Syntrichia norvegica Shoots Exhibit a Complex Inducible Response to Desiccation: separating the effects of Rate of Drying and Water Content

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***Syntrichia norvegica* Shoots Exhibit a Complex Inducible Response
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Content

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running head: Desiccation Tolerance in *Syntrichia norvegica*

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Abstract: Plants in the moss genus *Syntrichia* are considered to be constitutively desiccation tolerant (DT) – able to tolerate a rapid drying event without incurring significant damage upon rehydration. However, few workers have considered the separate effects of rate of drying and water content and incorporated fully dehardened (to DT) plants in the experiments. Plants of *S. norvegica* were cultured under conditions of suprasaturation and adult shoots were exposed to a range of drying rates and equilibrating relative humidities, rehydrated and assessed for chlorophyll fluorescence and regeneration potential. Adult shoots exhibited severe damage across all drying rates when equilibrated at RHs <30%. However, an inducible response to desiccation was present across all water contents as the rate of drying was extended from 0 to 4 d. The least desiccation damage occurred at longer drying times and higher water contents (8 d at 75% RH). A constitutive phenotype for DT was not strictly evident in *S. norvegica*. Rather, we observed an incomplete pattern of environmentally inducible DT coupled with heavy shoot damage at lower water contents. Rate of drying and equilibrating RH clearly interact in producing the pattern and strategy of DT for this species.

Key words: constitutive, ectohydry, chlorophyll fluorescence, regeneration, suprasaturation, mosses

INTRODUCTION

Desiccation tolerance (DT) in bryophytes is a reversible plastic trait (Gabriel 2005), i.e., a phenotype that is induced in a drying environment (hardening or acclimation) protects the plant both upon immediate rehydration and also against a future rapid drying event, but which is lost if the plants remain hydrated for more than a few days (dehardening to DT). Although DT is normally conceived as having two potential ecological strategies (constitutive and inducible DT), each species, plant structure, or life history stage is likely to exhibit a tolerance strategy along a gradient of inducibility (Stark and Brinda 2015). Further, such a strategy of surviving desiccation is likely to vary with the conditions attending drying, namely the rate at which plants are dried and the water content at which the plants are equilibrated.

The moss genus *Syntrichia* has been a historical focus for studies on the desiccation tolerance of plants, with the species *S. ruralis* and *S. caninervis* (especially the former) receiving the most attention (see references in Stark et al. 2014). The scientific consensus regarding the response strategy for tolerating desiccation in *Syntrichia* species points toward a system of constitutive protection (CDT) coupled with rehydration-induced repair (Proctor et al. 2007). This consensus of CDT protection is based on unchanging levels of dehydrins throughout a drying/rehydrating cycle (Bewley 1995); the ability of photocenters to reactivate in minutes or very quickly upon rehydration (Wood 2007; Zhang et al. 2011); the sequestration of desiccation-active transcripts during drying that immediately undergo translation upon rehydration (Wood & Oliver 1999); and the inability to deharden to DT (Stark et al. 2012).

As presented by Green et al. (2011) and Brinda et al. (2016), the principal factors of desiccation can be considered as (1) rate of drying (*RoD*), (2) equilibrating relative humidity (*RHeq*, which equates to a specific water content, WC), and (3) duration dry (how long the plants continuously experience the desiccated state). Historically, the factors *RoD* and *RHeq* are considered together as “intensity” of desiccation, and normally comprise the main desiccating treatment used in most experiments on bryophyte DT. Potential variation in the phenotype of DT may be lost when *RoD* and *RHeq* are combined as “intensity” of DT, especially if each factor has a unique effect or if the factors interact. Thus an important factor in understanding DT in *Syntrichia* remains unresolved: the relationship between the rate at which plants are desiccated and the equilibrating relative humidity (RH) experienced by the plants, the latter of which determines water content (WC). Separating these two factors may assist in understanding the relative effects of each factor of DT to the biological response, recovery, adaptation, and evolutionary trajectories of each species.

Traditionally a plant is considered to be DT if it can survive equilibration with air at ~50% RH (Alpert 2006). At approximately 50% RH plants have desiccated to a point that they retain only about 10% of their original water content (Alpert 2005). Intracellular vitrification of the cytoplasm is predicted to occur between 10% and 30% of water content (Wolfe & Bryand 1999; Hoekstra et al. 2001) and is a part of the desiccation tolerance program plants employ to minimize structural damage during water loss. There are valid reasons, however, for expanding the definition of DT to tolerances above 50% RH. First, most bryophyte species reach subturgor (slightly below full turgor) at equilibrating RHs of ~98% (water potentials $< \sim -2.0$ MPa, Proctor 2008), and second, during a dry-down the leaves of mosses begin to curl up (contort) and exhibit the

“desiccation morphology” characteristic of the species at ~86% RH (~ -20 MPa), and at this point most photosynthetic and metabolic process are markedly reduced (Dilks & Proctor 1979; Schonbeck & Bewley 1981; Proctor 2001; Walters et al. 2005; Buda et al. 2013; Brinda et al. 2016 and references therein). Thus there are grounds for including as DT those species that can survive when equilibrated with RHs of up to 85%, even though biological glass formation has not yet occurred.

