Generational Differences in Response to Desiccation Stress in the Desert Moss Tortula inermis

Lloyd R. Stark 1,*, Melvin J. Oliver 2, Brent D. Misher 3 and D. Nicholas McLetchie 4

1 School of Life Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, USA, 2 US Department of Agriculture, 205 Curtis Hall, University of Missouri, Columbia, MO 65211, USA, 3 Department of Integrative Biology, 1001 Valley Life Sciences Bld, #2465, University of California, Berkeley, CA 94720-2465, USA and 4 Department of Biology, 101 Morgan Bld, University of Kentucky, Lexington, KY 40506-0225, USA

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INTRODUCTION

Desiccation of plant cells causes cell membrane leakage of ions and electrolytes, pH changes, solute crystallization, protein denaturation, and the formation of reactive oxygen species capable of damaging intracellular organelles (Smirnoff, 1992; Mayaba and Beckett, 2003). These processes are manifested in reduced growth rates, loss of chlorophyll, and reduced regeneration potential (Schonbeck and Bewley, 1981a, b; Barker et al., 2005). However, photosystems in desiccation-tolerant bryophytes suffer little or no damage following desiccation, essentially returning to a functional state within minutes, which may be related to electrolyte retention during rewetting (Deltoro et al., 1998; Proctor and Smirnoff, 2000).

Desiccation tolerance (DT) is the ability of an organism to dry to equilibrium with the ambient air and then recover and return to normal metabolism on remoistening (Proctor and Pence, 2002). Vegetative DT evolved early in the land plants, and while subsequently lost during tracheophyte evolution, was retained in the bryophytes (Oliver et al., 2000). Bryophytes employ a mechanism of DT that includes both constitutive cellular protection coupled with a rehydration-induced repair–recovery system (Oliver et al., 2000). In Syntrichia ruralis, during rehydration from a desiccated state, gene expression is altered through translational control (Oliver, 1991). In addition, during a slow drying event, transcripts encoding rehydrins are sequestered in messenger ribonucleoprotein particles (mRNP) for storage in the dried state (Wood and Oliver, 1999). During rapid drying, such sequestration of transcripts is negligible, and this is why rapid drying presents a formidable stress even for desiccation-tolerant plants such as desert Pottiaceae. Desiccation damage to bryophytes is greatest when the period of hydration is the shortest (Bewley, 1979; Bewley and Krochko, 1982; Oliver and Bewley, 1997); i.e. when plants are dried rapidly (<2 h). Recent evidence points to a high frequency of short hydration intervals for bryophytes, even in relatively humid, mesic environments (Proctor, 2004), implying a high frequency of rapid drying events. When Syntrichia is dried rapidly, the plants are compromised in their ability to repair damage incurred when next rehydrated. Most critically, net carbon gain is not possible under repetitive RD conditions (Alpert, 2000). Upon rehydration, rapidly dried plants of S. ruralis suffer more extensive damage than slowly dried plants, as exhibited by (a) higher rates of resaturation respiration, (b) leakage of more solutes from their external membranes, (c) slower rates of protein synthesis, (d) a longer time to repair damage to mitochondria and chloroplasts, and (e) a greater rehydration from a desiccated state, gene expression is altered through translational control (Oliver, 1991). In addition, during a slow drying event, transcripts encoding rehydrins are sequestered in messenger ribonucleoprotein particles (mRNP) for storage in the dried state (Wood and Oliver, 1999). During rapid drying, such sequestration of transcripts is negligible, and this is why rapid drying presents a formidable stress even for desiccation-tolerant plants such as desert Pottiaceae. Desiccation damage to bryophytes is greatest when the period of hydration is the shortest (Bewley, 1979; Bewley and Krochko, 1982; Oliver and Bewley, 1997); i.e. when plants are dried rapidly (<2 h). Recent evidence points to a high frequency of short hydration intervals for bryophytes, even in relatively humid, mesic environments (Proctor, 2004), implying a high frequency of rapid drying events. When Syntrichia is dried rapidly, the plants are compromised in their ability to repair damage incurred when next rehydrated. Most critically, net carbon gain is not possible under repetitive RD conditions (Alpert, 2000). Upon rehydration, rapidly dried plants of S. ruralis suffer more extensive damage than slowly dried plants, as exhibited by (a) higher rates of resaturation respiration, (b) leakage of more solutes from their external membranes, (c) slower rates of protein synthesis, (d) a longer time to repair damage to mitochondria and chloroplasts, and (e) a greater
loss of chlorophyll from tissues (reviewed in Bewley, 1995). The principal reason postulated for the acute stress of rapid desiccation is that cells have insufficient time to sequester repair transcripts in specialized mRNPs for storage in the dried state (Wood and Oliver, 1999). Alternatively, when plants are slowly dried (approx. 6 h), an adequate stock of rehydrin transcripts is manufactured and stored in mRNPs for use when rehydration occurs.

