

## Sporophyte and Gametophyte Generations Differ in their Thermotolerance Response in the Moss *Microbryum*

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• **Background and Aims** Actively growing post-embryonic sporophytes of desert mosses are restricted to the cooler, wetter months. However, most desert mosses have perennial gametophytes. It is hypothesized that these life history patterns are due in part to a reduced thermotolerance for sporophytes relative to gametophytes.

• **Methods** Gametophytes with attached embryonic sporophytes of *Microbryum starckeanum* were exposed whilst desiccated to thermal episodes of 35 °C (1 hr), 55 °C (1 hr), 75 °C (1 hr) and 75 °C (3 hr), then moistened and allowed to recover for 35 d in a growth chamber.

• **Key Results** All of the gametophytes survived the thermal exposures and produced protonemata, with the majority also producing shoot buds. Symptoms of gametophytic stress (leaf burning and discoloration of entire shoots) were present in lower frequencies in the 55 °C exposure. Sporophyte resumption of growth and maturation of meiosis were significantly negatively affected by thermal treatment. Not a single sporophyte exposed to the two higher thermal treatments (75 °C for 1 h and 75 °C for 3 h) survived to meiosis, and those sporophytes exposed to 75 °C that survived to the post-embryonic phenophase took significantly longer to reach this phase. Furthermore, among the thermal treatments where some capsules reached maturity (35 °C and 55 °C), maternal shoots that produced a meiotic capsule took longer to regenerate through protonemata than maternal shoots aborting their sporophyte, suggestive of a resource trade-off between generations.

• **Conclusions** Either (1) the inherent sporophyte thermotolerance is quite low even in this desert moss, and/or (2) a gametophytic thermal stress response controls sporophyte viability.

**Key words:** Bryophyte, thermal stress, regeneration, sporophyte, gametophyte, protonema, *Microbryum starckeanum*.

### INTRODUCTION

Bryophyte gametophytes exhibit marked differences in survival of brief periods of heat shock depending upon plant hydration status, with greater thermal tolerance at lower tissue water content (Clausen, 1964; Volk, 1984; Meyer and Santarius, 1998). Whereas the lethal thermal limit for metabolically active (hydrated) gametophytes is 51 °C (with one record at 100 °C for *Fontinalis* allowed to recover for an extended duration under field conditions; Glime and Carr, 1974), when gametophytes are desiccated this limit rises to 110 °C at the extreme (Kappen, 1981; Meyer and Santarius, 1998; Proctor and Pence, 2002), with most species suffering injury at much lower temperatures (Furness and Grime, 1982; Hearnshaw and Proctor, 1982; Larcher, 1995; Liu *et al.*, 2003). In general, terrestrial bryophytes exhibit higher thermal optima and thermal tolerance than aquatic bryophytes (Carballeira *et al.*, 1998). The acquisition of dry heat tolerance in bryophytes is expected to be more sharply selected than wet heat tolerance since under most conditions heat stress is encountered when gametophytes are desiccated (Hearnshaw and Proctor, 1982; Meyer and Santarius, 1998). This contrasts with vascular plants, the vast majority of which are incapable of complete desiccation and which exhibit the highest wet heat tolerance among land plants (Nobel *et al.*, 1986).

At high temperatures, lethality is attributed to damage (including increased fluidity) to external and internal cell

membrane systems including the photosystem pigment apparatus (Larcher, 1995; Meyer and Santarius, 1998). This leads to increased membrane permeability and eventual cell death, possibly caused by a compromised plant defensive reaction leaving tissues vulnerable to viral and fungal infections (Kappen, 1981).

Sporophytes of mosses from xeric habitats exhibit a suite of correlated morphological features, including short setae, erect and broad capsules, and reduced/absent peristomes (Vitt, 1981). Very little is known of the stress tolerance (in particular thermotolerance or desiccation tolerance) of developing sporophytes. Sporophytes of mosses may be more susceptible to thermal stress than gametophytes: a lower thermal optimum was found in sporophytes relative to gametophytes in *Funaria* (15–20 °C vs. 25 °C, respectively; Dietert, 1980). Nevertheless, sporophytic embryos of at least two desert mosses exhibit a phenological pattern of over-summering, thus tolerating long periods of thermal stress while desiccated (Stark, 1997; Bonine, 2004). To our knowledge, and unlike many bryophyte species in cooler regions, sporophytes of desert species during the summer are never found in the presumably more stress-vulnerable physiological stages of development (phenophases) of seta elongation through to premeiosis, but rather only as embryos or postmeiotic capsules. Low-elevation warm desert species uniformly develop from embryonic through to meiotic phenophases during the cooler and wetter winter/spring months, at least in the Mojave Desert (Stark, 2002a). Therefore, we hypothesized that

