

## DO THE SEXES OF THE DESERT MOSS *SYNTRICHIA CANINERVIS* DIFFER IN DESICCATION TOLERANCE? A LEAF REGENERATION ASSAY

Lloyd R. Stark,<sup>1,\*</sup> Lorenzo Nichols II,\* D. Nicholas McLetchie,<sup>†</sup> and Mary L. Bonine\*

\*Department of Biological Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, Nevada 89154-4004, U.S.A.; and <sup>†</sup>Department of Biology, 101 Morgan Building, University of Kentucky, Lexington, Kentucky 40506-0225, U.S.A.

Disparate sex ratios are a widespread pattern in dioecious bryophytes, with female-biased ratios especially prevalent in arid environments. The absence of male plants in environments experiencing high desiccation pressure prompted the hypothesis that male plants may be less desiccation tolerant than female plants in the desert moss *Syntrichia caninervis*. This hypothesis was investigated by exposing detached leaves to consecutive wet/rapid-dry treatments and monitoring viability, protonemal emergence time, shoot production, growth rate of secondary protonemata, and microbial infection frequency over a 56-d period. The desiccation treatment consisted of exposure of mature 1-yr-old leaves to zero, two, four, and six wet/rapid-dry cycles. Hydrated leaves were then allowed to regenerate. Desiccation stress level was significantly correlated to reduced protonemal emergence, reduced growth rates, and reduced shoot production. Female detached leaves produced protonemata more quickly, and these protonemata grew twice as rapidly and eventually produced more shoots than male detached leaves. Male leaves were also more subject to mortality and microbial infection, although these trends were not statistically significant. No sex × desiccation stress interactions occurred in the stress responses measured. The disparity in growth rates between female and male leaf regeneration, under both stressed and nonstressed conditions, may play a significant role in male rarity. We conclude (i) that the leaf regeneration assay works well as a response variable for desiccation tolerance (DT) studies and (ii) that sex-based DT, at least with respect to responses to rapid drying cycles in the lab, while not indicated in *Syntrichia*, may yet operate under field conditions.

**Keywords:** desiccation tolerance, bryophyte, desert, leaf regeneration, sex dimorphism, sex ratio, protonema.

### Introduction

A common attribute among many bryophytes is the quick and fully reversible switch from the hydrated, metabolically active state to the dry, inactive, fully desiccated state (Proctor 2001). This form of vegetative desiccation tolerance (DT) evolved early in the land plants; while subsequently lost during tracheophyte evolution, it was retained in the bryophytes (Oliver et al. 2000) and actually represents a highly evolved strategy of coping with desiccation (Proctor and Tuba 2002). This initial evolution of vegetative DT was thus critical in the colonization of land by plants, albeit metabolically costly, with a diverse array of repair proteins synthesized both constitutively and inducibly that likely function to repair membrane damage incurred on rewetting (Oliver 1991). However, photosystems of desiccation-tolerant mosses essentially survive desiccation intact, returning to a functional state in minutes and without significant mediation from repair proteins (Proctor and Smirnov 2000).

A variety of assessments of DT among bryophytes has been recently employed, including viability, chlorophyll concentration or carotenoid content (Hearnshaw and Proctor 1982; Seel et al. 1992), protein synthetic response (Dhindsa and Bewley 1977; Oliver et al. 1993), color degradation (Glime

1971; Sollows et al. 2001), chlorophyll fluorescence (Hamerlynck et al. 2000; Robinson et al. 2000; Takács et al. 2000; Cleavitt 2002), photosynthetic or dark respiration rate (Arscott et al. 2000), rate of loss of antioxidant metabolites (Dhindsa 1987; Takács et al. 2001), membrane leakage (Dhindsa and Bewley 1977), cell ultrastructure and pigment composition (Gerdol et al. 1996), and sugar content (Robinson et al. 2000). The ability of bryophyte leaves to regenerate new plants led Egunyomi (1979) to suggest that leaf regeneration be used to comparatively assess DT among species of bryophytes. This approach has value because it focuses on the ability of a propagule, the detached leaf, to be a likely primary colonization agent among bryophytes, producing new individuals after a stress event. Indeed, as noted by Cleavitt (2002), fragments of shoots and leaves probably serve as the dominant means of asexual reproduction in mosses (Longton and Schuster 1983; Longton 1994, 1997).

Most hydrating events in the desert are short, especially during the warmer portions of the year (Alpert 1979), which may span 9 mo in hot deserts (Smith et al. 1997). Rapid desiccation, along with tolerance of high temperatures, is therefore likely to represent one of the most severe stresses faced by desert mosses and may thus serve as a major evolutionary selection force in harsh climates. Rapidly dried plants of *Syntrichia ruralis*, when rehydrated, suffer more extensive damage than slowly dried plants, as exhibited along at least five avenues: (i) they exhibit higher resaturation rates of

<sup>1</sup> Author for correspondence; e-mail lrs@unlv.nevada.edu.

metabolism; (ii) they leak more solutes from their external membranes; (iii) they exhibit slower rates of protein synthesis; (iv) they take longer to repair membrane damage to mitochondria and chloroplasts; and (v) they suffer greater loss of chlorophyll from tissues (reviewed in Bewley 1995). Following prolonged desiccation in *S. ruralis*, recovery of the photosynthetic system on rewetting is efficient, with recovery of  $F_v/F_m$  (fluorescence) nearly instantaneous (Csintalan et al. 1999; Proctor 2001) and a positive carbon balance regained within 1–2 h of rewetting (Proctor 2002). Although the complete recovery of membrane systems and cellular constituents on the basis of protein synthetic patterns may take up to 24 h (Oliver 1991), there is evidence that reassembly and reactivation (rather than repair) of internal cell machinery is as critical as repair-mediated processes, especially in photosynthetic pathways (Proctor and Smirnov 2000).