Bryophytes exhibit widely varying degrees of DT, ranging from species of Sphagnum, which cannot tolerate a single drying event (Schipperges and Rydin, 1998), to desert species of mosses that can recover maximal photosynthetic rates within 1 h of rehydration (Di Nola et al., 1983). Desiccation-tolerant bryophytes can tolerate both repeated wet/dry/wet cycles (Stark et al., 2005) and months to years of desiccation (Oliver et al., 1993). A diversity of assessments of bryophyte DT has been employed, including membrane permeability (Beckett et al., 2000), pigment concentrations (Seel et al., 1992), protein synthetic response (Oliver et al., 1993), and chlorophyll fluorescence (Takács et al., 2000). Shoot regeneration as a stress-response variable allows repetitive, noninvasive observations over the course of an experiment; it also constitutes a significant process of clonal expansion and establishment, which is most likely to be the dominant form of reproduction in bryophytes (Newton and Mishler, 1994). The DT of bryophyte sporophytes has (as far as is known) never been assessed, in part because a developing sporophyte is connected to and dependent upon the maternal gametophyte, thus making intrinsic comparisons difficult. Correlations between massive sporophyte abortions and unusually heavy summer precipitation events (followed by rapid drying; Stark, 2001, 2002a) suggest that sporophytes are less desiccation-tolerant than their maternal gametophytes. It is therefore hypothesized that a reduced recovery from desiccation in sporophytes is relative to their maternal gametophytes, and it is predicted that growth resumption in attached sporophytes will be more adversely affected than regeneration of maternal gametophytic shoot tissue.

MATERIALS AND METHODS

Selection of sporophytic plants

Sporophytic patches of Crossidium, Tortula, Pterygoneurum, Funaria and Microbryum were collected on 11 December 2004 from the foothills of the River Mountains in southern Nevada, USA (Clark County, Henderson, 760 m a.s.l., N36° 03′ 80.0″ N, 114° 55′ 79.7″ W). Recent record rainfall in this region had resulted in hundreds of patches of a variety of species having developing sporophytes, an uncommon event in the Mojave Desert. Hydrated patches were allowed to desiccate slowly in the laboratory over a 24-h period. Shoots were stored dry in the laboratory until use on 30 January 2005. A series of pilot experiments on several species was conducted to determine ease of manipulation in the laboratory (disposition of the calyptra can affect sporophyte viability), phenophase (a clearly marked developmental phase during development) variation in the sporophytes, and resumption of growth of sporophytic plants. For desiccation tolerance, Tortula inermis (Brid.) Mont. showed the most promise in that sufficient numbers of sporophytes were present in a single phenophase (early seta elongation) and these sporophytes were capable of resuming growth upon placement into culture. This species is known from throughout the American south-west and adjacent Mexico (Flowers, 1973; Mishler, 1994), and becomes a dominant species at lower elevations in the Mojave Desert (Stark, 1997).

