



UNIVERSITY
OF WOLLONGONG
AUSTRALIA

University of Wollongong
Research Online

Faculty of Science - Papers (Archive)

Faculty of Science, Medicine and Health

2000

Desiccation tolerance of three moss species from continental Antarctica

Sharon A. Robinson

University of Wollongong, sharonr@uow.edu.au

J. Wasley

University of Wollongong

M. Popp

University of Vienna

C. E. Lovelock

University of Queensland

Publication Details

This article was originally published as Robinson, SA, Wasley, J, Popp, M and Lovelock, CE, Desiccation tolerance of three moss species from continental Antarctica, *Australian Journal of Plant Physiology*, 27, 2000, 379-388. Copyright CSIRO. Original journal available [here](#).

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Desiccation tolerance of three moss species from continental Antarctica

Abstract

Tolerance of desiccation was examined in three species of moss, *Grimmia antarctici* Card., *Ceratodon purpureus* (Hedw.) Brid. and *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer et Scherb. collected from two sites of contrasting water availability in the Windmill Islands, continental Antarctica. Physiological tolerance to desiccation was measured using chlorophyll fluorescence in plugs of moss during natural drying in the laboratory. Differences in relative water contents, rates of drying and the response of photosynthesis to desiccation were observed among the three species and between sites. Of the three species studied, *G. antarctici* showed the lowest capacity to sustain photosynthetic processes during desiccation, *B. pseudotriquetrum* had an intermediate response and showed the greatest plasticity and *C. purpureus* showed the greatest capacity to sustain photosynthesis during desiccation. These results fit well with the known distribution of the three species with *G. antarctici* being limited to relatively wet sites, *C. purpureus* being common in the driest sites and *B. pseudotriquetrum* showing a wide distribution between these two extremes. Levels of soluble carbohydrates were also measured in these samples following desiccation and these indicate the presence of stachyose, an oligosaccharide known to be important in desiccation tolerance in seeds, in *B. pseudotriquetrum*. Both gross morphology and carbohydrate content are likely to contribute to differences in desiccation tolerance of the moss species. These results indicate that if the Casey region continues to dry out, as a result of local geological uplifting or global climate change, we would expect to see not only reductions in the moss community but also changes in community composition. *Grimmia antarctici* is likely to become more limited in distribution as *C. purpureus* and *B. pseudotriquetrum* expand into drying areas

Keywords

Bryum pseudotriquetrum, *Ceratodon purpureus*, chlorophyll fluorescence, *Grimmia antarctici*, stachyose, soluble carbohydrates

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

This article was originally published as Robinson, SA, Wasley, J, Popp, M and Lovelock, CE, Desiccation tolerance of three moss species from continental Antarctica, *Australian Journal of Plant Physiology*, 27, 2000, 379-388. Copyright CSIRO. Original journal available [here](#).

© CSIRO Australia 1999

0310-7841

Aust. J. Plant Physiol., 1999, **26**, 000-000.

PP90000

Desiccation tolerance of three moss species from continental Antarctica

Sharon A. Robinson^{A,D}, Jane Wasley^A, Marianne Popp^B, Catherine E. Lovelock^C

^A*Plant Molecular Ecophysiology Group, Department of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia*

^B*Institut für Pflanzenphysiologie, Universität Wien, POB 285, A-1091 Wien, Austria*

^C*Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA.*

^DCorresponding author;

Dr Sharon Robinson

Tel (61) 242 21 5753

Fax (61) 242 21 4135

email sharonr@uow.edu.au

Running title

Desiccation tolerance of three Antarctic moss species

Keywords

Bryum pseudotriquetrum, *Ceratodon purpureus*, chlorophyll fluorescence, *Grimmia antarctici*, stachyose, soluble carbohydrates

Abbreviations

F_m, maximal fluorescence; F_v, variable fluorescence; WC, water content. RFO, raffinose family oligosaccharide.