A link between the often-observed phenomenon of male rarity (relative to the female) and desiccation stress was made by Newton (1972). Male rarity has also been indirectly linked to gender-specific DT in the liverwort *Riccia frostii* (Pettet 1967) and in the moss *Syrrhopodon texanus* (Reese 1984). Newton (1972) presented preliminary data prompting the hypothesis that the sexes of dioecious bryophytes respond differently to desiccation stress. Following leaf regeneration in the moss *Mnium undulatum*, the effect of desiccation on regenerants had a pronounced effect on sex; 1 mo of air desiccation killed all male leaves, but female leaves survived desiccation at a rate of 77%, prompting Newton to hypothesize that male leaves may be less desiccation tolerant than female leaves. Although we employ wet/dry cycles in place of extended desiccation periods and use field-collected rather than regenerant leaves, this work is the first attempt to follow up on her hypothesis. Could sex ratio patterns in *Syntrichia caninervis*, where female individuals routinely outnumber males by ratios in excess of six female : one male, reflect sex-specific DT in the clonal regeneration of individual plants? If females of a dioecious species are able to recover from damage more quickly than males and produce a greater carbon gain than males (the carbon balance hypothesis; Ried 1960; Alpert and Oechel 1985), this could help explain both the dominance of females in deserts and the restriction of males to favorable microsites (Bowker et al. 2000). In this article, we explore potential sex-specific regeneration rates following exposure to desiccation stress in this desert moss and pose the following hypothesis: detached mature female leaves exhibit more vigorous leaf regeneration in some traits, given that they are the majority sex, after exposure to levels of desiccation stress. We predict that female detached leaves, relative to male leaves, will produce protonemata earlier, eventually produce more shoots, and grow at a faster rate than male detached leaves and that this difference will be stress dependent.

## Material and Methods

### Patch Sampling and Sex Determination

On May 29, 2002, 15 sporophytic patches from a single metapopulation (*sensu* Husband and Barrett 1996) of *Syntrichia caninervis* were collected from the northern Mojave Desert at the Nevada Test Site, Frenchman Flat, adjacent to

the Mojave Global Change Facility (a study site investigating the effects of global change on Mojave Desert vegetation; lat. 36°45'36"N, long. 115°59'24"W). Sporophytic patches were intentionally targeted because the presence of sporophytes indicates that both sexes are likely to be present (Bowker et al. 2000). At this site, *S. caninervis* represents the dominant moss in the landscape. These patches (defined as a series of more or less contiguous clumps of stems separated from other such series) were physically separated from one another by at least 5 m. Ten of these patches yielded at least two male and two female shoots from a nonintrusive sexing of 25 shoots per patch carried out using a dissecting microscope. Two shoots of each sex per patch were randomly selected for use. In all, 20 female and 20 male shoots (ramets) were used in the experiment, with all 40 shoots kept in the lab air dry and in the dark before the experiment. From each hydrated shoot, the first four mature, chlorophyllous leaves were removed and allowed to air dry in micropackets. During the leaf removal, preexperimental hydration intervals were kept fairly constant at ca. 5 min. These leaves were located just proximal to juvenile leaves near the shoot apex (pictured in Stark et al. 2004) and were ca. 1 yr old at the time of collection, produced during the most recent growth period. To estimate initial leaf biomass, 10 shoots were randomly selected (one per patch) and hydrated, and up to five mature, 1-yr-old leaves were detached from each stem. These groups of leaves were dried to constant mass at 40°C for 3 d and weighed to the nearest microgram.

### Initial Hydration Period

Soil was collected from the native habitat, sieved through a 500- $\mu$ m mesh, dry autoclaved for 60 min at 131°C, and apportioned into 160 mini petri dishes (35 mm i.d.) to a level about half filled (substrate depth = 4–5 mm). Distilled water was applied to the edge of the dish, wetting the entire surface, and the water level was blotted down to the soil surface. Each leaf was hydrated and placed on the substrate, adaxial surface up, in the growth chamber for 72 h (Percival E30B, Boone, IA). This initial hydration period is consistent with Schonbeck and Bewley (1981b), who found that after 3 d of continuous hydration of *Syntrichia ruralis* ramets dehardening to DT occurs. Growth chamber settings were a 12-h photoperiod and day/night temperatures of 20°/8°C, approximating winter/early spring conditions in the Mojave Desert (National Weather Service 1994) and consistent with physiological optima for a variety of xeric and mesic bryophytes from the United States and the United Kingdom (Furness and Grime 1982; Alpert and Oechel 1987). Light levels ranged from 89 to 128  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> on the upper shelf and from 33 to 44  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> on the lower shelf (PAR sensor, LI-COR LI-250, Lincoln, NE).

### Desiccation Treatments

After 72 h of hydration, each leaf was rinsed in dH<sub>2</sub>O and transferred into a 48-hole wellplate (one leaf per well), which was placed inside a glass desiccation chamber wherein relative humidity was maintained at <10% using a desiccant (Drierite). Leaves treated in this manner desiccated in <30 min. Following leaf desiccation, individual leaves were rehydrated by pipetting 1.5  $\mu$ L of dH<sub>2</sub>O into each well, and

the wellplate was lidded and placed into the lighted growth chamber for 2 h. After this hydration period, the wellplate was removed from the growth chamber, the lid was removed, and the wellplate was placed into the desiccation chamber. In this manner, leaves were subjected to zero (control), two (low stress), four (middle stress), and six (high stress) repetitive cycles of wet/rapid drying/wet (termed a "rapid-dry," or RD, cycle). Intervening hydration periods of 2 h occurred between RD treatments, following Schonbeck and Bewley (1981b), who found that rapidly dried ramets of *S. ruralis* required ca. 2–3 h to recover to zero net oxygen exchange, experiencing a negative net oxygen exchange until 2–3 h had passed. These treatment levels were decided after four separate pilot trials in which hydration time and number of RD cycles were varied. After the appropriate number of RD cycles, individual leaves were hydrated in the wellplate, placed in petri dishes containing hydrated substrate (one leaf per dish), adaxial surface upward, and transferred into the growth chamber. On day 1, leaves were repositioned and rinsed of sand grains as necessary to ensure clear visibility of the basal and lateral leaf edges (where protonemata commonly emerge).

Dishes were moved from one shelf to a random position on the other shelf every day through the first 9 d of the experiment, every third day from day 10 to day 21, and twice a week thereafter (days 22–56). Each leaf was examined under a dissecting microscope at 60 $\times$ ; protonemal production, shoot number, and any fungal or algal contamination were noted on days 4, 5, 6, 7, 8, 9, 12, 15, 18, and 21 and once per week thereafter. Dishes were watered weekly with dH<sub>2</sub>O to bring the water level to the substrate surface. RH in the chamber was 60%–70% for the duration of the experiment.

At the conclusion of the experiment (56 d), dishes were allowed to air dry inside the chamber by removing the lids. On desiccation (ca. 24 h), the surface area colonized by secondary protonemata was determined using image analysis software (SPOT, Diagnostic Instruments, Sterling Heights, MI). Final dry biomass produced by each leaf was determined as follows: (i) the dry protonemal/shoot complex (hereafter called the "protonemal complex," or complex) was excavated as it was viewed under a dissecting microscope; (ii) the complex was rinsed up to 10 successive times in dH<sub>2</sub>O as it was viewed under the dissecting microscope, which removed most sand grains; (iii) the complex was blotted dry and placed in a micropacket made of weighing paper; (iv) the complex was dried to constant mass at 40°C for 3 d; and (v) the complex was weighed on a microbalance to the nearest 0.001 mg. Known average values for fully expanded leaves were then subtracted, yielding an approximation of biomass produced from each regenerated leaf.