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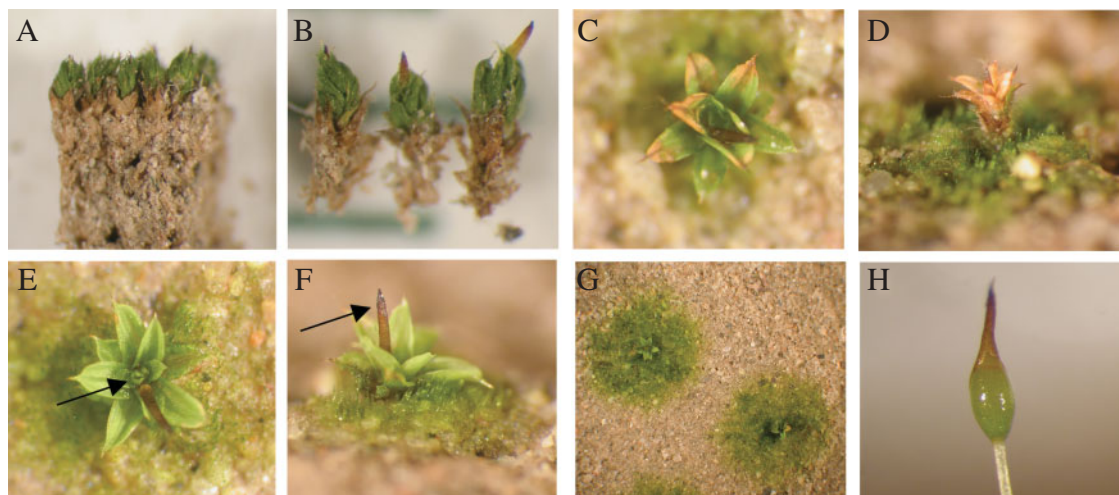


FIG. 1. Some of the phenophases of *Microbryum* gametophytes and sporophytes assessed following dry thermal exposures. (A) Portion of patch taken from field showing shoot packing; (B) three gametophytic shoots with attached embryonic sporophytes subjected to thermal exposures, scale in background is in millimetres; (C) plant with burned leaves; (D) plant entirely brown (and regenerating protonemata); (E) shoot bud (at arrow) subtending abortive sporophyte; (F) gametophyte producing protonemata while having an abortive sporophyte (at arrow); (G) protonemal areas of two gametophytes, each with a diameter of approx. 13 mm; (H) meiotic capsule.

the sporophytes of *Microbryum starckeanum*, a species found at lower elevations in the Mojave Desert, would exhibit reduced thermotolerance relative to gametophytes.

## MATERIALS AND METHODS

### *Selection of Sporophytic Plants*

Sporophytic patches of *Crossidium*, *Tortula*, *Pterygoneurum*, *Funaria* and *Microbryum* were collected on 19 Dec. 2004 from the foothills of the River Mountains in southern Nevada, USA (Clark County, Henderson, elevation 760 m, GPS coordinates N36, 03.880, W114, 55.797). Recent record rainfall in this region had resulted in hundreds of patches of a variety of species having relatively young embryonic or shortly post-embryonic sporophytes, an uncommon event in the Mojave Desert. Patches were allowed to desiccate slowly in the laboratory overnight. 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 variation in the sporophytes, and resumption of growth of sporophytic plants. *Microbryum starckeanum* (Hedw.) Zand. showed the most promise in that embryonic sporophytes were present at a single phenophase (Fig. 1B), and embryonic sporophytes resumed growth upon placement into culture. This species (= *Pottia starckeanum*) is characterized by an elongate seta, stegocarpous capsule and smooth spores, and is distributed in the south-western US, Mexico, Europe, northern Africa and Australia (Zander, 2005). One-hundred and twenty sporophytic plants were isolated from a single patch containing a dense population of sporophytes. A voucher specimen (*Stark s.n.*, 12-19-04 & 1-3-05) was determined by Richard Zander and deposited at UNLV and MO.

