occurred using sand and filter paper, and we selected filter paper as the spore germination substrate of choice; further details are found in Stark & dos Santos (2024). The 2011 field collection was opened and spread upon a chemical wipe. The first capsule encountered from the right edge of the plant material that was uninjured, operculate, reddish brown, and typically sized was detached a few mm below the theca and removed from the collection. The capsule was then immersed on a depression slide in a 2% commercial bleach solution for 3 min followed by a brief rinse (seconds) in sterile water. Spores were dry-applied to moistened filter paper as follows. A 35 mm (I.D., inner diameter) plastic Petri dish was half-filled

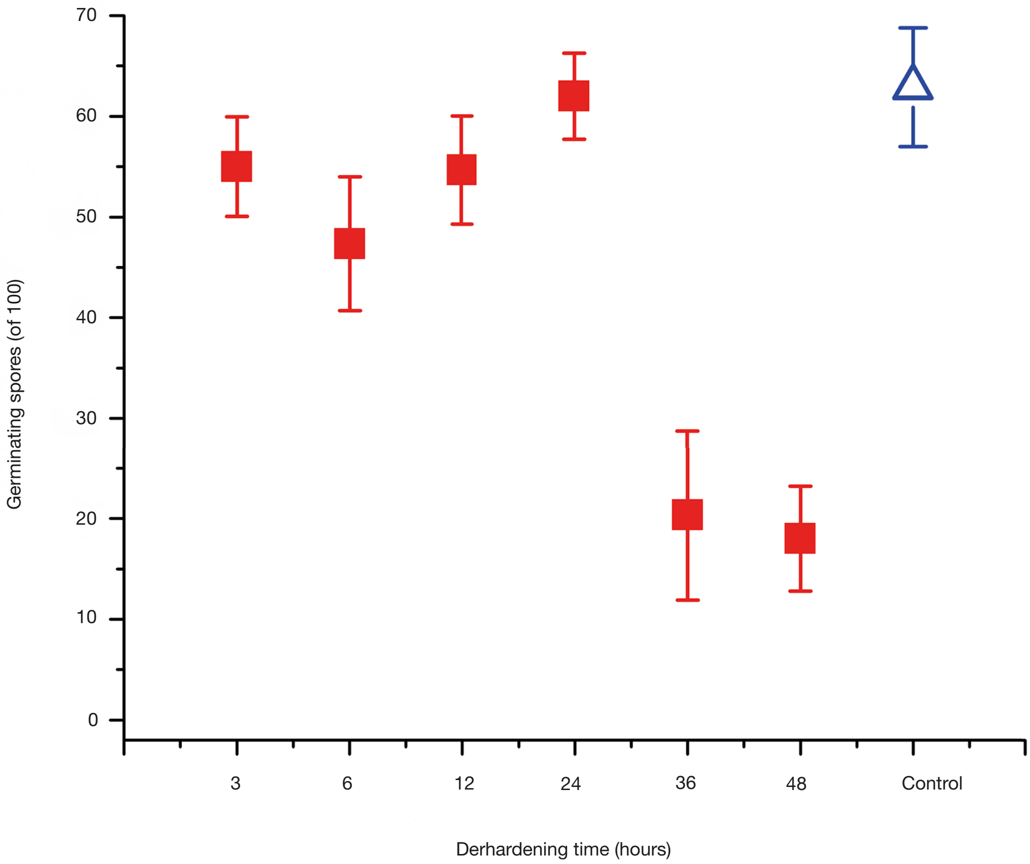

FIG. 3. — Germination counts of spores of *Tortula inermis* (Brid.) Mont. allowed to deharden for 3-48 h and then subjected to a rapid drying event (<1 h to equilibrate with a relative humidity of *c.* 34% at *c.* 22°C). Dehardening time is the number of hours spores from field collections were rehydrated prior to experimental rapid drying. Means ± one S.E. N = 3 for dehardening times 3, 6, 12 h, N = 6 for dehardening times 24, 36, 48 h, N = 9 for controls, with each N value corresponding to a count of 100 spores from a single capsule.