### Statistics

Analyses were performed to test for sex, cycle level (desiccation), and/or sex and desiccation interaction effects on (i) the time to emergence of protonema, (ii) protonemal extension rate after emergence, (iii) biomass accumulation rate after emergence, (iv) time to shoot production, (v) probability of shoot production, and (vi) number of shoots. For emergence (initial regeneration), we used a two-way ANOVA to determine the effect of sex, desiccation treatment, and the interac-

tion of sex and desiccation treatment on protonemal emergence time. Tukey's Student range test was used to compare desiccation treatment means. Protonemal extension and biomass accumulation rate for each plant were determined as the size of the protonemata (in square millimeters) or the amount of biomass (in milligrams) divided by the number of days from emergence to the end of the experiment (day 56). Biomass used in the above calculation was the final biomass minus an average initial biomass for mature leaves. Average initial biomass of mature leaves was estimated from weighing 37 mature leaves at one time, yielding a mean leaf mass of 0.014 mg. We used a two-way ANOVA to determine the effects of sex, desiccation, and the interaction of sex and desiccation on protonemal growth and biomass accumulation rates. Tukey's Student range test was used to compare desiccation treatment means. Shoot responses were treated in two ways. First, for the probability of shoot production, we used log-linear analysis to determine whether shoot occurrence was associated with sex and desiccation treatment; i.e., sex and desiccation treatment were considered explanatory variables for shoot occurrence. An association was indicated by a significant interaction between shoot occurrence and one or both of these factors (sex or desiccation). The statistical significance of an interaction was determined by assessing the change in the log-likelihood ratio ( $G^2$ ) after addition or deletion of that term from the model (Sokal and Rohlf 1995). This analysis was accomplished using categorical data analysis (CATMOD procedure; SAS 1994). Second, for the number of shoots, we used a two-way ANOVA to determine the effects of sex, desiccation treatment, and the interaction of sex and desiccation treatment on shoot production at day 56;  $\chi^2$  tests were employed to test differences between gender and stress level for infection and mortality. All statistical analyses were performed using the Statistical Analysis System (SAS 1994).

## Results

### Leaf Viability and Microbial Infection

At the conclusion of the experiment, some leaves failed to produce protonema, and some had microbial activity. Leaf mortality, as judged by an inability to produce protonema, was more likely to occur in the medium and high stress levels (four and six RD cycles) than in the control and low stress levels (zero and two RD cycles: 12.5% vs. 0%, respectively,  $\chi^2 = 9.26$ ,  $df = 1$ ,  $P < 0.005$ ; table 1). Although no female mortality occurred in the four-cycle treatment and three male leaves died, there was no significant relationship between sex and mortality. The incidence of leaves having microbial (usually fungal or algal) infection was higher under the medium- and high-stress treatments than in control and low stress levels (25% vs. 10%, respectively,  $\chi^2 = 6.2$ ,  $df = 1$ ,  $P < 0.125$ ). Across all treatments, males tended to have more microbial infections than females, a tendency that was marginally significant (24.7% vs. 13.0%, respectively,  $\chi^2 = 3.36$ ,  $df = 1$ ,  $P = 0.0667$ ).

### Time to Protonemal Emergence

Female leaves produced protonemata earlier than males (fig. 1;  $P < 0.05$ ), and there was a significant stress-level

**Table 1**  
**Leaf Viability of *Syntrichia caninervis* as a Function of Desiccation Treatment and Leaf Gender**

	Number of RD cycles			
	0 (control)	2	4	6
Male	20	20	17	16
Female	20	20	20	17
Total	40	40	37	33

Note. Values represent the number of leaves producing secondary protonemata after 56 d in the growth chamber (of 20). RD = rapid-dry (wet/rapid-dry/wet).

effect, with leaves subjected to higher stress levels taking longer to emerge ( $P < 0.0001$ ; table 2). There was no interaction effect between sex and stress.

#### *Protonemal Expansion Rate and Biomass Accumulation Rate*

Overall, female leaves produced protonemata that expanded in area and accumulated biomass at approximately twice the rate of males (figs. 2, 3;  $2.54 \pm 0.32$  vs.  $1.20 \pm 0.17$   $\text{mm}^2 \text{d}^{-1}$ ;  $0.019 \pm 0.002$  vs.  $0.008 \pm 0.001$   $\text{mg d}^{-1}$ ;  $P < 0.0001$ ; table 2). There was also a significant desiccation-level effect, with leaves subjected to higher stress levels (more cycles) having slower protonemal growth and biomass accumulation rates than controls (figs. 2, 3;  $P < 0.003$ ). However, only the highest stress level (six RD cycles) was significantly different from the control for both response variables. There was no interaction effect between sex and stress for protonemal growth and biomass accumulation rates.

#### *Shoot Production*

Female leaves were more likely to produce shoots (both from protonemata and directly from leaf tissue) than male leaves (0.48 vs. 0.18;  $P < 0.001$ ), and as the number of RD cycles increased, the occurrence of shoots decreased (zero cycles = 0.52; two cycles = 0.32; four cycles = 0.32; six cycles = 0.12;  $P < 0.01$ ; table 3). In addition, female leaves produced more shoots per leaf than males (fig. 4;  $P < 0.0002$ ). For both likelihood of producing a shoot and the number of shoots per leaf, there was a significant stress-level effect, with leaves subjected to higher stress levels having fewer shoots ( $P < 0.0002$ ; table 2). However, there was no interaction effect between sex and stress on shoot production. The mean time to produce the first shoot varied from 34 to 47 d and was unrelated to stress level or gender ( $P > 0.10$ ; data not shown).