Abstract. Tolerance of desiccation was examined in three species of moss, *Grimmia antarctici* Card., *Ceratodon purpureus* (Hedw.) Brid. and *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer *et* Scherb. collected from two sites of contrasting water availability in the Windmill Islands, continental Antarctica. Physiological tolerance to desiccation was measured using chlorophyll fluorescence in plugs of moss during natural drying in the laboratory. Differences in relative water contents, rates of drying and the response of photosynthesis to desiccation were observed among the three species and between sites. Of the three species studied, *G. antarctici* showed the lowest capacity to sustain photosynthetic processes during desiccation, *B. pseudotriquetrum* had an intermediate response and showed the greatest plasticity and *C. purpureus* showed the greatest capacity to sustain photosynthesis during desiccation. These results fit well with the known distribution of the three species with *G. antarctici* being limited to relatively wet sites, *C. purpureus* being common in the driest sites and *B. pseudotriquetrum* showing a wide distribution between these two extremes. Levels of soluble carbohydrates were also measured in these samples following desiccation and these indicate the presence of stachyose, an oligosaccharide known to be important in desiccation tolerance in seeds, in *B. pseudotriquetrum*. Both gross morphology and carbohydrate content are likely to contribute to differences in desiccation tolerance of the moss species. These results indicate that if the Casey region continues to dry out, as a result of local geological uplifting or global climate change, we would expect to see not only reductions in the moss community but also changes in community composition. *Grimmia antarctici* is likely to become more limited in distribution as *C. purpureus* and *B. pseudotriquetrum* expand into drying areas.

Introduction

Antarctica is the coldest, driest continent and plants that grow there are capable of withstanding severe desiccation. Wilkes Land, Antarctica has the most extensive and best developed terrestrial vegetation in continental Antarctica but the vegetation is restricted to six species of bryophytes and 27 lichen species (Lewis Smith 1988; Melick *et al.* 1994). The bryophytes are poikilohydric, depending on the presence of free water during the summer months for photosynthetic carbon gain and growth. Consequently Antarctic bryophyte communities are largely confined to the margins of melt lakes and streams and areas subject to snow accumulation. At Casey (the Australian Antarctic Base in the Windmill Island region (66° 17' S, 110° 32' E) the three dominant moss species *Ceratodon purpureus*, *Grimmia antarctici* and *Bryum pseudotriquetrum* are found in both pure and mixed communities (Selkirk and Seppelt 1987). The extent of these moss communities depends on the availability of summer melt water, and ranges from the extensive moss turves which occur around melt lakes and along melt streams to small isolated moss buttons which are found in moisture pockets on rocky outcrops. Of the three species *G. antarctici* is common within melt lakes and streams, *C. purpureus* is often associated with drier sites, and *B. pseudotriquetrum* co-occurs with both *G. antarctici* and *C. purpureus* (Selkirk and Seppelt 1987). From these distributions it has been proposed that *C. purpureus* and *B. pseudotriquetrum* have a greater tolerance of drier conditions (Selkirk and Seppelt 1987).

Recently, it has been suggested that periods of drying due to deglaciation and geological uplift of the region have resulted in a contraction in the extent of the moss beds at Casey, and a change to transitional moss-lichen communities, dominated by desiccation resistant lichens (Melick and Seppelt 1997). If the three moss species found in the Casey region do vary in their physiological tolerance to desiccation, then this drying trend is likely to result in changes to the relative distribution of the three species. Water availability is also likely to be highly dependent on global climatic fluctuations because of altered levels of snow accumulation (Morgan *et al.* 1991) and/or summer temperatures. We therefore wish to understand the relative responses of the three moss species to desiccation in order to understand the likely impact of global climate change on these communities. In areas near Antarctic Stations water availability may also be affected by changes in drainage (Wilson 1990) caused by construction on and around bryophyte communities.

In this study we have used chlorophyll *a* fluorescence as an indicator of physiological tolerance to desiccation. The chlorophyll fluorescence ratio F_v/F_m has been shown to correlate with the yield of photosynthetic oxygen evolution, or the efficiency with which light is used in photosynthesis (Björkman and Demmig 1987). Rates of water loss and the physiological tolerance of species to desiccation were assessed in the laboratory using replicate samples of moss for each species. Desiccation tolerance of the three moss species was also assessed at two sites with contrasting water availability where all three species co-occur. This enabled us to assess the degree of phenotypic plasticity in response to desiccation among species. The utilisation of a chlorophyll fluorescence parameter which can be rapidly measured on small samples is ideal for this type of experiment, and allowed sufficient replication to statistically compare samples of different species and sites. Previous techniques using gas exchange equipment have been too time consuming to allow this type of detailed analysis (see Oliver *et al.* 1993).