Bryophytes are poikilohydric, i.e., the plants are unable to regulate internal tissue water content and instead rapidly equilibrate their hydration status to the immediate environment. If external water is absent the plants desiccate, with their tissue weight dependent on the environmental relative humidity (RH). Many bryophytes are able to conduct and even store water externally, a trait known as ectohydry. In species like *Syntrichia*, external water is stored among shoots within a colony (Rice 2012). Additionally, leaves of many bryophytes strategically separate water storage from gas exchange. Water storage pockets persist in the concave reservoirs of the hydrophilic adaxial leaf surfaces while gas exchange occurs on the lower hydrophobic convex surface of the unistratose leaves (Proctor 2008; Green et al. 2011). Bryophytes must be hydrated in order to photosynthesize but because these plants rely on diffusion for CO₂ intake, too much external water has the potential to hinder gas exchange. Thus, this physical separation of water storage and gas exchange allows net photosynthesis to continue longer while water is continually accessed from the reservoir on the upper side of the leaf (figured for *Pseudocrossidium crinitum* in Rundel and Lange 1980), in many cases without a significant dropoff in net photosynthesis.

We approach the standing hypothesis that *Syntrichia* species are constitutively desiccation tolerant (incapable of dehardening) by assessing how shoots of *S. norvegica*,

maintained in a state of suprasaturation (defined as having a relative water content in excess of full turgor; Proctor 2008) that ensures the plants are entirely dehardened to DT, respond to a range of rates of drying and equilibrating relative humidities. The resulting pattern does not fall neatly into the dichotomy of constitutive or inducible DT, or even at a distinct point along a gradient of inducibility, but rather recovery from desiccation in *S. norvegica* depends upon the interacting factors of *RoD* and *RHeq* during drying.

MATERIALS AND METHODS

Species description

Syntrichia norvegica F. Weber is a locally common species that is widely distributed in the northern hemisphere and also occurs in southern Africa. It is generally a species of higher elevations and/or higher latitudes. The species is characterized by its larger laminal cell size, its brightly colored orange-reddish awns, and the weak dorsal stereid band. The stereid band is a group of thick-walled, fiberlike cells found in the costa (mid-vein) of the leaves of some moss species. In *S. norvegica*, the stereids are often entirely absent just below the leaf apex making the costa difficult to distinguish from the adjacent lamina.

Culture technique

Decontamination of *Brinda* 288 (USA, Nevada, Clark County, Bonanza Peak Trail, Spring Mountains, 36.377961°N, 115.753123°W, 2690.0 m, vouchered at MO, UNLV) was achieved by subculturing shoot apices until the associated protists/fungi/cyanobacteria were not observed. Plants were raised in Petri dishes (inner diameter = 35 mm) containing locally collected, sieved (355 µm), dry-sterilized, pH-neutral sand watered with 30% Hoaglands solution (Hoagland & Arnon 1938) under a

12-h photoperiod (20°C lighted, 8°C darkened) in a plant growth chamber (Percival E30B, Boone, Iowa, USA) with a photon flux density of $\sim 175 \mu\text{mol m}^{-2} \text{sec}^{-1}$ photosynthetic active radiation (PAR). When the shoots reached the lid of the Petri dish, the lower dish was transferred to a deeper watch glass, allowing the shoot population to reach at least 15 mm in length over the course of 3–4 months. Cultures were checked daily and watered with 30% Hoagland's solution in order to maintain the shoots in a suprasaturated condition (**Fig. 1a**).

Experimental design

Shoots were grown under suprasaturating conditions in order to remove any hardening to DT. Adult shoot apices of *S. norvegica* were dried in glass desiccators over salt solutions equating to equilibrating RHs (RH_{eq}) of 75, 54, 23, and 0%. Within each equilibrating RH, rate of drying (RoD , as the time from full turgor to leaf curling that occurs during a dry-down from 98% to $\sim 86\%$ RH) was varied from 30 min to ~ 200 h.