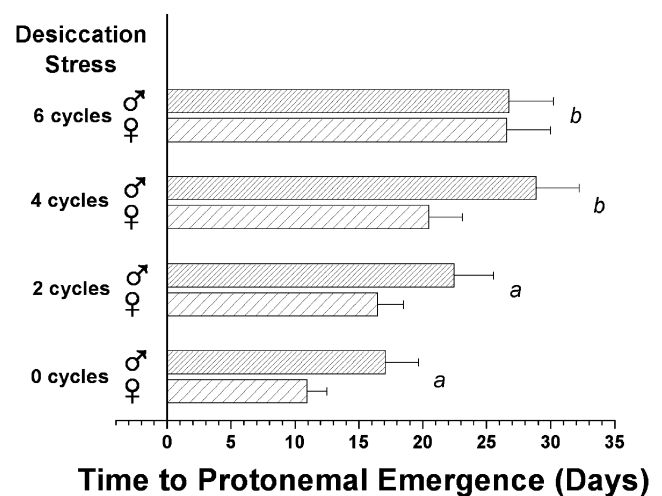
#### **Discussion**

*Syntrichia caninervis* exhibits both an extremely female-biased sex ratio and probable spatial segregation of the sexes, with males restricted to upper elevation sites that are shaded, moist, and of low light intensity (Stark et al. 1998; Bowker et al. 2000). Such habitat preferences indicated the possibility

of a gender-based difference in stress response, in particular the response to desiccation tolerance (DT), because low-elevation and exposed sites, where males are absent, typically desiccate more quickly than higher-elevation and shaded sites (Walker et al. 2001; Hamerlynck et al. 2002). Given the low frequency of sexual reproduction and the capacity of bryophytes to expand clonally (Newton and Mishler 1994), we elected to test the regenerational capacity of detached leaves of each sex after exposure to increasing levels of stress in the form of consecutive wet/rapid-dry events (RD cycles). We hypothesized that female detached leaves should respond to desiccation stress more favorably than male detached leaves and that this response would help us understand the unusually biased sex ratios and habitat preferences exhibited by this plant.

#### *Regeneration Response to Stress*

The ability of desiccation-tolerant bryophytes to recover from rapid drying events sets them apart from desiccation-tolerant vascular plants, which survive gradual drying rates but die when exposed to desiccation rates commonly experienced by aridland bryophytes (Bewley and Krochko 1982; Phillips et al. 2002). Even so, one can view DT among all plants as a continuum from low-inertia (bryophytes and lichens) to high-inertia (vascular plants) desiccation-tolerant plants (Proctor and Tuba 2002). Survival and regeneration following consecutive episodes of rapid desiccation are required of desert bryophytes and represent perhaps the most severe stress commonly experienced by these plants. As noted by Proctor and Pence (2002 p. 217), "Very short moist periods will lead to net carbon loss. Moist periods long enough for a positive net carbon balance may be insufficient for cell division and growth, but might perhaps allow significant DNA repair." In the Mojave Desert (classified as a hot desert),



**Fig. 1** Time to first protonemal emergence from leaves as a function of desiccation treatment and leaf sex in *Syntrichia caninervis*. Values are calculated from untransformed data (means  $\pm$  1 SE). Cycles with different letters are significantly different ( $P < 0.05$ , Tukey's treatment comparisons).

**Table 2**  
ANOVAs of the Effect of Sex and Desiccation from Leaves  
in *Syntrichia caninervis*

Source	df	F	P
Time to protonemal emergence (d): <sup>a</sup>			
Sex	1	6.14	0.0144
Desiccation	3	9.33	<0.0001
Sex × desiccation	3	0.89	0.4497
Error	141		
Protonemal expansion rate (mm <sup>2</sup> d <sup>-1</sup> ): <sup>a</sup>			
Sex	1	16.63	<0.0001
Desiccation	3	4.89	0.0029
Sex × desiccation	3	0.58	0.6306
Error	141		
Biomass accumulation rate (mg d <sup>-1</sup> ): <sup>a</sup>			
Sex	1	13.72	0.0003
Desiccation	3	4.88	0.0029
Sex × desiccation	3	0.79	0.5012
Error	141		
Shoot production (shoots leaf <sup>-1</sup> ): <sup>b</sup>			
Sex	1	14.89	0.0002
Desiccation	3	7.11	0.0002
Sex × desiccation	3	2.41	0.0696
Error	141		

<sup>a</sup> Data were log transformed before analysis.

<sup>b</sup> Data were square root transformed before analysis.

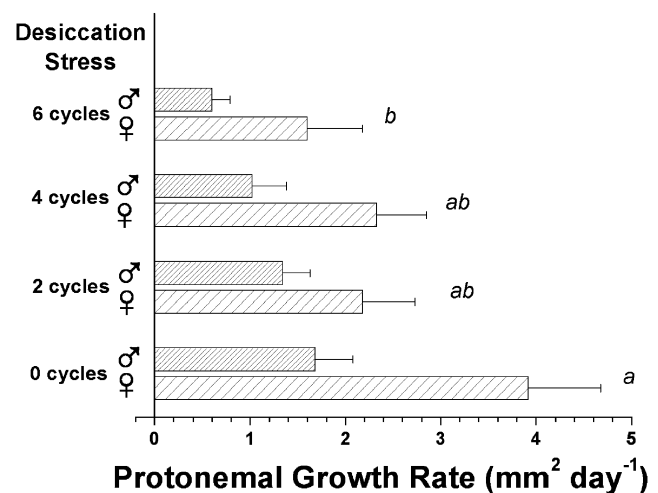
mosses are fully hydrated for only several consecutive days and can desiccate in a matter of minutes. The treatment employed in this experiment of a 2-h hydration period followed by rapid drying in under 30 min thus imposes a realistic stress on these plants.

Rapid drying is normally considered the complete desiccation of plant tissue within a 1–2 h period (Oliver and Bewley 1984). However, shortened drying times (e.g., 20–30 min) have been used (Dhindsa and Bewley 1977; Schonbeck and Bewley 1981a; Takács et al. 2001). As noted in the “Introduction,” desiccation damage to bryophytes is greatest when the rate of desiccation is fastest (Schonbeck and Bewley 1981b), when the period of hydration is shortest (Bewley 1979; Bewley and Krochko 1982; Oliver and Bewley 1997), and when temperatures are highest (Hearnshaw and Proctor 1982). Although recovery of positive carbon gain is rapid (in minutes; Phillips et al. 2002) and normal internal organelle structure is restored within a few hours (Proctor 1984), full cellular recovery from a drying event may take 24 h or longer (Oliver 1991). After a slow-dry event, respiration levels are several times higher than rates before desiccation for at least 2 h in *Syntrichia ruralis* (Tuba et al. 1996). This contrasts with rapidly dried ramets of *S. ruralis* requiring 2–3 h to recover to zero net oxygen exchange, as noted by Schonbeck and Bewley (1981b), who suggested that rapid drying in *Syntrichia* disrupts thylakoid membranes and ultimately causes a breakdown of chlorophyll *a* (degreening). Such rapidly dried ramets were allowed to grow under normal conditions for