In addition to their role as energy storage compounds simple sugars and polyols may perform multiple roles in plants. They are thought to act as cryoprotectants, as osmotic regulators in drought and salt stressed plants, and as antioxidants (Popp and Smirnov 1995). Various soluble carbohydrates (including sucrose and raffinose family oligosaccharides, RFO) are able to interact with the polar head groups of phospholipids, taking the place of water molecules and maintaining membrane integrity during desiccation (Crowe and Crowe 1986). In addition high sugar content promotes formation of a vitreous state in the cytoplasm of desiccated cells improving structural stability and minimising protein denaturation (Koster 1991; Sun and Leopold 1997). Polyols accumulate in higher plants in response to water stress. These molecules probably have multiple roles, as compatible solutes, scavengers of active oxygen species and as stabilisers of macromolecules (Loescher 1987; Smirnov and Cumbes 1989; Popp and Smirnov 1995). In contrast to the situation in higher plants, there is no evidence for accumulation of sugars during drying in mosses (Smirnov 1992) and it has been suggested that these plants should maintain sufficient levels of protectants at all times given the speed

with which drying occurs in moss species (Oliver 1996; Marschall *et al.* 1998). Soluble carbohydrate content of these Antarctic moss samples was therefore measured to determine if such molecules could explain any variation in desiccation tolerance observed between the three moss species.

Materials and Methods

Plant material

Samples of *Ceratodon purpureus* (Hedw.) Brid., *Grimmia antarctici* Card. and *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer *et* Scherb. were collected from moss turves at two sites at Casey Station on Bailey Peninsula (Windmill Islands, East Antarctica). Sites which contained all three species within a 4 m² area were chosen to ensure that relative water availability and other environmental conditions were as similar as possible for all species at each site. The moist site (66.283° S, 110.527° E) was on the edge of a melt stream behind the accommodation building. This site is covered with snow and ice during the winter months, but during the summer months it is permanently wet due to its low lying elevation and the substantial amount of melt water flowing off adjacent hills. Moss in this site forms substantial turves. The dry site (66.282° S, 110.522° E) was 100 m west on a small, elevated rocky area adjacent to the Science building. The moss at this site is relatively wet in the early summer months when overlying snow and ice melts, but after this initial thaw, it receives very little water. Moss at this site tends to form discrete cushions rather than extensive turves. At the time of these experiments, (December 1997-January 1998), surface snow had melted from the site several weeks previously and the latter site was dry. It should be noted that the dry site chosen for this study was one in which all species are found and as such is not an extremely dry site by the standards of the region.

Response to desiccation

Moss plugs of each species, approximately 1 cm diameter and 1.5 cm depth, were collected from each site and rehydrated in the laboratory overnight by placing the bases of the plugs in contact with water. At the start of the experiment excess surface water was removed by blotting samples on paper towel. The samples, which weighed approximately 1.5 g fresh weight, were placed in small pre-weighed foil capsules. Photosynthetic efficiency for each sample was determined as the ratio of variable to maximal fluorescence (F_v/F_m) measured after a 10 min. dark adaptation using a PAM 2000 chlorophyll fluorometer (H. Walz, Germany). Dark adapted F_v/F_m has been shown to be a good indicator of the maximum photon-use efficiency of photosynthesis in a broad range of plant taxa (Björkman and Demmig 1987; Lovelock *et al.* 1994). The settings on the PAM 2000 were optimised for the three species at the start of the experiment (measuring light 6, gain 4, damping 5, 0.8 s saturating pulse level 8). The moss samples were then allowed to desiccate slowly in the laboratory under low light conditions (approximately 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$), air temperature of 18°C and relative humidity of 22%.

Change in the water content of each sample was determined from changes in the weight of each sample. The weight of each sample plus capsule was determined immediately after measurement of chlorophyll fluorescence. The water content (WC, in grams water per gram dry weight of sample) was calculated for each measurement point after subtraction of the capsule weight. The WC of each moss sample at each time period was determined as:

$$\frac{\text{Fresh weight}(\text{time } x) - \text{Final dry weight}}{\text{Final dry weight}}$$

after Slatyer (1967). Expression of water content in moss is difficult because much of the water is stored in capillary spaces outside the cells or in the cell wall apoplastic space (for a detailed discussion see Tuba *et al.* 1996). In higher plants where tissue is bound by an epidermis, water content of tissues can be expressed relative to the water content at full turgor. Determination of the water content at full turgor is difficult in mosses (Fowbert 1996); however, expression of water contents on a dry weight basis has been found to be repeatable, and thus is used here (Fowbert 1996; Tuba *et al.* 1996).