with sterilized local (Nevada, United States) sand, hydrated with sterile water, and a circular sheet of sterile filter paper (Whatman #1) placed atop the sand and slightly moistened. A capsule was moved to near the center of the filter paper and the lid and peristome were teased off using a probe and forceps. The deoperculated capsule was repetitively squeezed near its middle until a small clump of dry spores was released onto the filter paper. The capsule was moved to a different location on the filter paper, and another squeeze given, repeated several times until the capsule was nearly empty of spores and spores were spread across the filter paper in clumps. Theca dimensions (dried) were measured to the nearest 0.1 mm. Petri dishes with spores were placed on a lab counter under grow lights producing 24-90 µmol m^{-2} s^{-1} (PAR), under a 12-h photoperiod, and temperatures from 18-24°C. Watering was daily as needed with sterile water to ensure continuous hydration, deliberately watering the periphery of the filter paper so as not to move the spores during watering.

Spore desiccation treatment

Once the experimental dehardening time was reached (e.g. 3 h postrehydration) in the Petri dish containing spores, 9 thin sections of filter paper underlying spore clumps (for three replicate observations on days 3, 7, and 14 postrehydration) were peeled from the single-ply filter paper of the spore culture, using fine forceps. These spore peels (thin sections of filter paper overlain by spores) were placed on 2-ply filter paper in a 35 mm unlidded Petri dish, along with an *iButton* (recording temperature and RH every h), in the head space over a saturated solution of $MgCl_2$, creating a RH of *c.* 34% at temperatures *c.* 22°C. RH adjacent to the spore peels reached *c.* 34% in <1 h, and the spore peels remained at equilibration in the desiccator for 3 days under dim light (≤5 µmol m^{-2} s^{-1} PAR). The spore peels were rehydrated by transferring each spore peel to a Petri dish containing 1-ply filter paper overlying native sterilized sand, watered and allowed to germinate under conditions in *Spore rehydration and germination* above.

Rehydration response variables

Each day for the control, and on days 3, 7, and 14 for the dehardening treatments, spores were checked for germination at magnifications up to 400× using a light microscope. Beyond day 14 postrehydration, the tangle of protonemata did not allow reliable counts of germination. The germination phenophase of the first 100 spores encountered was assessed by: 1) transferring the spore peels to a gridded microscope slide and mixing the spore masses in a drop of water; and

TABLE 1. — The effects of dehardening on 13-year-old *Tortula inermis* (Brid.) Mont. spores, using germination levels and abortive spore frequency. Dehardening consisted of rehydrating spores for 0, 3, 6, 12, 24, 36, and 48 h followed by a rapid drying to equilibration with *c.* 34% relative humidity at *c.* 23°C, and then rehydrating and assessing germination on days 3, 7, and 14 postrehydration. Abbreviations: **GP**, germination percentage; **N**, number of spores assessed, mean ± one S.E., N = 600 spores for day 14 postrehydration germination percentage and abortive spore counts.

Dehardening time (h)	Spore no. (N)	GP Day 3	GP Day 7	GP Day 14	Abortive spores Day 14
0 Control	900	30.2 ± 4.9	58.0 ± 4.9	62.9 ± 5.9	13.0 ± 3.4
3	300	7.7 ± 3.7	36.7 ± 7.2	55.0 ± 4.9	27.0 ± 2.0
6	300	9.0 ± 4.04	39.7 ± 13.1	47.3 ± 6.6	10.3 ± 2.3
12	300	18.7 ± 2.0	38.3 ± 6.4	54.7 ± 5.4	9.7 ± 0.9
24	300	16.3 ± 12.8	45.7 ± 8.9	62.0 ± 4.3	23.7 ± 4.8
36	300	5.3 ± 2.8	4.7 ± 0.9	20.3 ± 8.4	58.3 ± 16.4
48	300	9.3 ± 7.4	31.0 ± 11.8	26.3 ± 8.1	71.7 ± 8.4

2) observing the spores at higher magnification (100 and 400×). Spores under germination tests were classified with reference Valanne's (1966) categories of spore germination (unswollen, swollen, distended, germ tube present) with one exception. Due to the small size of the spores and a range of spore sizes during swelling, in practice it was difficult to reliably discern the difference between a swollen and an unswollen spore. As a result, our spore germination categories were: 1) abortive (collapsed and/or brown, off-green, or clear, often under microbial attack); 2) swollen or swelling (greenish small or larger spores); 3) distended (spore bulging at one or both ends and departing from a spherical shape); and 4) protonemal germ tube present (Fig. 1). Spores were counted as germinating if they reached the distended or germ tube phenophase (Valanne 1966; Silva *et al.* 2010). The presence of distinctly cellular protonemal germ tubes could not be accurately discerned in many cases at 400×. We note that, whereas germination (as a distended spore or a spore producing a germ tube) is fairly straightforward to assess, distinguishing an abortive spore from a green unswollen spore on day 14 postrehydration was difficult; some uncertainty is present for the latter assessment.