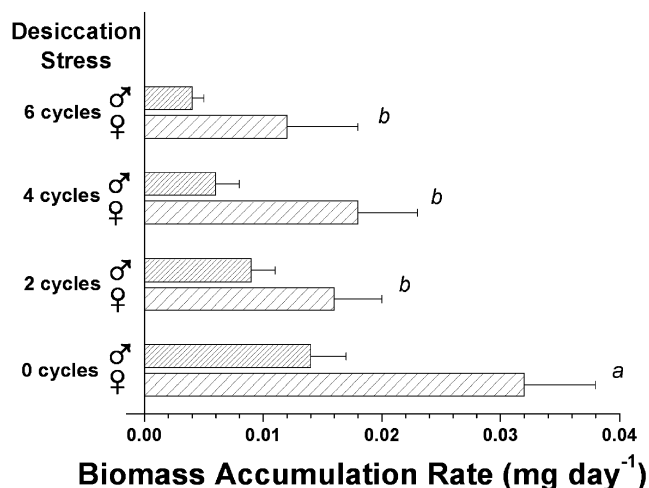
14 d and exhibited an 84% inhibition of growth relative to controls (Schonbeck and Bewley 1981a). Therefore, our treatment of consecutive RD episodes with an intervening hydration period of only 2 h compounds the damage incurred by interrupting the repair processes. This may explain the relatively few consecutive wet/RD events tolerated by detached leaves of *S. caninervis* in this experiment and the strong correlation found between stress level and reduced regenerational vigor.

#### Sex Differential Response to Stress

Previous evidence for a gender-specific response to DT in bryophytes is preliminary. Following Briggs's (1965) suggestion that male plants of *Dicranum scoparium* may prove to be more drought sensitive than the female plants, Newton (1972) presented the first experimental evidence that male regenerant leaves of *Mnium* are more vulnerable to desiccation stress than female leaves. In addition, sex differential desiccation stress was postulated as responsible for the higher mortality observed in expressing males of *Polytrichum commune*: all 50 of the plants that had recently expressed maleness (produced an antheridial cup) died by the end of the study (1 yr), compared with a 34% survival rate of nonexpressing stems; however, female stems were not assessed, and sex-specific comparisons were limited. In *P. commune*, mortality reached a maximum during the summer drought in Connecticut (Watson 1975). Quite possibly, the production of a relatively expensive antheridial cup may compromise the ability of the plant to withstand severe desiccation stress. Furthermore, male rarity (relative to the female) has been indirectly linked to gender-specific DT in the liverwort *Riccia frostii* (Pettet 1967) and in the moss *Syrrhopodon texanus* (Reese 1984). Similarly, in species of bryophytes known only from



**Fig. 2** Protonemal expansion rates from regenerating leaves as a function of leaf desiccation and leaf sex in *Syntrichia caninervis*, after 56 d in culture. Values are calculated from untransformed data (means ± 1 SE). Cycles with different letters are significantly different ( $P < 0.05$ , Tukey's treatment comparisons).



**Fig. 3** Biomass accumulation rates of regenerating leaves as a function of leaf desiccation and leaf sex in *Syntrichia caninervis*. Values are calculated from untransformed data (means  $\pm$  1 SE). Cycles with different letters are significantly different ( $P < 0.05$ , Tukey's treatment comparisons).

a single sex in the arid southwestern United States, all five such species are female-only (*Syntrichia chisosa*, *Syntrichia bartramii*, *Syntrichia pagorum*, *Pseudocrossidium crinitum*, and *Didymodon nevadensis*), consistent with the hypothesis of the male's becoming either extinct or unable to disperse into regions of high desiccation stress.

Although female leaves of *S. caninervis* responded significantly better than male leaves in all measures of regeneration used here, the absence of a sex  $\times$  stress interaction for any regenerative response indicates against an association between gender and DT (we thus reject our hypothesis). However, it is possible that the stress levels we used were not stressful enough compared with field conditions such that at extremely high stress levels females are much more likely to recover than males compared with the female advantage at the stress levels tested here or that so few regenerations occur at extreme stress levels that the sex with the slightest regeneration advantage will more likely survive. Thus, high stress levels are also ecologically relevant to explain male rarity, as are the sex differences in regeneration.

Clearly, females are at an intrinsic advantage in regenerative growth rate over males, and this advantage is preserved when plants are exposed to increasing desiccation stress in the form of RD cycles. Sex differences in growth rates are not unprecedented among dioecious plants. In dioecious seed plants, female plants, relative to male plants, generally have lower growth rates (Jing and Coley 1990; Garcia and Antor 1995; Obeso et al. 1998). Such patterns have been associated with the higher cost of sexual reproduction in female plants relative to male plants. However, the reverse growth rate pattern is also known (Grant and Mitton 1979; Sakai and Burris 1985; Williams 1995). Some bryophyte species are characterized by extreme sexual size dimorphisms where male plants are dwarf and epiphytic on female plants (Loveland 1956; Wallace 1970; Une 1985) or, while not dwarf, obviously smaller than females,

as in the genus *Sphaerocarpos* and in some *Riccia* species (Schuster 1992a, pp. 811–826; 1992b, pp. 421–710). In these species, females are expected to have significantly higher growth rates than males to achieve such a size differential. In species that are not obviously dimorphic with respect to size, females are known to have higher growth rates, as indicated by more biomass production (Shaw and Gaughan 1993) or greater change in area (McLetchie and Puterbaugh 2000). In the two latter studies, male plants had either higher shoot production or higher asexual reproduction than female plants, respectively. Clearly, *S. caninervis* belongs in the latter group of plants, without obvious size dimorphism yet having higher growth rates in females. When this growth rate advantage is combined with a superior shoot production, it is apparent that females are potentially capable of locally displacing males.