Desiccation treatments

A few short-term pilot runs were carried out to determine suitable desiccation treatments. The early seta elongation phenophase was used instead of embryonic sporophytes because pilot trials indicated that embryonic sporophytes were less desiccation tolerant, despite a sporophyte phenopattern in several desert mosses that exposes embryonic sporophytes to long periods of desiccation (Stark, 2002b). One-hundred and sixty shoots having sporophytes in the early seta elongation phenophase (Fig. 1A) were removed from the collections, and 100 (90 experimental plants and ten excess plants to replace any plants damaged during handling) were randomly selected for the experiment. Individual plants with sporophytes were separated from co-joined shoots and 30 such plants were randomly assigned to each treatment and placed in a 48-hole (capacity) plastic well-plate, one plant per well-hole. Desiccation treatments consisted of a control [0 rapid-dry (RD) cycle], one RD cycle (RD×1) and two RD cycles (RD×2) as follows. Each sporophytic plant was hydrated in the well-hole by adding one to three drops of sterile water, a lid was placed on the well-plate and it was allowed to sit in the laboratory for 60 min (allowing shoots and sporophytes to fully hydrate). After 60 min, each plant was removed from its well-hole and placed on a microscope slide where shoots were cut at ground level and secondary shoots removed, then planted directly (control) or blotted dry, transferred to another unlidded well-plate and placed into a desiccation chamber (relative humidity approx. 15%, light intensity approx. 4.5 μmol m⁻² s⁻¹ (PAR sensor, Licor LI-250; Lincoln, NE, USA) overnight (RD×1 treatment). Plants were handled using forceps gently clasping a leaf or the base of the shoot.

The following day, plants were hydrated in the well-plate by adding one to three drops of sterile water, allowing 1 min to ensure partial turgor, and then transplanted upright into watch-glasses (inner diameter 45 mm) half-filled with premoistened, sieved (500 μm mesh), dry-autoclaved (60 min at 131 °C) field-collected sand at a substrate depth of approx. 5 mm. Three plants per watch-glass were installed, equally spaced, marking the watch-glass to allow observations on each plant, with care taken to ensure calyptrae remained undisturbed during planting. If a calyptra was dislodged during handling, that plant was discarded and another plant was selected from the ten excess plants. This process was repeated for the RD×2 treatment.
Growing conditions and observations

Lidded watch-glasses were randomly placed among 30 positions on a single shelf within a plant growth chamber (Percival model E30B, Boone, IA, USA) under recovery conditions of a 12-h photoperiod, 20 °C lighted, 8 °C darkened. Light intensity in the chamber ranged from 41 to 52 μmol m⁻² s⁻¹ and relative humidity ranged from 70 to 85 %. Watch-glasses were randomly rotated among the positions following each set of observations, with observations occurring daily from day 2 to day 14, and on days 16, 19, 22, 29 and 35. Sand in the watch-glasses was moistened with 35 % Hoaglands solution (Hoagland and Arnon, 1938) as needed to maintain full turgor without creating any standing water.

Gametophyte response variables included recording (a) the day when leaves discoloured (partial or complete browning of leaves; Fig. 1D), (b) the day when protonema emerged, (c) the day when new shoots were produced (Fig. 1C), and (d) the total number of shoots produced on days 14, 29 and 35. Shoots were produced both attached to the original plant and also at a short distance from the plant by subterranean or superficial protonema (Fig. 1B). Whereas observations made up to day 35 were made looking directly down upon the plants, on day 35 an effort was made to detect all shoot buds and protonema under the basal leaves of the main shoot, probing gently as necessary to get a clear view. Therefore, a few shoots and protonema may have gone undetected until day 35. Rhizoids were occasionally seen extending from the vaginular region, and these were not counted as protonema. Observations for sporophytes consisted of the days when (a) the seta length exceeded the calyptra length (‘seta elongation’, indicative of sporophyte elongation), (b) the calytra began to split up the side (‘premeiotic capsules’; Fig. 1E), (c) capsules became fully extended with opercular tissue differentiated (‘meiotic capsules’; Fig. 1F), and (d) when capsules began to turn from green to brown (‘post-meiotic capsules’; Fig. 1G). The experiment was concluded when all capsules that had resumed growth had reached the post-meiotic phenophase (day 35).