Statistics

To assess whether the number of germinated spores and the number of abortive spores varied among the different treatments (Control, 3 h, 6 h, 12 h, 24 h, 36 h, and 48 h), we applied the non-parametric Kruskal-Wallis test. Normality of the data was checked beforehand for both variables, finding the data do not follow a normal distribution, confirming the need for a non-parametric test. After the Kruskal-Wallis test, we performed Dunn's post-hoc test for multiple comparisons between treatment pairs. All analyses were conducted in RStudio Team (2023), and the Dunn package (Dinno 2017) was used to carry out the *post hoc* test.

RESULTS

Germination controls

Spores of *Tortula inermis* (13 years old) begin to germinate (distended shapes and germ tubes protruding) after 48 h, reaching 23% germination by day 3 postrehydration and increasing to 50-60% from day 7 postrehydration forward (Fig. 2).

Spore dehardening

Significant differences were found for spore germination across dehardening treatments (Kruskal-Wallis chi-squared = 22.07, df = 6, $P<0.001$). Using day 14 germination levels, spores showed no signs of dehardening to a desiccation stress for the first 24 h of the pregermination period, showing similar germination percentages as the controls ($P>0.05$; Fig. 3). However, if treated with a rapid-dry event 36-48 h following the initial rehydration, spore germination declined significantly below control levels ($P<0.05$), indicative of a majority of spores dehardening to desiccation stress later in the pregermination period. On days 3 and 7 postrehydration, germination levels of dehardened spores were generally lower than controls. However, by day 14 spores that were dehardened for up to 24 hours germinated at similar rates to controls (Table 1).

Theca dimensions and abortive spores

Theca size in the control (N = 3) and dehardened (N = 6) treatments was similar (control 1.11 ± 0.22 mm^{-2}; dehardened 0.95 ± 0.09 mm^{-2}, mean ± S.E., standard error). Significant differences were found for spore abortion across dehardening treatments (Kruskal-Wallis chi-squared = 25.0225, df = 6, $P<0.001$). The Dunn *post hoc* comparisons revealed that spore abortion rates at 48 h dehardening time were significantly higher compared to the control ($P<0.01$) and 6-h treatment ($P<0.01$). At 36 h dehardening time, the abortion levels were significantly higher compared to the control ($P = 0.01$; Table 1). Approximately 65% of the spores aborted in the 36 and 48 h dehardening treatment.

DISCUSSION

Hardening (acclimation) to desiccation stress in bryophytes occurs when plants or structures dry out slowly, are allowed to experience partial drying in humid atmospheres, or when exposed to alternating episodes of hydration and (slow) drying (Schonbeck & Bewley 1981a, b; Beckett 1999; Stark *et al.* 2022). This hardened condition may persist to varying degrees for ≥7 days in the five taxa studied (see references in Introduction), and during this time the plant can tolerate drying out rapidly without suffering mortality or severely reduced regenerational vigor (Werner *et al.* 1991; Hájek & Vicherová 2014;

Stark *et al.* 2014). Dehardening from thermal stress is shorter, 2-3 days (Meyer & Santarius 1998). Under prolonged periods of hydration, plant structures may undergo dehardening (deacclimation) to desiccation stress, and will suffer mortality or reduced regenerational vigor upon rehydration following a severe (rapid) drying event (Schonbeck & Bewley 1981a; Hellwege *et al.* 1994; Marks *et al.* 2016; Hájek & Beckett 2008; Brinda *et al.* 2016). In bryophytes, dehardening to desiccation tolerance normally occurs during rapid growth and in juvenile phenophases (e.g. Werner *et al.* 1991), when growth and stress tolerance may trade-off due to the high metabolic costs of both growth and stress tolerance (Alpert 2006), creating challenges for the structure to both grow rapidly and maintain stress tolerance readiness. The conventional test for the presence of dehardening phenomena is the administration of a rapid drying event after a prolonged period of hydration. This is also the approach to determining a desiccation tolerance survival strategy (Stark 2017). Thus species and structures that exhibit dehardening have adopted an inducible strategy of desiccation tolerance, and those that do not deharden have adopted a constitutive strategy of desiccation tolerance.