Water content

Water content (WC) was calculated on a dry weight (DWt) basis as:
$$[(\text{Experimental Wt} - \text{DWt}) / \text{DWt}] \times 100$$
. Groups of 4 shoots were selected, cropped at 8–10 mm in length, and blotted on a chemical wipe until the external water was mostly absent while the shoots were yet at full turgor. Fresh weight (FW) was taken of individual shoots to the nearest μg using a microbalance (Sartorius M2P, Göttingen, Germany). Shoots were then placed in the appropriate glass desiccator for 48 h in unlidded Petri dishes with 2 sheets of Whatman #1 filter paper that were preequilibrated at least 24 h at the designated RH, which allowed complete equilibration with the desiccator RH. Each shoot was weighed directly from the desiccator (Experimental Wt), then oven-dried at 80°C for 3 d to yield DWt, and re-weighed. For suprasaturation WC (WC of shoots that

are growing in a supersaturated condition), each shoot was cut and lifted directly from the culture and into the weigh dish. For cutting, we used fine forceps and a straight edge, cutting at just above the water level in the culture dish.

Drying treatment

Shoots of *S. norvegica* that were available (3–5 months old), at least 12 mm in length, and unbranched, were removed from a culture in groups of four, placed in a drop of sterile water, cleaned and cut to 8–10 mm in length using a straight edge, and blotted free of visible external water for ~30 sec (or until external water had dissipated while shoots were at full turgor, **Fig. 1B**) on a chemical wipe. The drying technique employed is derived from Werner et al. (1991) and Pressel and Duckett (2010), as modified in Stark et al. (2013). Immediately upon blotting, the contiguous group of four shoots was transferred into a Petri dish and the Petri dish placed in a glass desiccator containing a saturated salt solution producing atmospheres of 0% ($\text{CaSO}_{4(s)}$), 23% (KCH_3CO_2), 54% ($\text{Mg}(\text{NO}_3)_2$), or 75% (NaCl) RH. Petri dishes were fitted with circular sheets of pre-equilibrated Whatman #1 filter paper (using one sheet per 100 μL pipetted sterile water) and an iButton (Maxim, San Jose, CA, USA) positioned adjacent to the shoots to record T and RH adjacent to the drying shoots. The Petri dish was unlidded within the desiccator for rapid dry-downs (RD) and lidded for slower drying times (SD). All manipulations were carried out in an environmental room set to 20°C, 50% RH, under continuous low light of 2–4 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ PAR. Prolonging the drying times at lower RHs of 0 and 23% required parafilming the Petri dish and making a ~5 mm incision in the parafilm in order to allow a reduced air flow in the Petri dish; this elevated the RH inside the Petri dish at the onset of drying to close to 98% in all treatments.

Once the shoots exhibited desiccation morphology (leaves contorted about the stem, visible through the glass desiccator), we waited 4 d for the shoot WC to equilibrate at the desiccator RH, checking the iButton every 2 d until at least 12 h at constant RH had been reached. At this point, the desiccator was opened and the shoots placed horizontally on premoistened sand media in a Petri dish and placed into the growth chamber under conditions described above under *Culture technique*. These shoots were watered as needed on a daily basis using sterile distilled water.

Response variables

Seven d postrehydration *visual leaf damage* was assessed by examining the shoot at 60× and assigning each leaf along a shoot to one of 3 categories: entirely chlorophyllose (green, value=100%), partially chlorophyllose (value=50%), or entirely chlorotic (brown, value=0%), with means calculated for each group of 4 shoots treated together. Fourteen d postrehydration the shoot apices were examined and assessed for *resumption of apical growth* if the shoot apex exhibited phototropism (turning upward). Twenty-one d postrehydration the *number of regenerating lateral shoots* was counted. For *chlorophyll fluorescence*, groups of 4 desiccated shoots were rehydrated in a drop of sterile water, cut to 2.5 mm sections (essentially the shoot crown) and placed in a fluorescence clip on a chemical wipe dipped in water allowing the shoots to remain hydrated and dark adapted for 24 h, after which a modulated chlorophyll fluorometer (FMS2, Hansatech, King's Lynn, UK) incorporating the saturation pulse method (Bilger et al. 1995) to determine the maximum photochemical efficiency of dark-adapted photosystem II (F_v/F_m) in clips sealed from incident light. Subsequently, the effective quantum yield of PSII photochemistry (Φ_{PSII}) was determined from the following light program on the same shoots: emission of an actinic light ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) with

thirty second equilibration periods prior to delivering saturating light pulses (initial 8,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) at increasing light intensities (Genty et al. 1989). F_v/F_m is a measure of the general condition of the photocenters, Φ_{PSII} represents the fraction of excitation energy flowing through PSII and hence an indicator of active photosynthesis (Green & Proctor 2016). Additionally, we used the value F_m (maximal fluorescence) to provide a rough comparative measure of the amount of photocenter activity, and thus of the total potential photosynthetic activity and indication of cell damage incurred by the plant material (Proctor 2003). Sample size (number and size of shoots) and distance from the sample (1 cm) was standardized during measurement. For the chlorophyll fluorescence analyses, three drying treatments were used equating to 30 min (a rapid-dry), ~100 h (a slow-dry), and ~200 h (a very slow-dry) at each of the experimental *RHeqs*.