Dark adapted F_v/F_m and sample weights were determined for each sample every 3 h for the first 20 h and then every 2 h until constant weight was achieved. At the completion of the experiment moss samples were removed from foil capsules and microwaved at high power for 2 min to preserve organic compounds (Popp *et al.* 1996) prior to being dried, to constant dry weight, in an 80°C oven.

Carbohydrate analysis

Following determination of dry weight, samples were ground into 0.2 mm particles in a sample mill. Hot water extracts (40 mg sample in 1mL ddH₂O) were prepared and de-ionised on ion exchange resins. Low molecular weight carbohydrates and polyols in the neutral fraction were derivatised and analysed as their trimethylsilyl ethers using gas chromatography (Hewlett Packard HP 6890 Gas Chromatograph) as described in Richter *et al.* (1990). Calibration and data analysis were performed using the HP GC ChemStation computer program. Calibrations were performed with a wide range of available standards. For unidentified peaks the calibration curve of the standard with the closest retention time was used. It is difficult to differentiate mannitol, sorbitol and dulcitol using this method and the term hexitols refers generally to acyclic polyols with 6 carbon atoms.

Statistical analysis

In order to test for significant differences between species in their tolerance of desiccation over wet and dry sites, differences between species at the two sites were tested for (1) the WC at which F_v/F_m declined to 50% of its maximum value, (2) the WC at the start of the experiment (initial WC), and (3) the rate of change of WC over time.

The WC at which F_v/F_m declined to 50% of its maximum value was determined graphically for each individual moss sample. Maximum F_v/F_m was not necessarily equal to the initial F_v/F_m values as maximum F_v/F_m was often reached below full hydration. The WC at 50% of maximum F_v/F_m , initial WC and soluble carbohydrate concentrations were analysed as a two way ANOVA with site (wet or dry) and species as fixed effects in the model. Prior to the analysis the WC at 50% of maximum F_v/F_m , initial WC and soluble carbohydrate concentrations were logarithmically transformed to normalise the variance of the data and thereby satisfy the assumptions of the ANOVA model.

The rate of change of WC over time (ΔWC) was calculated for each measurement interval as $(WC_{t_2} - WC_{t_1})/(t_2 - t_1)$, where t_1 and t_2 are the initial and final times of each measurement interval. Data were transformed ($\sqrt{\text{arc-hyperbolic sine}}$) prior to analysis to normalise the variance, and then analysed using a generalised linear model where time was a random continuous variable, and site and species were fixed effects in the model. This model essentially fits a linear function to the transformed ΔWC over time, and then tests whether the slopes or intercepts are altered by site or species.

Results

Differences in the response of three Antarctic moss species to desiccation, and the phenotypic plasticity in their responses was assessed by testing for differences in three parameters (1) the WC at full hydration, (2) the rates of drying over time, and (3) the relationship between the decline in chlorophyll fluorescence and relative water content, over two sites that differed in water availability. The concentration of soluble carbohydrates in all species was also measured to determine if differences in these molecules could explain variation in desiccation tolerance.

Rates of water loss

Figure 1 shows the decline in WC over the duration of the experiment for all three moss species at both wet and dry sites (Fig. 1; note different scale for the wet site, Fig. 1A, and dry site, Fig. 1B). Species from the moist site had a higher water content at the start of the experiment than those from the dry site ($F_{1,39} = 508.87$, $P=0.0001$). There were differences among the species in the WC when they were fully hydrated ($F_{2,39} = 46.02$, $P=0.0001$). In addition, for the two sites the rank order of the WC of the species was different (Site x Species, $F_{2,39} = 12.34$, $P=0.0001$). For moss from the wet site, *B. pseudotriquetrum* and *G. antarctici* had similar WC values that were double that of *C. purpureus*. In moss from the dry site, *G. antarctici* had the highest WC, followed by *B. pseudotriquetrum* and *C. purpureus*.

Initial rates of water loss were faster in moss obtained from the wet site compared to those from the dry site (Fig. 2; note the different scales for the wet site, Fig 2A, and the dry site, Fig 2B, Site effect $F_{1,623}$, $P<0.0001$). In moss from the wet site, rates of water loss were similar in *B. pseudotriquetrum* and *G. antarctici*, but much lower in *C. purpureus*. In moss sampled from the dry site, *G. antarctici* had higher rates of water loss initially than *B. pseudotriquetrum* and *C. purpureus*. However, after approximately 20 h of drying, rates of water loss by *B. pseudotriquetrum* and *G. antarctici* were similar, while those of *C. purpureus* were lower. The rank order of species in rates of drying tends to follow the same pattern apparent in the WC when moss was fully hydrated (Figs 1 and 2).