**Table 3**

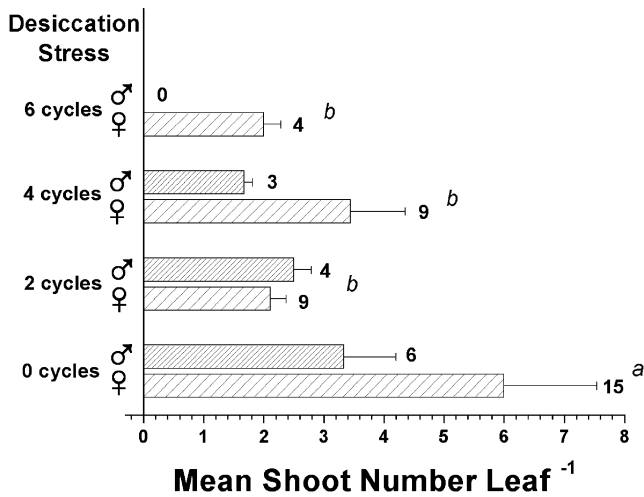
**Results of Log-Linear Analysis of Leaf Desiccation and Sex Associations with Shoot Production of *Syntrichia caninervis***

Association tested with shoot occurrence/model	df	$G^2$
Sex (SO):		
DS, O	7	30.64
–DS, SO	–6	–15.83
Total	1	14.81 <sup>***</sup>
Desiccation (DO):		
DS, O	7	30.64
–DS, DO	–4	–17.66
Total	3	12.98 <sup>**</sup>
Sex within desiccation (SO):		
DS, DO	4	17.66
–DS, DO, SO	–3	–1.12
Total	1	16.54 <sup>***</sup>
Desiccation within sex (DO):		
DS, SO	6	15.83
–DS, SO, DO	–3	–1.12
Total	3	14.71 <sup>**</sup>
Desiccation sex combinations (DSO):		
DS, DO, SO	3	1.12
DSO	–0	–0
Total	3	1.12

Note. The statistical significance of an interaction is determined by assessing the change in the log-likelihood ratio  $G^2$  after addition or deletion of that term from the model. Models include all lower-order terms, e.g., the model DS, O includes D, S, O and DS, where D = desiccation level ( $n = 4$ ), S = sex ( $n = 2$ ), O = occurrence/absence of shoots ( $n = 2$ ), and DS = interaction of desiccation level and sex. See text for further descriptions of categorical data. The difference between the two models (DS, O) ( $G^2 = 30.64$ ) and (DS, SO) ( $G^2 = 15.83$ ) is the interaction term SO ( $G^2 = 14.81$ ), which is the effect of sex on occurrence of shoots. Analyses with the three interaction terms in the models test the effect of one explanatory variable while controlling for the other.

<sup>\*\*</sup>  $P < 0.01$ .

<sup>\*\*\*</sup>  $P < 0.001$ .



**Fig. 4** Shoot production by regenerating leaves as a function of leaf desiccation and leaf sex in *Syntrichia caninervis*. Values are calculated from untransformed data (means  $\pm$  1 SE). Data were log transformed before analysis. Cycles with different letters are significantly different ( $P < 0.05$ , Tukey's treatment comparisons).

The general pattern of higher growth rates of males in seed plants relative to females and the reverse trend in bryophytes is not inconsistent and relies on the same conceptual argument of sex-specific differences and costs of reproduction. In seed plants, females are expected to have higher costs because of the combination of sex expression (flower production in the case of angiosperms) and offspring (fruit) maturation. This cost is reflected as lower growth and survival rates in females relative to males (Delph 1999 and references therein). Growth rate differences are predicted and usually found after reproduction episodes (Lloyd and Webb 1977; Delph 1999). In dioecious bryophytes, females are less likely to produce sexual offspring because of sperm limitation, and in fact, many dioecious species have low levels of sexual reproduction because of a lack of males. Thus, females only incur their total cost if males are present (and even then very rarely; Stark et al. 2000). However, sex-expressing males incur their total cost in the presence or absence of females, and thus on average, males, relative to females, are expected to have higher costs of sexual reproduction, successful or not (Stark et al. 2000). Two studies on bryophytes (Shaw and Gaughan 1993; McLetchie and Puterbaugh 2000) and this study used parts (fragments or asexual propagules) from plants that were once expressing sex. We may consider these derived plants as juveniles, i.e., plants that have not yet incurred the cost of sexual reproduction. Nevertheless, these juvenile, nonexpressing individuals manifest sex differences in growth rates. These results, coupled with the extreme size-dimorphic pattern in other bryophytes, indicates that there may be underlying sex-specific selection that favors larger females and thus higher growth rates, assuming similar germination times, whereas in males, selection favors higher production in ramets (shoots/new asexual offspring, as documented in Shaw and Gaughan 1993 and

McLetchie and Puterbaugh 2000 but not this study). This sex-specific selection might be driven by an advantage for females to be competitive in occupied sites and/or a size advantage to mature offspring and for males to increase gamete dispersal by producing more ramets, each capable of fragmentation/asexual reproduction and subsequent dispersal (McLetchie et al. 2002). However, we are not aware of data documenting a positive correlation of female size with female fitness or male regeneration/asexual reproduction capacity with male fitness. Such data will be needed to support these hypotheses. The key point is that for both seed plants and bryophytes the main driving forces for sex-specific patterns in growth rates ultimately link to sex-specific selection for trait values that increase female and male fitness, given that the sexes play different roles in the sexual process. Previous assessments of shoot extension rates in field patches of *S. caninervis* yielded equivalent male and female growth rates (Stark et al. 2001; Bonine 2004). However, in this study, the leaf regenerational growth rates widely differed between the sexes, suggesting that a qualitative difference may be operating between conventionally measured growth rates and the rate of fragment regeneration. Clearly, a need exists to determine shoot extension rates among regenerant plants in the lab.

In an earlier experiment on the regeneration of detached leaves of different ages in *S. caninervis*, we found shorter emergence times for mature male leaves and less of a difference in biomass accumulation rate between the sexes under conditions of no desiccation stress (Stark et al. 2004). Although constants between the two experiments include patch, time of collection, and observer, leaves in this experiment were subjected to 12 mo of additional dry storage. Such extended periods of desiccation negatively affect recovery (Alpert and Oechel 1985); data from this experiment are consistent with a slight negative effect on regenerational behavior of males (especially). Although we found no evidence of a sex differential stress response in this study, it is possible that such a response may yet lie along avenues of stress alternative to the consecutive RD cycles explored here. Therefore, future experiments will address additional aspects of DT, such as hardening and dehardening to DT and exposure of both regenerant shoots and protonemata to DT, as well as the effects of heat exposure and nutrient depletion on the regeneration potential of each sex.

### Acknowledgments

We thank Derek Babcock, Lynn Fenstermaker, Eric Knight, and Stan Smith for assistance at the Nevada Test Site; Melvin Oliver and Brent Mishler for discussions on desiccation tolerance; and Peter Starkweather for use of his laboratory equipment. L. Nichols was funded by a McNair scholarship and a Western Alliance to Expand Student Opportunities (WAESO) scholarship. Materials and supplies were funded in part by WAESO. Travel funds were provided by an Aridlands Seed Grant through the Interdisciplinary Science of Nevada's Unique Environments Program (National Science Foundation [NSF]-Experimental Program to Stimulate Competitive Research). D. N. McLetchie was supported by NSF grants DEB 99074086 and 0219762.