### *Thermal Exposures*

Experimental exposure times of 30 or 60 min have commonly been used to assess thermotolerance in plants (e.g. Nörr, 1974). By assessing dry plants, we avoid the problems of evapotranspirational cooling of surface plant tissues (Kappen, 1981). A few short-term pilot experiments were carried out in order to determine suitable thermal treatments. Desiccated plants were cut to roughly 5 mm in length, and 30 plants were randomly assigned to each of four treatments: 35 °C (1 h), 55 °C (1 h), 75 °C (1 h), and 75 °C (3 h). The surface of desert soils can reach 70–80 °C (Kappen, 1981 and unpubl. data, LRS); our selection of 75 °C as the high temperature treatment coincides with expected exposures in the natural habitat, allowing that patches of *Microbryum* occur in shaded soil recesses seldom exposed to direct sunlight. Pilot experiments pursuant to this experiment indicated no differences in sporophyte and gametophyte viability between exposures of 25 and 35 °C. Plants were placed into a 48-well (capacity) plastic wellplate, one plant per well. The plate was covered with a lid and placed into a preheated gravity convection oven (Yamato DX 300, Integrated Services, Palisades Park, NJ, USA) for 60 or 180 min. After the thermal exposure, the wellplate was allowed to cool on a lab bench for 15 min with the lid on, whereupon one drop of sterile water was added to each well and the plants allowed to hydrate for 5 min. Each plant was subsequently transferred into a water droplet on a microscope slide, secondary branches if present were removed, and each plant was cut to a length of 2.5 mm using a straight-edge.

### *Growing conditions and observations*

Each 2.5-mm plant was transplanted into a plastic Petri dish (inner diameter 35 mm) containing pre-moistened,

sieved (500  $\mu\text{m}$  mesh) and dry-autoclaved (60 min at 131 °C) field-collected sand at a substrate depth of 4–5 mm. Three sporophytic plants were transplanted upright into each dish and placed into the growth chamber (Percival model E30B, Boone, IA, USA) in dishes covered with lids under recovery conditions of a 12-h photoperiod, 20/8 °C light/dark. Light intensity in the chamber ranged from 59 to 72  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PAR sensor, Licor LI-250, Lincoln, NE, USA) and relative humidity ranged from 70–85 %. Observations and random repositioning of Petri dishes on the shelf were made on a daily basis from day 3 to day 16, and subsequently on days 19, 21, 28 and 35. Sand in the dishes was moistened with 35 % Hoaglands solution (Hoagland and Arnon, 1938) as needed to maintain full turgor without creating any standing water.

A variety of stress response variables has been used to assess survivability in bryophytes following thermal stress, including pigment indices (Carballeira *et al.*, 1998), chlorophyll content (Hearnshaw and Proctor, 1982), membrane leakage (Liu *et al.*, 2003), shoot production (Glime and Carr, 1974), and enzyme activity (Liu *et al.*, 2001). Here we select the regenerational ability of gametophytes along with the capability of growth resumption in sporophytes. Observations for gametophytes consisted of (1) leaf burning (partial or complete browning of at least two leaves; Fig. 1C, D), (2) day of protonemal emergence, (3) day of shoot bud appearance (subtending a perichaetium or lateral along the shoot; Fig. 1E), (4) number of shoots produced, and (5) protonemal area produced upon completion of the experiment (Fig. 1G). For the latter, dishes were uncovered and allowed to dry overnight in the chamber, and then the circumference of the superficial protonemal area was determined using image analysis software (Spot, Diagnostic Instruments, Sterling Heights, MI, USA); protonemal area is a good estimate of biomass production in *Syntrichia caninervis* (Stark *et al.*, 2004). Observations for sporophytes consisted of the days when (1) the embryonic phase terminated (post-embryonic), (2) the seta length exceeded the calyptra length (seta elongation), (3) the calyptra began to split up the side (pre-meiotic capsules), (4) capsules became fully extended with opercular tissue differentiated (meiotic capsules; Fig. 1H), and (5) when capsules began to turn from green to brown as noted by appearance of brown longitudinal theca ridges (post-meiotic capsules). Sporophytes that turned hyaline or brown and failed to resume growth were classified as abortive (Fig. 1F).