Declines in chlorophyll fluorescence during desiccation

The chlorophyll fluorescence parameter F_v/F_m remained high until WC had declined to approximately 30% of their initial values; thereafter, F_v/F_m declined rapidly with further reductions in WC (Fig. 3). Photosynthetic efficiency of *G. antarctici* was the most sensitive to reductions in WC. At the wet site, the rapid decline in F_v/F_m began at a WC of approximately 5 g g⁻¹ dry weight in *G. antarctici*, while in *B. pseudotriquetrum* and *C. purpureus* the rapid decline in F_v/F_m began at a WC of approximately 4 and 2 g g⁻¹ dry weight respectively. At the dry site, declines in F_v/F_m were not experienced until *G. antarctici* desiccated to a WC of approximately 2 g g⁻¹ dry weight.

Bryum pseudotriquetrum and *C. purpureus* were much more tolerant of low water content with F_v/F_m remaining high until WC declined below 1 g g^{-1} dry weight.

Analysis of the WC at which F_v/F_m had declined to 50% reflected the above trends. In all three species photosynthetic efficiency declined at a higher WC in the wet site than the dry site (Fig 4, Site effect $F_{1,39} = 230.8$, $P < 0.0001$). In both sites the decline in F_v/F_m to 50% of maximum value occurred at significantly higher WC in *G. antarctici* than in *B. pseudotriquetrum* and *C. purpureus* (Species effect $F_{2,39} = 55.4$, $P < 0.0001$). In the wet site *B. pseudotriquetrum* also showed a decline in photosynthetic efficiency at a significantly higher WC than *C. purpureus*, whereas in the dry site these two species are similar in their response to desiccation (Species x Site interaction $F_{2,39} = 7.15$, $P = 0.0023$). Thus *B. pseudotriquetrum* showed the greatest range in its photosynthetic tolerance of desiccation over the two sites, with WC at which F_v/F_m was reduced to 50% varying between 2.89 g g^{-1} dry weight in moss from the wet site to 0.56 g g^{-1} dry weight in moss from the dry site (Fig. 3).

Species differences in carbohydrates

Soluble carbohydrate concentration was determined in each species at the end of the desiccation treatment. The concentration of known sugars and polyols is shown in Figure 5, whilst the percentage contribution of soluble carbohydrates (including various unidentified carbohydrates) to the total pool, as well as the total pool size, are shown in Table 1. In all three species, total soluble carbohydrate concentration was higher in the wet site samples than the dry site (Table 1, Site effect $F_{1,39} = 42.33$, $P < 0.0001$). Across both sites *B. pseudotriquetrum* had a higher carbohydrate concentration than *G. antarctici* and *C. purpureus*. Total soluble carbohydrate concentration was highest in *B. pseudotriquetrum* collected from the wet site, and lowest in dry site *C. purpureus* (Table 1, Species effect $F_{2,39} = 44.14$, $P < 0.0001$). Amongst the wet site samples, only *B. pseudotriquetrum* and *C. purpureus* were significantly different from each other ($P = 0.0004$), whilst in the dry site *C. purpureus* had a significantly lower soluble carbohydrate content than either *B. pseudotriquetrum* or *G. antarctici* ($P < 0.0001$). Samples collected from the dry site had consistently lower concentrations of soluble carbohydrates than samples of the same species collected from the wet site. In both *B. pseudotriquetrum* and *C. purpureus* this difference was significant ($P = 0.008$ and < 0.0001 respectively). *Grimmia antarctici* samples showed the smallest difference among sites, with dry site samples containing more than 75% of the total soluble carbohydrate of the wet site samples. In *B. pseudotriquetrum* the dry site samples contained 50% of the total soluble carbohydrate found in the wet site. *Ceratodon purpureus* showed the largest variation, with the samples from the dry site samples containing less than 30% the soluble carbohydrates of those from the wet site (Table 1, Species x Site interaction $F_{2,39} = 7.662$, $P = 0.0016$).