Statistics

Leaf proportion damage was calculated as described under *Response variables*. Because of the inherent censoring in these data, Tobit regression models (Tobin 1958) were used to test for the effects of rate of drying (*RoD*) and equilibrating relative humidity (*RHeq*) on leaf damage using the R package VGAM (Yee 2015). The effects of *RoD* and *RHeq* on regenerative shoot counts were evaluated with negative binomial models using the R package MASS (Venables & Ripley 2002). One-way ANOVAs were used to partition the effects of *RoD* and *RHeq* on the three fluorescence parameters: F_v/F_m , F_m , and Φ_{PSII} . ANOVAs were created for each *RoD* to compare the fluorescence responses between shoots exposed to different *RHeq* levels (three per fluorescence parameter). Additional ANOVAs evaluated the effect of *RoD* within each *RHeq* level (four per fluorescence parameter). All analyses were performed using R version 3.2.5 (R Core Development Team, 2016).

RESULTS

Water content (WC), subturgor RH, and equilibrating RH

On a dry weight basis, WC ranged from 13.45% at an equilibrating RH of 75% to 3.08% at an equilibrating RH of 0% (**Table 1**). Suprasaturated shoots had a WC = $3391.50 \pm 331.96\%$ (N=4), and freshly blotted shoots had a WC = $652.21 \pm 19.19\%$ (N=16). Equilibrating RHs experienced by shoots were ~1–5% above the target RHs predicted by saturated salt solutions (**Table 1**), likely influenced by the filter paper upon which the plants rested. Subturgor RH experienced by the shoots during drying was high (excepting the rapidly dried shoots), approaching 98% for all salt solutions (**Table 1**). A sample drying curve for a 4-day dry at each of the equilibrating RHs is given in **Fig. 2**. When the RH inside the Petri dish equilibrates near the target RH of the saturated salt solution, the WC of the plants is also equilibrated (shown in Stark & Brinda 2015) and not repeated here.

Visual leaf damage

Leaf damage was significantly affected by both rate of drying (*RoD*; $z=2.76$, $df=2$, $P=0.017$) and equilibrating RH (*RHeq*; $z=16.25$, $df=2$, $P<0.0001$; **Figs. 3, 4**). Heavier damage was incurred by shoots dried at lower *RHeq* relative to higher *RHeq* in a graded series from 0% to 75% *RHeq*. In addition, longer *RoD* resulted in less damage than shorter *RoD*, except at *RHeq* of 54%, where a very slow drying period (8 d) resulted in more damage than a 4 d drying period, more closely approximating damage from a rapid-dry (RD).

Resumption of apical growth

All shoots equilibrated at 75% RH exhibited positive shoot apex phototropism regardless of rate of drying (**Table 2, Fig. 5a**). For shoots equilibrated at 0 and 23% RH, a tendency for shoot apex positive phototropism occurred at longer rates of drying. However, for a $RHeq = 54\%$, a tendency for reduced shoot apex viability was present at the longer drying rates.

Regenerating lateral shoots

At drying times up to ~200 h, the number of shoots regenerating from the desiccation treatments was significantly influenced by the $RHeq$ ($z=7.73$, $df=122$, $P<0.0001$), with greater regeneration observed at higher $RHeq$ (**Figs. 5b, 6**). RoD did not have the same influence on shoot regeneration as $RHeq$ within these same drying times ($z=1.204$, $df=122$, $P=0.229$). However, when the RoD was extended to >200 h, the effects of $RHeq$ and RoD on shoot regeneration were not significant as the treatments converged on a regeneration shoot number from ~3–5 ($RHeq$: $z=0.819$, $df=50$, $P=0.413$; RoD : $z=-1.419$, $df=50$, $P=0.156$). All but 14 shoots (of a total of 172 tested) regenerated by forming new lateral shoots, with 12 of these non-regenerating shoots equilibrated at 0% RH.