#### Statistical analysis

For gametophytes, 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, protonemal growth rate, and number of shoots. Within each analysis, treatment means were compared using Tukey's mean comparisons. Days to protonemal emergence and new shoot production were square-root transformed and protonemal area was log transformed. We tested for an association between the stress treatment and the probability that leaves turned brown. A similar test was done for plants that became completely brown. The

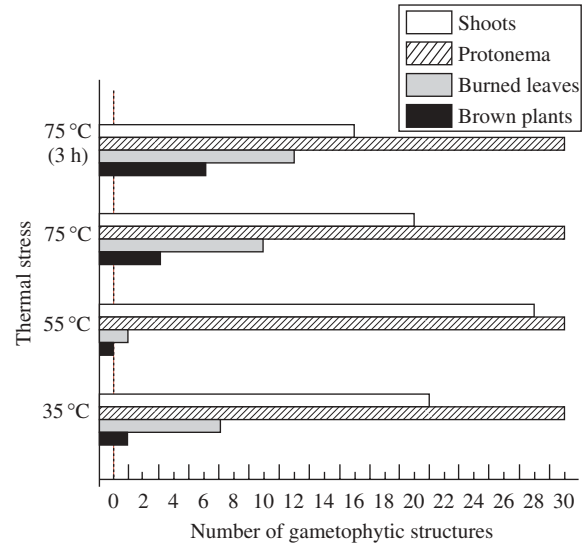


FIG. 2. Number of *Microbryum* gametophytes that became entirely chlorotic (brown plants), exhibited partial burning of leaves (burned leaves), produced shoot buds (shoots), and produced protonemata (protonema) after exposure to thermal stress when desiccated. Exposure was for 1 h unless stated.

critical  $\chi^2$  value at  $\alpha = 0.05$  was adjusted as each pairwise treatment comparison was made (Sokal and Rohlf, 1995). For sporophytes, we tested for an association between stress treatment and the probability of the sporophyte leaving one phenophase and entering another phenophase. The critical  $\chi^2$  value at  $\alpha = 0.05$  was adjusted for each pairwise treatment comparison among the four phenophase transitions (embryonic to post-embryonic, post-embryonic to seta elongation, seta elongation to pre-meiotic capsules, and pre-meiotic capsules to meiotic capsules). Two-way nested ANOVAs (dishes within treatment) were used to analyse for treatment differences in days to post-embryonic sporophytes and capsule expansion. Due to some treatments having no or very low phenophase transitions, the number of possible comparisons among treatments was necessarily reduced in the later phenophase transitions.

To investigate gametophytic–sporophytic interactions, we tested for a relationship between gametophytic vigour and sporophyte development, within treatment, to determine if plants aborting sporophytes at the embryonic phenophase had a different time to gametophytic emergence relative to plants that produced a post-meiotic capsule. A one-way ANOVA was used to test for these patterns. Days to emergence were square-root transformed. All statistical analyses were done using SAS (1994).

## RESULTS

### Gametophytic recovery

All 120 gametophytes regenerated (Fig. 2), with mean time to protonemal emergence ranging from 14.5 to 17.2 d across treatments (Table 1). Protonemata took significantly longer ( $P < 0.05$ ) to emerge from plants subjected to 55 °C for 1 h compared with all other thermal exposures. Growth rate

TABLE 1. Times (in days) to protonemal emergence, production of first shoot buds, termination of the embryonic phenophase, and capsule expansion after thermal exposures (1 h unless stated) in *Microbryum*

Thermal exposure (°C)	Protonema	Shoot bud	Post-embryonic sporophytes	Capsule expansion
35	14.47 ± 0.63 <sup>a</sup>	17.62 ± 1.11 <sup>a</sup>	4.93 ± 0.30 <sup>a</sup>	12.88 ± 0.74 <sup>a</sup>
55	17.20 ± 0.36 <sup>b</sup>	16.50 ± 0.41 <sup>a</sup>	5.00 ± 0.21 <sup>a</sup>	14.13 ± 0.90 <sup>a</sup>
75	15.50 ± 0.39 <sup>a</sup>	16.43 ± 0.65 <sup>a</sup>	7.00 ± 1.13 <sup>b</sup>	–
75 (3 h)	14.73 ± 0.53 <sup>a</sup>	17.94 ± 1.49 <sup>a</sup>	–	–

Values are mean ± 1 s.e.; different letters indicate a significant difference ( $P < 0.05$ ). Dashes indicate phenophase was not reached.