Statistics

For gametophytes, an association between treatment and the probability of shoot production was tested using a 3 × 2 contingency table. A two-way nested ANOVA (dishes within treatment) was used to analyse for treatment differences in days to protonemal emergence, days to first shoot production (for plants that produced shoots), and for these plants, the number of shoots at day 14 and day 35. Within each analysis, treatment means were compared using Tukey’s mean comparisons. Also an association between treatment and leaves of the gametophyte turning brown (burning) or the entire gametophyte turning brown was tested. In this test of association, the critical $\chi^2$ value at $P = 0.05$ was adjusted for each pairwise comparison (Sokal and Rohlf, 1995). Days to protonemal emergence, new shoot production, and numbers of shoots were square root transformed. For sporophytes, an association between treatment and the probability of the sporophyte leaving one phenophase and entering another phenophase was tested. Among the four phenophase transitions (embryonic to post-embryonic, post-embryonic to seta elongation, seta elongation to premeiotic capsules, and premeiotic capsules to meiotic capsules) the critical $\chi^2$ value at $P = 0.05$ was adjusted for each pairwise
treatment comparison (Sokal and Rohlff, 1995). Due to the RD×2 treatment having a very low n in the last two phenophase transitions, the only comparison made was between RD×0 and RD×1. A two-way nested ANOVA (dishes within treatment) was used to analyse for treatment differences in days to seta elongation, capsule expansion, meiotic capsules and post-meiotic capsules. Days to these events were square-root transformed. Only the first phenophase included all treatments. Given that only one sporophyte of the RD×2 treatment survived past the first phenophase, this treatment was dropped from analyses of the remaining phenophases.

To investigate gametophytic–sporophytic interactions, a relationship between gametophytic vigour (days to shoot production) and the resumption of sporophyte development (seta elongation) within treatment was tested. This was to assess if plants aborting sporophytes had a shorter time to gametophytic regeneration relative to plants that initiated sporophyte development. A one-way ANOVA was used to test for these patterns. Days to shoot production were square-root transformed. Most of the control plants began sporophyte development, leading to a very low n in the category of plants that produced shoots but which did not resume sporophyte development. Thus, the stress treatments were only analysed to test for this relationship. All statistical analyses were done using SAS (1994).

**RESULTS**

Gametophytic recovery

Of the 90 maternal shoots used in this experiment, 84 (93 %) resumed growth in culture, either by producing shoot buds and/or protonemata from the base of the maternal shoot (Fig. 1B). Symptoms of stress included the ‘burning’ of leaves on the maternal shoots, i.e. where leaves become partially discoloured (Fig. 1D), or the browning of the entire maternal shoot. These symptoms increased significantly with increasing desiccation stress (see Table 3 and Fig. 2). Twenty-one of 30 maternal shoots exhibited partial discoloring in the RD×2 treatment compared with three of 30 in the control group (RD × 0). There was a significant association between treatment level and probability of producing shoots (d.f. = 2, Χ² = 19.72, P < 0.0001) with RD×2 less likely to produce shoots (63.3 %) than RD×1 (96.7 %) and the control (100 %). Of plants that produced shoots, plants from the RD×2 treatment took longer to produce shoots than plants from RD×1 (26.00 ± 2.51 and 15.79 ± 2.37 d, P < 0.05, Tukey’s means test). The control treatment (20.60 ± 1.97) did not differ from the other two treatments. For plants that produced shoots, the mean number of shoots on day 14 ranged from 1.18 ± 0.12 (RD × 0) to 1.40 ± 0.16 (RD × 1) and on day 35 the range was 2.36 ± 0.41 (RD × 2) to 3.16 ± 0.30 (RD × 0; Table 1). On days 14 and 35, there were no statistically significant differences in shoot production among the treatments. In general, maternal shoots of Tortula tended to produce clonal shoot buds prior to producing protonemata (20.12 ± 1.00 days vs. 29.60 ± 0.62 days, respectively), and when protonemata were produced they were often subterranean. Days to protonemal emergence did not differ among the treatments (Table 2).

Sporophytic recovery

Sporophytes were more adversely affected by increasing desiccation stress than maternal gametophytes. Whereas in the control group 22 of 30 sporophytes reached meiosis, only seven reached meiosis in the low-stress treatment, and one in the high-stress treatment (Fig. 3). The controls were more likely (P < 0.05) to successfully complete the first two pheno-phase transitions (embryonic to post-embryonic, post-embryonic to seta elongation) than the stress treatments (Table 3). In the subsequent pheno-phase transitions (seta elongation to premeiotic capsules, and premeiotic capsules to meiotic capsules) successful development for all treatments was at or near 100 % (Table 3). Of sporophytes that resumed development, there were no treatment effects on time to development. However, there was a strong trend that control plants took longer to reach the capsule expansion phase than