Chlorophyll fluorescence

Overall the fluorescence parameters were significantly influenced individually by RoD (F_m $F=26.09$, $df=3$, $P<0.0001$; F_v/F_m $F=47.73$, $df=3$, $P<0.0001$; Φ_{PSII} $F=51.28$, $df=3$, $P<0.0001$) and $RHeq$ (F_m $F=7.40$, $df=2$, $P=0.001$; F_v/F_m $F=12.86$, $df=3$, $P<0.0001$; Φ_{PSII} $F=10.45$, $df=2$, $P=0.0001$; **Figs 7, 8, 9**). For F_m , the combined influence of these two factors was marginally significant ($RoD \times RHeq$: F_m $F=1.98$, $df=6$, $P=0.08$). However, for F_v/F_m and Φ_{PSII} the combined influence of RoD and $RHeq$ was significant ($RoD \times RHeq$: F_v/F_m $F=3.55$, $df=6$, $P=0.005$; Φ_{PSII} $F=1.04$, $df=6$, $P=0.002$). Control

levels of F_v/F_m (quantum yield) for unstressed shoot tips were ~ 0.60 , and these levels were only approached for the very slow rate of drying (*RoD*) period (8 d) at an equilibrating RH (*RHeq*) = 75% (**Fig. 7**). Additionally, other than control values, maximal Φ_{PSII} (quantum efficiency of Photosystem II) and F_m (maximal fluorescence) values were only attained by shoots dried at 75% RH at the most rapid *RoD* (30 min: Φ_{PSII} $F=40.006$, $df=3$, $P<0.0001$; F_m $F=7.986$, $df=3$, $P=0.0001$) and at the slowest *RoD* (8 d: Φ_{PSII} $F=8.200$, $df=3$, $P=0.0009$; F_m $F=10.68$, $df=3$, $P=0.0002$). Shoots dried at 54% RH had fluorescence values similar to those dried at lower *RHeq* when dried rapidly (30 min) or slowly (8 d). However, the *RoD* mitigated F_v/F_m and Φ_{PSII} values at *RHeq* of 54% RH making them indistinguishable from the 75% RH values under the same *RoD* regime of 4 d (F_v/F_m $F=1.835$, $df=1$, $P=0.2027$; Φ_{PSII} $F=0.8927$, $df=1$, $P=0.365$).

DISCUSSION

Constitutive hypothesis not supported

We hypothesized that *S. norvegica* would exhibit an ecologically constitutive response pattern to desiccation stress, meaning that upon exposure to various rates of drying and water contents that shoots would exhibit minimal damage upon rehydration. This hypothesis is consistent with (1) literature reports from at least three species in the genus (*S. caninervis*, *S. pagorum*, *S. ruralis*; Zhang et al. 2011, Oliver et al. 2005, Stark et al. 2012, 2016a) and (2) reports of 6 species of *Syntrichia* (including *S. norvegica*) tolerating equilibration with $\leq 30\%$ RH (Wood 2007). However, most previous studies implemented a desiccation treatment based on “intensity”, which combines the factors of rate of drying (*RoD*) and equilibrating relative humidity (*RHeq*). When these factors are separated, a response pattern that cannot be easily characterized as either constitutively or

inducibly desiccation tolerant (DT) emerges for *S. norvegica*. Both *RoD* and *RHeq* significantly affected chlorophyll fluorescence parameters on an individual basis, and, importantly, *RoD* and *RHeq* interacted significantly for F_v/F_m and Φ_{PSII} . These two parameters relate to the efficiency of photosynthesis, indicating that the biochemistry of PSII is affected by the combined stresses of *RoD* and *RHeq* historically regarded as desiccation intensity. When shoot apices are rapidly dried (<30 min from full turgor to desiccation morphology), damage is severe at all equilibrating RHs (*RHeq*) except 75%. However, as *RoD* is extended to 4 d, a clear inducible response across WCs is present. Extending the *RoD* to ~8 d further improved plant health upon rehydration except at an intermediate WC (*RHeq* 54%). Based upon F_m values, regardless of the *RoD* or the *RHeq*, some rehydration damage is present (that was not detected visually), although based upon F_v/F_m and Φ_{PSII} values, a drying regime resulting in negligible damage occurs at a *RoD* of 8 d at a *RHeq* of 75%. When the ability to regenerate through shoot production 21 d postrehydration is considered, equilibrating RH is senior to *RoD*, and no combination of *RoD* $\times$ *RHeq* approaches the regeneration level of control shoots. We conclude that in the high elevation species *S. norvegica*, the response to desiccation is generally that of inducible desiccation tolerance (IDT), but the response pattern depends upon the interaction between *RoD* and *RHeq*. At *RHeqs* below 50%, shoots are significantly damaged based on chlorophyll fluorescence, visual leaf damage, and regeneration potential, although even in these cases actual shoot death was rare (3 instances). At these depressed WCs, a slight inducible effect is observed at long drying times, but heavy damage is the general pattern observed. At a *RHeq* ~54%, when the *RoD* is extended from 4 d to 8 d, shoots are severely damaged, a puzzling result confirmed by

visual assessment of leaf damage, shoot apex viability, and independent fluorescence assessments, but not reflected in shoot regeneration numbers 21 d postrehydration.