TABLE 2. Shoot number, final protonemal area produced, and growth rate after thermal exposures (1 h unless stated) in *Microbryum*

Thermal exposure (°C)	Shoot number	Protonemal area (mm <sup>2</sup> )	Growth rate (mm <sup>2</sup> d <sup>-1</sup> )
35	1.00 ± 0.15 <sup>ab</sup>	127.60 ± 7.39 <sup>a</sup>	6.13 ± 0.34 <sup>a</sup>
55	1.33 ± 0.13 <sup>a</sup>	125.42 ± 4.15 <sup>a</sup>	7.08 ± 0.24 <sup>a</sup>
75	1.30 ± 0.21 <sup>ab</sup>	133.62 ± 4.78 <sup>a</sup>	6.88 ± 0.23 <sup>a</sup>
75 (3 h)	0.73 ± 0.17 <sup>b</sup>	93.28 ± 7.81 <sup>b</sup>	4.62 ± 0.39 <sup>b</sup>

Values are mean ± 1 s.e.; indicate a significant difference ( $P < 0.05$ ).

after emergence ranged from 4.62 to 7.08 mm<sup>2</sup> d<sup>-1</sup> and was significantly less in plants subjected to 75 °C for 3 h compared with all other stress treatments (Table 2;  $P < 0.05$ ). This pattern was also reflected in final protonemal area (Table 2). Mean shoot production ranged from 0.73 to 1.33 shoots per plant, with shoot production from plants subjected to 55 °C for 1 h significantly higher than plants exposed to 75 °C for 3 h (Table 2;  $P < 0.05$ ). The probability of having burned leaves (partial chlorosis) was less in shoots subjected to 55 °C for 1 h than in 75 °C for 1 or 3 h.

#### Sporophytic recovery

Sporophytes were more adversely affected by the thermal exposures than gametophytes. Sporophytes exposed to 35 °C had a higher probability of transitioning from embryonic to post-embryonic (rupturing the vaginula) phenophases than sporophytes exposed to 75 °C for 1 h (46 % vs. 0 %, respectively, Fig. 3;  $P < 0.05$ ). Similarly, sporophytes exposed to 55 °C had a higher probability of transitioning from embryonic to post-embryonic phenophases than sporophytes exposed to 75 °C (1 h and 3 h exposures, 63 % vs. 20 % and 0 %, respectively, Fig. 3;  $P < 0.05$ ). Since none of the sporophytes from the 75 °C/3 h treatment became post-embryonic, comparisons involving these plants in later transitions are not possible. Sporophytes exposed to 35 °C and 55 °C had a higher probability of transitioning from post-embryonic to seta elongation phenophase than sporophytes exposed to 75 °C for 1 h (100 % and 100 % vs. 17 %, respectively, Fig. 3;  $P < 0.05$ ). Sporophytes from the 75 °C/1 h treatment were not included in subsequent analyses because only a single sporophyte survived the transition to seta elongation. In all other phenophase transitions the two remaining treatments (35 °C, 55 °C) did not differ from each other (Fig. 3;

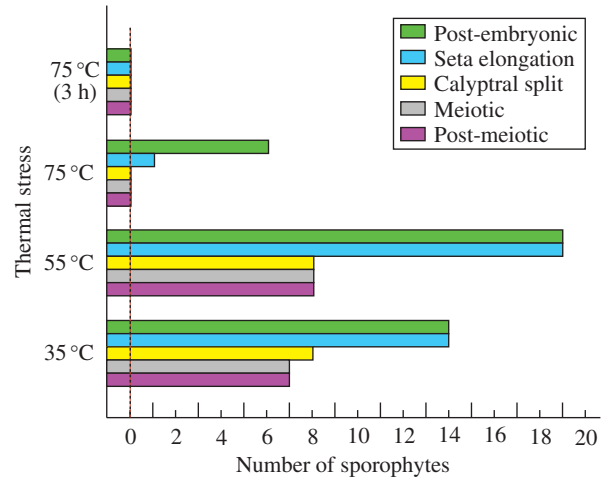


FIG. 3. Number of *Microbryum* sporophytes that resumed sporophyte growth and terminated the embryonic phenophase (post-embryonic), extended in length to where the seta exceeded the calyptra (seta elongation), initiated capsule expansion (calyptral split), became meiotic (meiotic), and completed meiosis (post-meiotic) after exposure to thermal stress when desiccated. Exposure was for 1 h unless stated.