Overall, the spore dehardening data for *Tortula inermis* highlight the impact of desiccation stress on spore germinability during the pregermination period. Spores of bryophytes had never been assessed for dehardening, although Newton (1972) slowly dried spores and sporelings of *Plagiomnium undulatum* and found a delay in germination at a lower temperature along with decreased germination levels (but still 55-80%) at higher (20°C) and lower (10°C) temperatures. As reasoned by Glime (2017a), it can be fatal to interrupt spores during germination by lack of water or light. If hydrated spores of the desert moss *T. inermis* are interrupted by a rapid drying event, the spores maintain a constitutive response to desiccation stress over the first 24 h of rehydration (control levels of germination), but then become vulnerable to a rapid-dry after 36-48 h of rehydration, suffering significant mortality upon rehydration. This potentially vulnerable period of dehardening late in the pregermination period was predicted to occur in mosses that release spores in the hydrated condition (Mogensen 1978), and may coincide with the "recovery" phase preceding spore distension (Olesen & Mogensen 1978). However, a desiccation stress at the point of dispersal in species dispersing hydrated spores contrasts with the stress administered here during the early stages of rehydration of spores dispersed in the desiccated condition.

Tortula inermis populations release spores from the same cohort over a period spanning three years, with the falling of opercula occurring during intervening dry periods of winter and spring. Thus in this species spores are expected to be initially dispersed in a desiccated condition (Stark 2001). During the course of the current experiment, we observed an inhibition of spore germination when spores were in physical contact with the exothecial cells of the theca, the operculum, peristome, and/or columella tissue (all of these tissues dead), with germination abundant on filter paper when spores were adjacent but not touching these sporophytic tissues (in the same Petri dish). These observations are consistent with: 1) the inhibition of spore (and fragment) germination in parental colonies of *Syntrichia* and *Dicranum* Hedw. (Mishler & Newton 1988); and 2) inhibition of gemma germination on media where conspecific gametophores had grown in *Tetraphis pellucida* Hedw. (Schneider & Sharp 1962). After a rain, sandy soils are expected to dry out rapidly in the Mojave Desert, where rainfall is very low and unpredictable (Stark 1997). Spores of this species are adapted to withstand the pressures of a fast drying event to equilibration with low relative humidity during the first 24 h of rehydration. While most bryophyte IDT species deharden gradually over at least 7 days (discussed in Introduction), *T. inermis* spores lose this ability after 1.5 days. The loss of hardening in *T. inermis* spores over a period of *c.* 12 h (between hour 24 and hour 36 postrehydration) suggests that the switch in ecological strategy of constitutive to inducible protection can be rapid and thus perhaps physiologically simple. Although the effect of wet/dry cycling on spore germination was not taken up here, in *Sphagnum* L. species (occupying much wetter habitats than *T. inermis*) wet/dry cycling was shown to reduce spore viability and germination levels (Fan *et al.* 2023).

A logical followup experiment in *T. inermis* is to slowly dry the spores during dehardening and test germination rates, with the prediction that a slow dry event should improve germination and even protect the spore from a future rapid dry event, unlikely as that event may be given that germination begins on day 3. We note that Newton's (1972) data would suggest otherwise, i.e., a slow dry event may reduce or delay spore germination. The inducibly DT spore strategy probably extends to the sporeling and protonema phases of *T. inermis*, given that protonemata of the bryophyte species studied to date are IDT and thus vulnerable to a rapid dry event (Werner *et al.* 1991; Pressel & Duckett 2010; Greenwood *et al.* 2019). In bryophyte spores hardened to desiccation tolerance, the hydration time required to complete the dehardening physiological processes may be longer than two days, vary by species, or be nonexistent (constitutive protection). Although not tested here, we predict that a rapid dry administered 72 h postrehydration (initial stages of germination) would result in similarly high levels of spore and sporeling abortion.

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