![Fig. 2. Number of Tortula inermis gametophytes that produced shoot buds, produced protonemata, became entirely discoloured (shoots brown), and which exhibited partial burning of leaves (leaves burned), after exposure to a desiccation stress of none, one or two rapid-dry cycles.](https://academic.oup.com/aob/article-abstract/99/1/53/2769255/suppl/FIG2)

**Table 1. Number of shoot buds produced per maternal shoot on days 14 and 35 following exposure to none, one and two rapid-dry (RD) cycles in Tortula inermis (no significant differences found)**

<table>
<thead>
<tr>
<th>Desiccation treatment</th>
<th>n</th>
<th>Day 14</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD × 0</td>
<td>30</td>
<td>1.18 ± 0.10</td>
<td>3.38 ± 0.41</td>
</tr>
<tr>
<td>RD × 1</td>
<td>30</td>
<td>1.40 ± 0.16</td>
<td>3.54 ± 0.42</td>
</tr>
<tr>
<td>RD × 2</td>
<td>30</td>
<td>1.25 ± 0.13</td>
<td>2.64 ± 0.48</td>
</tr>
</tbody>
</table>
sporophytes in the RD×1 treatment (10-78 d to 9-43 d, $F = 4.14$, d.f. = 1, $P = 0.059$; Table 2).

Gametophytic and sporophytic relationships

For the RD×1 treatment, there was no significant relationship between gametophytic vigour and resumption of sporophyte development. However, for the RD×2 treatment, plants that resumed sporophyte development produced shoots earlier than plants that did not resume sporophyte development (20.25 ± 4.08 d and 30.18 ± 1.6 d, respectively; d.f. = 1, $F = 6.89$, $P < 0.02$).

**DISCUSSION**

The extreme variation in DT among bryophytes is based on research of the gametophyte generation. This research found that DT ranges from non-tolerance of a single drying event (Schipperges and Rydin, 1998), to desert mosses that can recover photosynthetic potential from a drying event (Schipperges and Rydin, 1998), to desert species that can also tolerate both repeated wet/dry/wet cycles (Stark et al., 2005) and months to years of desiccation (Oliver et al., 1993). However, even in these species, as the number of wet/dry cycles increases, photosynthetic rates and regeneration rates decline significantly (Davey, 1997; Stark et al., 2005). This pattern of DT is expected in the desert because hydration periods are very short (hours to several days). In the present study, both the gametophyte and sporophyte showed a high level of recovery after rehydration in the controls (100% and 73%, respectively). After a single dying cycle, control gametophytes still had 100% recovery. However, after two cycles of rapid desiccation, recovery was compromised in gametophytes (63%). In sporophytes, a single drying cycle resulted in 23% recovery while two drying cycles resulted in 3% recovery. These results indicate that the sporophyte is more negatively affected by desiccation than the gametophyte. In pilot studies it was found that embryonic sporophytes were less desiccation tolerant than post-embryonic sporophytes, i.e. once the embryonic phase transitions into the post-embryonic phase, resumption of development is more likely to occur, a puzzling result given that embryonic sporophytes are capable of over-summering through long dry periods (Stark, 1997), but perhaps understandable given that embryos were collected in a dehardened state.

The shoot (gametophyte) of bryophytes is expected to have higher DT than the sporophyte for at least two reasons. First, most gametophytes are perennial and thus exposed to seasonal and yearly variation in moisture. Individuals having traits (such as DT) to survive this environmental variation will be favoured over individuals that do not have these traits. Relative to the gametophyte, the sporophyte is short-lived (1–2 years). While sporophytes are exposed to seasonal variation in moisture, they are not as likely to experience long periods of desiccation during development. Additionally, sporophyte initiation will be low to nonexistent in dry years due to the requirement of free water for fertilization. Consequently selection for DT will be stronger in the gametophyte relative to the sporophyte. Also, throughout sporophyte development, a physiological connection exists at the sporophyte/gametophyte junction, where resources are transported from haploid to diploid tissues (Ligrone and Gambardella, 1988). Therefore sporophyte growth and fitness, and likely responses to stresses such as desiccation, are dependent upon the gametophyte to which it is attached, termed the
Table 3. Analysis of the probability of development through the four sporophytic phenophase transitions and of the probability of leaves and entire gametophytes turning brown in Tortula inermis