$P > 0.05$ ). At the two lower thermal exposures, 25 % (15/60) of the sporophytes reached meiosis, compared to none in the two highest thermal exposures (Fig. 3). The time to reach the post-embryonic phenophase was significantly longer in the 75 °C/1 h treatment, and the time to reach the capsule expansion phenophase did not differ among treatments (Table 1).

#### Gametophytic and sporophytic relationships

Only sporophytes from the 35 °C and 55 °C exposures reached meiosis; therefore the relationship between gametophytic regeneration and sporophytic development was restricted to these two treatments. In both of these exposures, plants that produced meiotic sporophytes took longer times to regenerate gametophytically when compared with those plants that aborted their sporophytes. In the 35 °C exposure, the number of days to protonemal emergence was 15.8 ± 1.0 d vs. 12.7 ± 0.3 d (matured and abortive sporophytes, respectively, d.f. = 1,  $F = 13.37$ ,  $P = 0.0015$ ), and for the 55 °C treatment the number of days to protonemal emergence was 18.5 ± 0.7 d vs. 15.8 ± 0.4 d (matured and abortive sporophytes, respectively, d.f. = 1,  $F = 10.62$ ,  $P = 0.0049$ ).

## DISCUSSION

In this study, short-term thermal exposures on desiccated maternal shoots containing embryonic sporophytes of the moss *Microbryum starckeanum* resulted in generational differences in survival. While all maternal gametophytes survived exposures up to 75 °C for 1–3 h by retaining the ability to regenerate through protonemal production, no embryonic sporophyte remained viable (capable of reaching meiosis) after exposure to 75 °C for 1–3 h. Maternal gametophytes exposed to 75 °C for 3 h responded less vigorously in growth rate and had non-significant tendencies to produce fewer shoots, to have burned (partially chlorotic) leaves, and to have more entirely chlorotic shoots and leaves than maternal gametophytes from other exposures. A puzzling result was the regeneration response of maternal gametophytes exposed to 55 °C for 1 h. These gametophytes were less likely to be thermally stressed, as indicated by the proportion of shoots with burned leaves, and yet these shoots had significantly delayed emergence times relative to other exposures. Longer times to protonemal emergence suggest reduced vigour. Why would maternal gametophytes subjected to lower temperature exposures be less vigorous relative to more highly stressed maternal gametophytes? The answer to this question may involve the interactions among the stress response and the apparent trade-off between emergence times of the gametophyte and maturation of the sporophyte to meiosis (discussed below).

To our knowledge, thermotolerance, while fairly well studied in gametophytes, is virtually unstudied in the sporophytes of bryophytes. Known phenological patterns of sporophyte maturation clearly indicate that sporophytes are capable of surviving elevated temperatures of summer in temperate regions, with four of the five patterns including partial sporophyte development during the warmer portions of the year. Desert mosses in particular achieve fertilization during the winter and the embryonic sporophytes persist through the summer, doubtless exposed to high soil-surface temperatures (Stark, 2002a). In this experiment, no sporophytes matured following the higher thermal exposures (75 °C for 1 or 3 hr), while only 15 out of 60 sporophytes matured following the 35 °C/1 h and 55 °C/1 h exposures. After thermal exposures, maternal shoots with attached embryonic sporophytes were rehydrated, whereupon shoots experience elevated levels of respiration ('resaturation respiration') as part of the recovery from desiccation stress (Oliver and Wood, 1997). Thus, shoots of *Microbryum* were simultaneously recovering from desiccation (where synthesis of repair proteins can be costly) and also from thermal stress.