## Literature Cited

- Alpert P 1979 Desiccation of desert mosses following a summer rainstorm. *Bryologist* 82:65–71.
- Alpert P, WC Oechel 1985 Carbon balance limits the microdistribution of *Grimmia laevigata*, a desiccation-tolerant plant. *Ecology* 66: 660–669.
- 1987 Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting microdistributions. *Am J Bot* 74:1787–1796.
- Arcott DB, WB Bowden, JC Finlay 2000 Effects of desiccation and temperature/irradiance on the metabolism of 2 arctic stream bryophyte taxa. *J N Am Benthol Soc* 19:263–273.
- Bewley JD 1979 Physiological aspects of desiccation tolerance. *Annu Rev Plant Physiol* 30:195–238.
- 1995 Physiological aspects of desiccation tolerance: a retrospect. *Int J Plant Sci* 156:393–403.
- Bewley JD, JE Krochko 1982 Desiccation tolerance. Pages 325–378 in OL Lange, PS Nobel, CB Osmond, H Ziegler, eds. *Physiological ecology. II. Encyclopedia of plant physiology*. Vol 13. Springer, Berlin.
- Bonine ML 2004 Growth, reproductive phenology, and population structure in *Syntrichia caninervis*. MS thesis. University of Nevada, Las Vegas.
- Bowker MA, LR Stark, DN McLetchie, BD Mishler 2000 Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). *Am J Bot* 87:517–526.
- Briggs D 1965 Experimental taxonomy of some British species of the genus *Dicranum*. *New Phytol* 64:366–386.
- Cleavitt NL 2002 Stress tolerance of rare and common moss species in relation to their occupied environments and asexual dispersal potential. *J Ecol* 90:785–795.
- Csintalan Z, MCF Proctor, Z Tuba 1999 Chlorophyll fluorescence during drying and rehydration in the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Anomodon viticulosus* (Hedw.) Hook. & Tayl. and *Grimmia pulvinata* (Hedw.) Sm. *Ann Bot* 84:235–244.
- Delph LF 1999 Sexual dimorphism in life history. Pages 149–173 in MA Geber, TE Dawson, LF Delph, eds. *Gender and sexual dimorphism in flowering plants*. Springer, Berlin.
- Dhindsa RS 1987 Glutathione status and protein synthesis during drought and subsequent rehydration in *Tortula ruralis*. *Plant Physiol* 83:816–819.
- Dhindsa RS, JD Bewley 1977 Water stress and protein synthesis. V. Protein synthesis, protein stability, and membrane permeability in a drought-sensitive and a drought-tolerant moss. *Plant Physiol* 59: 295–300.
- Egunyomi A 1979 Autecology of *Octoblepharum albidum* in western Nigeria. II. Phenology and water relations. *Nova Hedwigia* 31: 377–389.
- Furness SB, JP Grime 1982 Growth rate and temperature responses in bryophytes. II. A comparative study of species of contrasted ecology. *J Ecol* 70:525–536.
- Garcia MB, RJ Antor 1995 Sex ratio and sex dimorphism in the dioecious *Borderea pyrenaica* (Dioscoreaceae). *Oecologia* 101: 59–67.
- Gerdol R, A Bonora, R Gualandri, S Pancaldi 1996 CO<sub>2</sub> exchange, photosynthetic pigment composition, and cell ultrastructure of *Sphagnum* mosses during dehydration and subsequent rehydration. *Can J Bot* 74:726–734.
- Glime JM 1971 Response of two species of *Fontinalis* to field isolation from stream water. *Bryologist* 74:383–386.
- Grant MC, JB Mitton 1979 Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. *Evolution* 33:914–918.
- Hamerlynck EP, Z Csintalan, Z Nagy, Z Tuba, D Goodin, GM Henebry 2002 Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. *Oecologia* 131:498–505.
- Hamerlynck EP, Z Tuba, Z Csintalan, Z Nagy, G Henebry, D Goodin 2000 Diurnal variation in photochemical dynamics and surface reflectance of the desiccation-tolerant moss, *Tortula ruralis*. *Plant Ecol* 151:55–63.
- Hearnshaw GF, MCF Proctor 1982 The effect of temperature on the survival of dry bryophytes. *New Phytol* 90:221–228.
- Husband BC, SCH Barrett 1996 A metapopulation perspective in plant population biology. *J Ecol* 84:461–469.
- Jing SW, PD Coley 1990 Dioecy and herbivory: the effect of growth rate on plant defense in *Acer negundo*. *Oikos* 58: 369–377.
- Lloyd DG, CJ Webb 1977 Secondary sex characters in plants. *Bot Rev* 43:177–216.
- Longton RE 1994 Reproductive biology in bryophytes: the challenge and the opportunities. *J Hattori Bot Lab* 76:159–172.
- 1997 Reproductive biology and life-history strategies. *Adv Bryol* 6:65–101.
- Longton RE, RM Schuster 1983 Reproductive biology. Pages 386–462 in RM Schuster, ed. *New manual of bryology*. Vol 1. Hattori Botanical Laboratory, Nichinan, Japan.
- Loveland HF 1956 Sexual dimorphism in the moss genus *Dicranum* Hedw. PhD diss. University of Michigan, Ann Arbor.
- McLetchie DN, G García-Ramos, PH Crowley 2002 Local sex-ratio dynamics: a model for the dioecious liverwort *Marchantia inflexa*. *Evol Ecol* 15:231–254.
- McLetchie DN, MN Puterbaugh 2000 Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort, *Marchantia inflexa*. *Oikos* 90:227–237.
- National Weather Service 1994 Local climatological data: monthly summaries. McCarran International Airport, Las Vegas, NV.
- Newton AE, BD Mishler 1994 The evolutionary significance of asexual reproduction in mosses. *J Hattori Bot Lab* 76:127–145.
- Newton ME 1972 Sex ratio differences in *Mnium hornum* Hedw. and *M. undulatum* Sw. in relation to spore germination and vegetative regeneration. *Ann Bot* 36:163–178.
- Obeso JR, M Alvarez-Santullano, R Retuerto 1998 Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). *Am J Bot* 85:1602–1608.
- Oliver MJ 1991 Influence of protoplasmic water loss on the control of protein synthesis in the desiccation-tolerant moss *Tortula ruralis*. *Plant Physiol* 97:1501–1511.
- Oliver MJ, JD Bewley 1984 Plant desiccation and protein synthesis. IV. RNA synthesis, stability, and recruitment of RNA into protein synthesis during desiccation and rehydration of the desiccation-tolerant moss, *Tortula ruralis*. *Plant Physiol* 74:21–25.
- 1997 Desiccation-tolerance of plant tissues: a mechanistic overview. *Hortic Rev* 18:171–212.
- Oliver MJ, BD Mishler, JE Quisenberry 1993 Comparative measures of desiccation-tolerance in the *Tortula ruralis* complex. I. Variation in damage control and repair. *Am J Bot* 80:127–136.
- Oliver MJ, Z Tuba, BD Mishler 2000 The evolution of vegetative desiccation tolerance in land plants. *Plant Ecol* 151: 85–100.
- Pettet A 1967 Sex ratios of *Riccia frostii* in Khartoum Province, Sudan. *Trans Br Bryol Soc* 5:332–337.
- Phillips JR, MJ Oliver, D Bartels 2002 Molecular genetics of desiccation and tolerant systems. Pages 319–341 in M Black, HW Pritchard, eds. *Desiccation and survival in plants: drying without dying*. CABI, Wallingford, UK.