The variation we observed when shoots were dried over a period of 8 d at ~54% RH may arise from the influence of WC on membrane stability. At water contents approaching $0.1 \text{ g g}^{-1} \text{ DWt}$ (=10%) associated with a $RH_{eq} \sim 50\%$, membranes transition from a liquid crystalline to gel phase (Crowe & Crowe 1982). Damage to the lipid bi-layer during this phase change can allow imbibitional leakage during rehydration (Hoekstra et al. 1999). Previous research indicates that the related *S. ruralis* and *S. caninervis* do not experience membrane damage during desiccation, suggesting a constitutive strategy of DT (Singh 1984; Wu et al. 2012). The current study suggests that *S. norvegica* may rely more on inducible protection conferred during slow drying episodes; we caution that previous studies did not always fully dehydrate plants, opening the door to a hardened effect that mimics CDT (Stark et al. 2014). Additionally, critical enzymatic activity relevant to tolerating desiccation in mosses depends upon the phase state of the membrane (Fernández-Marin et al. 2013). It is possible that some shoots in our experiment experienced a membrane phase change or a partial phase change, and this may have produced some of the wide variation we observed with this particular treatment.

Water content

Water content (WC), as varied through equilibrating RH (RH_{eq}), appears to be of senior importance to postrehydration health of *S. norvegica* shoot apices. When shoots are dried to $RH_{eq} < \sim 75\%$ (equating to WCs <13%) and dried rapidly, upon rehydration all shoots are heavily damaged. Even slowly drying shoots to WCs <7% DWt ($RH_{eq} < 25\%$) resulted in severe shoot damage upon rehydration. Vulnerability at low WCs is

likely due to (1) a sudden membrane phase change upon rehydration as noted earlier and (2) failure to reorganize the photosynthetic apparatus upon rehydration, which is less likely to occur at $RH_{eqs} > 50\%$ (Fernández-Marin et al. 2013). This effect was not observed in the related *S. ruralis*, where “slow drying” (only ~1 h) to equilibrating RHs below 22% produced minimal damage to shoot apices upon rehydration (Schonbeck & Bewley 1981). Clearly the level of inherent DT in *S. ruralis* is greater than that observed in the higher elevation *S. norvegica*, with data from the latter species consistent with mosses normally occurring in shaded habitats (Oliver et al. 1993; Proctor et al. 2007).

Suprasaturation effects

By varying drying rates (as time from full turgor to leaf curling) from 30 min to 8 days across a range of equilibrating RHs, we attempted to approximate the minimum rate of drying at specific water contents that resulted in minimal shoot damage upon rehydration. Only one set of drying conditions was found where the shoot health approached that of undried controls: an 8 d drying time at a RH_{eq} of ~75%, equating to a WC of 13.4% on a DWt basis. Reasons for this rather restrictive finding may lie in the techniques used to assess DT in this species. First, cultures of *S. norvegica* were intentionally maintained in a suprasaturated condition, where external water persists along the shoots and especially in the concave pockets of adaxial leaf surfaces. Suprasaturated conditions do not appear to appreciably decrease net photosynthesis in *Syntrichia* (Seel et al. 1992) and in several other species of bryophytes (~20% reduction; Dilks & Proctor 1979) for reasons detailed in Proctor (2008) and Green et al. (2011). Cultures of *S. norvegica*, with a WC at suprasaturation of ~3400%, were vigorous and healthy as assessed visually and through chlorophyll fluorescence. Nevertheless, cushion WCs of ~700% decreased net photosynthesis in *Grimmia pulvinata* by nearly half (Zotz