Although unstudied in bryophytes, the mechanism of recovery from heat shock in its basics is nearly universal in organisms and probably similar to that found in seed plants. Thermal recovery in seed plants enlists both constitutive and inducible production of heat stress proteins (HSPs), which are molecular chaperones essential for maintenance and/or restoration of protein homeostasis. The transcription of genes encoding HSPs is controlled by cytoplasmic regulatory proteins called heat stress transcription factors (HSFs), of which there are three classes in

plants. Organisms of all kinds respond to thermal stress by synthesizing HSPs, which assist in the normalization of functions during the recovery period. Specifically, these HSPs assist in the folding, intracellular distribution, assembly and degradation of proteins, and prevent unproductive aggregation of proteins. Critically, the HSPs serve to stabilize damaged proteins and facilitate renaturation in the recovery period (reviewed in Baniwal *et al.*, 2004).

Abortion of bryophyte sporophytes may be due to either extrinsic or intrinsic factors. Extrinsic factors known to operate include frost (Hancock and Brassard, 1974; Longton, 1990), shoot density in the patch (Kimmerer, 1991), physical damage (Moyá, 1992), atmospheric pollution (Sagmo Solli *et al.*, 2000) and desiccation (Johnsen, 1969). In arid-land regions, where moisture availability restricts the duration of patch hydration to the cooler months (Alpert and Oechel, 1985), summer rainstorms serve to hydrate the patch temporarily and thus expose hydrated shoots to thermal stress, and may trigger sporophyte abortions (Stark, 2002b). The situation is complicated by the dependence of the sporophyte upon the maternal gametophyte, with the latter known to withstand long periods of desiccation under cool and hot thermal regimes (Oliver *et al.*, 1993). Intrinsic causes are based on the resource pool of the maternal gametophyte that can be made available to the maturing sporophyte, especially during capsule expansion (Proctor, 1977; Ligrone and Gembardella, 1988; Yip and Rushing, 1999). Thus in bryophytes, the nature of the physiological connection between sporophyte and gametophyte that is present throughout sporophyte development predicts that sporophyte vigour depends upon the 'phenotype (and genotype) of the gametophyte to which it is attached' (Shaw and Beer, 1997). Therefore, the maternal gametophyte is intricately coupled to the stress environment of the embryonic and post-embryonic sporophyte, presenting a significant nurturance effect and exerting control over sporophyte size and developmental vigour.

Although it is known that sporophytic maturation is dependent upon gametophytic resources (Proctor, 1977), differentiating the roles of these generational responses may be difficult due to their organic inseparability, the phenomenon of apical dominance, and their likelihood to function in synchrony. In *Microbryum*, the relatively small gametophytes (in comparison with their sporophytes) probably places a further strain on maternal shoot resources. Thus sporophyte thermotolerance is best viewed as a combination of extrinsic stresses imposed on the sporophyte through temperature elevation and desiccation recovery, linked with dependence upon the physiological state of the maternal gametophyte. This hypothesis is consistent with observations in seed plants that external stress tends to slow vegetative growth and yet terminates reproductive structures (Chiariello and Gulmon, 1991). In *Microbryum* the severe thermal stress of 75 °C not only terminated all embryos but noticeably damaged (but did not kill) the maternal gametophytes. The dual nature of the imposed stress (thermal and desiccation) probably compromises the gametophytic resource pool to be made available to the maturing sporophyte, causing the gametophyte to abort the sporophyte.

While we are unable to distinguish between strict thermal tolerance on the part of the sporophyte and abortion triggered by inadequate allocation of resources by the maternal gametophyte, we did detect a possible trade-off between gametophytic response and sporophytic response. For both the 35 °C/1 h and 55 °C/1 h exposures, maternal gametophytes that produced mature sporophytes had significantly slower protonemal emergence compared to maternal gametophytes with sporophytes aborting as embryos. Thus the development of the sporophyte through meiosis may have led to delayed protonemal emergence from the (stressed) maternal gametophyte. This apparent trade-off needs further study, and is consistent with findings in *Dicranum*, where Bisang and Ehrlén (2002) found (1) segments bearing sporophytes were of lower biomass and (2) segment length and sporophyte production reduced the probability of future sex expression and innovation production. In the future it should be possible to explore this trade-off further by experimentally manipulating the resource environment of the maternally gametophyte.

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