- Proctor MCF 1984 Structure and ecological adaptation. Pages 9–37 in AF Dyer, JG Duckett, eds. The experimental biology of bryophytes. Academic Press, London.
- 2001 Patterns of desiccation tolerance and recovery in bryophytes. *Plant Growth Regul* 35:147–156.
- 2002 Ecophysiological measurements on two pendulous forest mosses from Uganda, *Pilotrichella ampullacea* and *Floribundaria floribunda*. *J Bryol* 24:223–232.
- Proctor MCF, VC Pence 2002 Vegetative tissues: bryophytes, vascular “resurrection plants” and vegetative propagules. Pages 207–237 in M Black, H Pritchard, eds. Desiccation and survival in plants: drying without dying. CABI, Wallingford, UK.
- Proctor MCF, N Smirnoff 2000 Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. *J Exp Bot* 51:1695–1704.
- Proctor MCF, Z Tuba 2002 Poikilohydry and homoihydry: antithesis or spectrum of possibilities? *New Phytol* 156:327–349.
- Reese WD 1984 Reproductivity, fertility, and range of *Syrrhopodon texanus* Sull. (Musci: Calymperaceae), a North American endemic. *Bryologist* 87:217–222.
- Ried A 1960 Stoffwechsel und Verbreitungsgrenzen von Flechten. II. Wasser- und Assimilationshaushalt, Entquellungs- und Submersionsresistenz von Krustenflechten benachbarter Standorte. *Flora* 149:345–385.
- Robinson SA, J Wasley, M Popp, CE Lovelock 2000 Desiccation tolerance of three moss species from continental Antarctica. *Aust J Plant Physiol* 27:379–388.
- Sakai AK, TA Burris 1985 Growth in male and female aspen clones: a twenty-five-year longitudinal study. *Ecology* 66:1921–1927.
- SAS 1994 SAS/STAT user’s guide, version 6, 4th ed., vol I. SAS Institute, Cary, NC.
- Schonbeck MW, JD Bewley 1981a Responses of the moss *Tortula ruralis* to desiccation treatments. I. Effects of minimum water content and rates of dehydration and rehydration. *Can J Bot* 59:2698–2706.
- 1981b Responses of the moss *Tortula ruralis* to desiccation treatments. II. Variations in desiccation tolerance. *Can J Bot* 59:2707–2712.
- Schuster RM 1992a The Hepaticae and Anthocerotae of North America east of the hundredth meridian. Vol 5. Columbia University Press, New York.
- 1992b The Hepaticae and Anthocerotae of North America east of the hundredth meridian. Vol 6. Columbia University Press, New York.
- Seel WE, GAF Hendry, JA Lee 1992 The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. *J Exp Bot* 43:1023–1030.
- Shaw AJ, JF Gaughan 1993 Control of sex ratios in haploid populations of the moss, *Ceratodon purpureus*. *Am J Bot* 80:584–591.
- Smith SD, RK Monson, JE Anderson 1997 Physiological ecology of North American desert plants. Springer, Berlin. 286 pp.
- Sokal RR, FJ Rohlf 1995 Biometry: the principles and practice of statistics in biological research. 3rd ed. WH Freeman, New York.
- Sollows MC, KA Frego, C Norfolk 2001 Recovery of *Bazzania trilobata* following desiccation. *Bryologist* 104:421–429.
- Stark L, N McLetchie, B Mishler 2001 Sex expression and sex dimorphism in sporophytic populations of the desert moss *Syntrichia caninervis*. *Plant Ecol* 157:183–196.
- Stark LR, BD Mishler, DN McLetchie 1998 Sex expression and growth rates in natural populations of the desert soil crustal moss *Syntrichia caninervis*. *J Arid Environ* 40:401–416.
- 2000 The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. *Am J Bot* 87:1599–1608.
- Stark LR, L Nichols II, DN McLetchie, SD Smith, C Zundel 2004 Age and sex-specific rates of leaf regeneration in the Mojave Desert moss *Syntrichia caninervis*. *Am J Bot* 91:1–9.
- Takács Z, HK Lichtenthaler, Z Tuba 2000 Fluorescence emission spectra of desiccation-tolerant cryptogamic plants during a rehydration-desiccation cycle. *Plant Physiol* 156:375–379.
- Takács Z, Z Tuba, N Smirnoff 2001 Exaggeration of desiccation stress by heavy metal pollution in *Tortula ruralis*: a pilot study. *Plant Growth Regul* 35:157–160.
- Tuba Z, Z Csintalan, MCF Proctor 1996 Photosynthetic responses of a moss, *Tortula ruralis*, ssp. *ruralis*, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: a baseline study at present-day CO<sub>2</sub> concentration. *New Phytol* 133:353–361.
- Une K 1985 Sexual dimorphism in the Japanese species of *Macromitrium* Brid. (Musci: Orthotrichaceae). *J Hattori Bot Lab* 59:487–513.
- Walker LR, DB Thompson, FH Landau 2001 Experimental manipulations of fertile islands and nurse plant effects in the Mojave Desert, USA. *West N Am Nat* 61:25–35.
- Wallace MH 1970 Developmental morphology and sexual dimorphism in *Homalothecium megaptulum* (Sull.) Robins. PhD diss. Washington State University, Pullman.
- Watson MA 1975 Annual periodicity of incremental growth in the moss *Polytrichum commune*. *Bryologist* 78:414–422.
- Williams SL 1995 Surfgrass (*Phyllospadix torreyi*) reproduction: reproductive phenology, resource allocation, and male rarity. *Ecology* 76:1953–1970.