et al. 2000) and decreased NP in *S. ruralis* by 38% (Zotz & Rottenberger 2001). Control F_v/F_m values are on the low side (~ 0.6), but well within what is considered normal for unstressed bryophytes, including *Syntrichia* (e.g., Csintalan et al. 1999; Hamerlynck et al. 2000; Beckett et al. 2005). The high suprasaturation WC of *S. norvegica* shoots even exceeds that reported for species of *Sphagnum* (Schipperges & Rydin 1998; Hájek & Beckett 2008). Growing plants in the suprasaturated condition prevented any hardening to DT during the growth of the cultures through partial dehydration, which may occur during the normal course of growth when shoot apices lose turgor, even at $RHeq$ s near 98% (Hájek & Vicharová 2014), and thus allowed an assessment of the inherent DT in the shoot apices. Second, shoots were rehydrated directly from the desiccators into relatively cool ($\sim 2\text{--}4^\circ\text{C}$) liquid water, without an opportunity to prehydrate. Although prehydration (exposure to high RH prior to liquid water) is rarely part of the protocol in plant DT studies, there is some evidence that exposing desiccated bryophyte shoots to high RHs for several hours prior to immersing in liquid water mitigates some desiccation induced damage in *S. ruralis* (e.g., Schonbeck & Bewley 1981 and reviewed in Stark et al. 2016b), and prehydration is likely to occur in natural colonies of mosses.

Stem tissue and shoot apices are highly DT

In the related *S. ruraliformis* and *S. ruralis*, the shoot apex is often the most resistant to desiccation stress (Dilks & Proctor 1974; Schonbeck & Bewley 1981). Our findings for *S. norvegica* reflect this pattern, with most visual leaf damage occurring on proximal leaves. This phenomenon is well known among mosses (e.g., Glime & Carr 1974; Gupta 1977; Beckett 2001; Proctor & Pence 2002; Stark et al. 2013 and references therein) and is likely a widespread phenomenon. Despite the severe shoot damage associated with lower water contents in *S. norvegica*, in all cases but 14, upon

rehydration shoots regenerated through lateral shoots, and of these 11 regenerated solely through protonemata. In other words, even though experiencing a range of drying times across a range of WCs, 169 of 172 shoots were capable of regeneration (the 3 shoots that died were rapidly dried). This speaks to a constitutive DT mechanism of at least some stem tissues. We hypothesize that axillary leaf buds are phenotypically CDT and that such an adaptation may greatly assist in withstanding severe desiccation events through asexual regeneration.

Conclusion

In this study we showed that the high elevation species *S. norvegica* departs from the pattern (or assumption) of a strategy of inherent phenotypic constitutive DT in the genus. By separating the factor of “desiccation intensity” into its component parts of rate of drying and equilibrating relative humidity (or water content here), the pattern of DT for this species is found to vary within a specific water content based on how fast the shoots were desiccated. As the drying time is extended, an incomplete pattern of inducible DT was observed across all water contents tested. At the highest WC tested, recovery of shoots approached control undried shoots. However, at lower WCs, even the longest rates of drying did not translate into full recovery of shoots. Separation of environmental factors experienced by plants during desiccation may open potential avenues to investigate a proliferation of strategies adopted by mosses to tolerate desiccation. Species may exhibit a diversity of responses through an integration and coordination of physiological, morphological, and reproductive DT strategies. Future studies should factor in the process of prehydration of shoots prior to rehydration with liquid water, and compare the DT in suprasaturated shoots vs. shoots maintained at full turgor without significant external water.

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Table 1. Salt solutions and their target relative humidities, equilibrating relative humidities (*RHeq*) experienced by plants, subturgor RH experienced by plants during a representative drying trial, temperatures (T) experienced by plants during an equilibration, and water contents (WC) attained in the desiccators containing shoots of *Syntrichia norvegica*; *RHeq* and T represent means of the last 12 h in the desiccator, subturgor RH represents means of the first 24 h in the desiccator during a ~8 d drying event, and WC is based on dry weight (DWt, = 100× [(Experimental Wt – Dry Wt) / Dry Wt]. Means with one SE, N=44–47 for *RHeq* & T, N=24 for Subturgor RH, N=4 for WC.

Salt Solution (target RH at 20°C)	RHeq	Subturgor RH	T during Equilibration	WC at Equilibration (% DW)
NaCl (75%)	79.01 ±0.12	99.61 ±0.13	20.19 ±0.02	13.45 ±1.16
Mg(NO ₃) ₂ (54%)	59.36 ±0.16	98.46 ±0.23	20.16 ±0.00	11.40 ±0.20
KCH ₃ CO ₂ (23%)	25.95 ±0.16	98.69 ±0.20	20.17 ±0.04	6.98 ±0.48
CaSO ₄ (s) (0%)	00.64 ±0.22	97.90 ±0.14	20.55 ±0.03	3.08 ±0.72

Table 2. Percentage of *Syntrichia norvegica* shoot apices exhibiting positive phototropism (indicating viability of apical meristem) 14 days postrehydration based on equilibrating RH (*RHeq*) and rate of drying (*RoD*); shoot number (N) given parenthetically.