<table>
<thead>
<tr>
<th>Phenophase transition or effect</th>
<th>Probabilities (n)</th>
<th>$\chi^2$ (d.f.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic to post-embryonic</td>
<td>$0.93 (30)$</td>
<td>$0.033 (30)$</td>
</tr>
<tr>
<td>Post-embryonic to seta elongation</td>
<td>$0.82 (28)$</td>
<td>$0.10 (30)$</td>
</tr>
<tr>
<td>Seta elongation to premeiotic capsule</td>
<td>$0.95 (22)$</td>
<td>$1 (22)$</td>
</tr>
<tr>
<td>Premeiotic capsule to meiotic capsule</td>
<td>$0.67 (30)$</td>
<td>$0.20 (30)$</td>
</tr>
<tr>
<td>Leaf browning (browning)</td>
<td>$0.70 (30)$</td>
<td>$0.083 (30)$</td>
</tr>
<tr>
<td>Gametophyte browning</td>
<td>$0.351 (3)$</td>
<td>$6.4 (3)*$</td>
</tr>
<tr>
<td>Embryonic to post-embryonic</td>
<td>$0.27 (30)$</td>
<td>$1 (22)$</td>
</tr>
<tr>
<td>Post-embryonic to seta elongation</td>
<td>$0.351 (3)$</td>
<td>$0.20 (30)$</td>
</tr>
<tr>
<td>Seta elongation to premeiotic capsule</td>
<td>$0.44 (16)$</td>
<td>$1 (22)$</td>
</tr>
<tr>
<td>Premeiotic capsule to meiotic capsule</td>
<td>$0.083 (12)$</td>
<td>$1 (1)$</td>
</tr>
<tr>
<td>Leaf browning (browning)</td>
<td>$1.176 (3)$</td>
<td>$1 (1)$</td>
</tr>
<tr>
<td>Gametophyte browning</td>
<td>$19.07 (3)*$</td>
<td>$1 (1)$</td>
</tr>
</tbody>
</table>

RD, Rapid dry cycles.
Comparisons involving the RD×2 treatment were not made for the latter two transitions since RD×2 had a very low n (n = 1) after the second transition. The critical $\chi^2$ value at $P = 0.05$ was adjusted for each pairwise treatment comparison (Sokal and Rohlf, 1995). *$P < 0.05$.

‘nurturance effect’ (Shaw and Beer, 1997). As a result, if a gametophyte carrying a developing sporophyte does not recover from desiccation, then the sporophyte will not develop. Selection should not favour a scenario where the sporophyte is more desiccation tolerant than the gametophyte. If recovery from stress requires resources such as the manufacture of repair proteins, then a reasonable assumption is that the life history stage that is independent is likely to ensure its own survival by reducing resources allocated to nonessential sinks (like the sporophyte).

Thus sporophytes are expected to have lower stress tolerances relative to maternal shoots (as seen in thermotolerance; McLetchie and Stark, 2006). More detailed experiments are needed to determine contributions of the maternal shoot versus the physiology of the sporophyte in determining sporophyte recovery.

As the number of wet/dry cycles increases, photosynthetic rates and regeneration rates in bryophytes are known to decline significantly (Davey, 1997; Stark et al., 2005). Following just a single wet/dry/wet cycle, desiccation-tolerant bryophytes suffer little or no damage to the photosynthetic apparatus, which is correlated to lower electrolyte leakage (Deltoro et al., 1998). However, a single rapid-dry cycle can produce a 50% leaf bleaching effect and an 84% reduction in shoot growth in Syntrichia (Schonbeck and Bewley, 1981a). In deserts where hydration periods are very short (hours to several days), net carbon gain in the gametophyte is severely restricted (Alpert and Oechel, 1985; Alpert, 2000). While the photosystem, respiratory chain, dark reactions of C-assimilation, and protein synthesis also seem to have constitutive protection (Proctor and Smirnoff, 2000), for long-term recovery including regeneration and growth, they are just as likely to be dependent upon the repair-based processes that are initiated upon rehydration (Oliver et al., 2000).