<u><i>RHeq</i> (%)</u>	<u><i>RoD</i> (h)</u>		
	<u>≤50</u>	<u>50–149</u>	<u>150+</u>
0	0 (20)	0 (12)	33 (12)
23	0 (8)	40 (20)	50 (8)
54	94 (16)	94 (16)	69 (16)
75	100 (16)	100 (12)	100 (16)

Fig. 1. Appearance of cultured shoots of *Syntrichia norvegica* exhibiting (A) the suprasaturated condition, whereby free water is stored in the shoot apices and concave spaces of the adaxial leaf surfaces, and (B) (the same) shoots without appreciable external water.

Fig. 2. Relative humidity (RH) experienced by shoots of *Syntrichia norvegica* during an experimental 4-day rate of drying at four targeted equilibrating RHs. Dotted line at ~86% RH, where leaf curling begins. When the RH equilibrates at the targeted level, shoot water content also equilibrates. Photo insets indicate a shoot at full turgor (no external water) at the initiation of the experimental dry-down, and the same shoot that has equilibrated in a desiccated condition.

Fig. 3. Range of shoot damage experienced by shoot apices of *Syntrichia norvegica* on day 7 postrehydration from a desiccation treatment. (A) undried control, (B) dried 126 h and equilibrated at 75% RH (minor damage), (C) dried 127 h and equilibrated at 54% RH (moderate damage), and (D) rapidly dried (<30 min) and equilibrated at 23% RH (severe damage).

Fig. 4. Visual leaf damage (as the percentage of leaves along a shoot that are chlorophyllous) day 7 postrehydration on shoots of *Syntrichia norvegica* subjected to a range of drying times at targeted equilibrating relative humidities of 0, 23, 54, and 75%. Each point represents a mean and one S.E. (N=4) with fitted unweighted polynomial curves.

Fig. 5. Examples of positive or no shoot apex phototropism and lateral shoot regeneration as seen on day 21 postrehydration from a desiccation treatment in *Syntrichia norvegica*.

(A) four shoots rapidly dried (<30 min) and equilibrated at 75% RH, all shoot apices exhibiting positive phototropism and abundant lateral shoot production, (B) dried 38 h and equilibrated at 23% RH, 0 shoot apices viable yet some lateral shoot regeneration (at arrows).

Fig. 6. Lateral shoot regeneration day 21 postrehydration on shoots of *Syntrichia norvegica* subjected to a range of drying times at targeted equilibrating relative humidities of 0, 23, 54, and 75%. Each point represents a mean and one S.E. (N=4) with fitted unweighted polynomial curves.

Fig. 7. Maximum photochemical efficiency of dark-adapted photosystem II as F_v/F_m at 24-h postrehydration on shoot apices of *Syntrichia norvegica* subjected to a range of drying times at targeted equilibrating relative humidities of 0, 23, 54, and 75%. Each point represents a mean and one S.E. (N=6) with fitted unweighted polynomial curves. Mean drying time $\pm$ one S.E. for each relative humidity treatment: 0% 4d = 114 ± 9 h, 0% 8d = 204 ± 18 h, 23% 4d = 106 ± 7 h, 23% 8d = 212 ± 6 h, 54% 4d = 99 ± 8 h, 54% 8d = 183 ± 2 h, 75% 4d = 92 ± 6 h, 75% 8d = 194 ± 10 h.

Fig. 8. Maximal chlorophyll fluorescence as F_m at 24-h postrehydration on shoot apices of *Syntrichia norvegica* subjected to a range of drying times at targeted equilibrating relative humidities of 0, 23, 54, and 75%. Each point represents a mean and one S.E.

(N=6) with fitted unweighted polynomial curves. For mean drying time $\pm$ one S.E. for each treatment N see caption Fig. 7.

Fig. 9. Effective quantum yield of PSII photochemistry as Φ_{PSII} at 24-h postrehydration on shoot apices of *Syntrichia norvegica* subjected to a range of drying times at targeted equilibrating relative humidities of 0, 23, 54, and 75%. Each point represents a mean and one S.E. (N=6) with fitted unweighted polynomial curves. For mean drying time $\pm$ one S.E. for each treatment N see caption Fig. 7.

Draft

Figure 01

165x222mm (300 x 300 DPI)

Figure 02

274x209mm (300 x 300 DPI)

Figure 03

215x149mm (300 x 300 DPI)

Figure 04

274x209mm (300 x 300 DPI)

Figure 05

165x222mm (300 x 300 DPI)

Figure 06

274x209mm (300 x 300 DPI)

Figure 07

274x209mm (300 x 300 DPI)

Figure 08

274x209mm (300 x 300 DPI)

Figure 09

274x209mm (300 x 300 DPI)