Regeneration of shoots from pre-existing detached shoots and leaves is more rapid than regeneration of shoots from spores. In Macromitrium, while it took $>50–70$ d to produce shoots from spores depending on the sex, detached leaves produced first shoots in 30 d (Une, 1985). Indeed, models for Marchantia revealed that specialized asexual reproductive rates (gemma production) had stronger effects on patch dynamics than those parameters associated with sexual reproduction (McLetchie et al., 2002). This has significance in that asexual regeneration probably plays the major role in local site recolonization (Kimmerer, 2005). Given an environmental stress such as rapid desiccation, maternal shoots are thus more valuable as far as local regeneration potential is concerned than are spores, and the selection for superior DT ability in gametophytic tissues is expected.

In bryophyte gametophytes, during rehydration episodes membranes leak cations (K$^+$, Mg$^{2+}$), but these ions are subsequently bound by cell wall exchange sites, which function in trapping and then recycling these important ions (Bates, 2000). This helps to explain the high DT observed in gametophytes, but not necessarily sporophytes, which are less studied with respect to membrane leakage/reabsorption. Although the last cells to become desiccated in Polytrichum were axial meristematic cells of the shoot apex (based on the 48-h delay in resumption of normal mitosis and DNA synthesis), the shoot apex may be the most affected region of the plant following a desiccation event (Mansour and Hallet, 1981). Bryophyte shoots that are stressed in the distal region may manifest this stress in proximal regions (Proctor and Pence, 2002), and, in the case of maternal shoots, in the sporophyte. This finding is also consistent with recovery estimates, where at least 24 h is required before gametophytes resume normal protein synthetic patterns (Oliver, 1991). In Tortula inermis, because the shoot apical meristem is consumed during sporophyte formation, the only regeneration option available to maternal plants is through lateral branching and protonemal production, where lateral apical meristems are expected to experience a similar delay in mitosis and DNA synthesis. In the present study the first signs of asexual regeneration (shoot production) appeared in about 20 d.

The ability of gametophytes to tolerate desiccation varies with season. Using ion leakage assays, DT is highest (lower leakage) during the dry portion of the year and lowest (greater leakage) during the wet portion of the year in South Africa (Beckett and Hoddinott, 1997). In addition, spring maxima and autumn minima for DT
are reported from Antarctica (Davey, 1997), which loosely conforms to this pattern in light of the coupling of freezing tolerance to DT. Sporophytes of *Tortula inermis* develop from seta elongation through meiosis during wet winters in the Mojave Desert (Stark, 1997). Considerable dehardening to DT is expected to occur after a few days of patch hydration (Schonbeck and Bewley, 1981b), no doubt leading to reduced DT on the part of the maternal gametophytes. Although sporophytes of *T. inermis* routinely tolerate prolonged desiccation during the embryonic phenophase, the active extension of sporophytes is always attended by a dehardened (hydrated) microenvironment. Additionally, plants of *T. inermis* were rehydrated under laboratory lights, and therefore subjected to a harsher desiccation treatment based on a comparison of resaturation respiration rates in lighted versus darkened environments (Seel et al., 1992; Mayaba et al., 2001).

The difference between bryophyte generations in DT may relate to inherent differences in the DT repair pathways. The key rehydrin Tr288 is known to accumulate during slow drying and most rapidly during the first 15 min after rehydration from a slow-dry episode in *Syntrichia ruralis* (Velten and Oliver, 2001). Should these gametophytic repair pathways and translational controls on rehydrin synthesis be less efficient or even absent in sporophytes, it would help explain the markedly lower DT observed in *Tortula inermis* sporophytes. Desiccation tolerance requires synthesis of an array of repair proteins, and is likely to be an energetically expensive process given the elevated respiration rates attending rehydration from a desiccated state (Tuba et al., 1996). As the maternal gametophyte recovers from rapid drying, the flow of resources from gametophyte to sporophyte may be interrupted, compromising the ability of the sporophyte to recover from desiccation injury. Unable to import an adequate store of resources from the gametophyte during the embryonic period, the sporophyte aborts while the maternal shoot is capable of surviving (Fig. 1J). This hypothesis is consistent with (a) the correlation of size between generations in *Funaria*, where larger maternal shoots produce larger sporophytes (Shaw and Beer, 1997), (b) density-dependent sporophyte abortion patterns in *Syntrichia* and other bryophytes (Stark et al., 2000), and (c) the widespread sporophyte abortion patterns and sporophyte size reductions coinciding with summer precipitation in the Mojave Desert (Stark, 2002a).

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**LITERATURE CITED**