Sucrose was the major sugar accounting for more than 40% of the total soluble carbohydrates, in all samples (Table 1 and Fig. 5). Fructose and glucose were also found at relatively high concentration in all species and these sugars showed significantly lower levels in the samples collected from the dry site ($P < 0.0001$). Despite the general decline in carbohydrate concentrations at the dry site certain sugars and polyols were maintained at relatively high levels. In *G. antarctici* sucrose was found at similar concentrations in the dry and wet sites. In *B. pseudotriquetrum* stachyose was both a major carbohydrate and was maintained in both wet and dry site samples, comprising 16% and 28% of the total identified sugars respectively. This is compared with a decline of 30-50% for all other measured carbohydrates for this species.

Hexitols were found in wet site samples of all species and glycerol was present in wet site *B. pseudotriquetrum* and *C. purpureus*. Myo-inositol was detectable in both *B. pseudotriquetrum* and *G. antarctici*, although the concentration declined to 73 % of wet site values in *G. antarctici* and 29% in *B. pseudotriquetrum* obtained from the dry site (Table 1 and Fig. 5).

Five unidentified soluble carbohydrates (labelled X1-5 in Table 1) were detected in wet site *B. pseudotriquetrum* samples. Of these X1 was unique to *B. pseudotriquetrum* (both wet and dry samples) whilst X2 was the only ubiquitous unknown carbohydrate. The concentration of X2 was also maintained across wet and dry habitats, most notably in *B. pseudotriquetrum* and *C. purpureus*. Carbohydrates X3-5 were also found at low concentration in wet site *G. antarctici*.

Discussion

These experiments, and others (Seel *et al.* 1992a; Lovelock *et al.* 1995) demonstrate that chlorophyll fluorescence is highly sensitive to moss relative water content, and that detailed relationships between desiccation and photosynthetic activity, measured here as photosynthetic efficiency, can be obtained. The method is much faster than alternative methods, such as measuring photosynthetic gas exchange, and also allows the use of much smaller samples of moss, thereby reducing the impact on moss communities, an important factor in any field work, but particularly in Antarctica where growth rates are slow and communities are limited in extent (Melick and Seppelt 1997).

This study shows that there are indeed differences in water contents, rates of drying and the response of photosynthesis to desiccation among the three species. The results in Figs 1 and 2 show that *G. antarctici* is able to maintain a high WC when water is plentiful but tends to lose water at a fast rate. Additionally, of the three species studied, *G. antarctici* showed the lowest capacity to maintain photosynthetic efficiency during desiccation, its F_v/F_m being affected by reductions in water content at WCs that were higher than the other two species (Fig. 3). *Grimmia antarctici* may therefore be restricted to wetter sites because, although it can take full advantage of high water availability, its

ability to photosynthesise under dry conditions is limited. *Bryum pseudotriquetrum* had an intermediate response that was highly dependent on the site from which the moss was obtained. It showed the greatest plasticity in terms of both the amount of water it can hold, its rate of water loss and the WC at which photosynthetic efficiency was critically affected, factors that may be important in explaining its wide distribution. *Ceratodon purpureus* had a lower WC at full hydration, a slower rate of water loss, and the ability to maintain its photosynthetic efficiency at lower WC than the other species, features which may make it less competitive in wetter sites but well suited to areas where desiccation is more common. In other ecosystems, species that grow in different environments have also been shown to differ in both their tolerance of desiccation and their rate of recovery following rehydration (e.g. in the comparatively milder maritime Antarctic, Davey 1997 and in the UK, Seel *et al.* 1992a, b). Additional studies have shown that these three Antarctic moss species recovered photosynthetic efficiency within minutes of rehydration (J. Wasley *pers. comm.*). In contrast in the study by Seel *et al.* 1992a, *B. pseudotriquetrum* was described as desiccation sensitive because it did not recover upon rehydration; a difference which would seem to suggest either an extreme degree of plasticity or evidence of divergence of this species.

More similarly to our study, differences in desiccation tolerance have been observed among co-occurring species of *Sphagnum* moss distributed along gradients in water availability (Titus *et al.* 1983; Titus and Wagner 1984; Gerdol *et al.*, 1996). In the former studies, tolerance of desiccation strongly reflected the distribution of the *Sphagnum* species along the water availability gradient. Thus it was concluded, that more favourable photosynthetic carbon balance under drying conditions in some species, is the likely mechanism leading to the distribution of moss species over gradients in water availability. (Titus *et al.* 1983; Titus and Wagner 1984) Additionally, in a study of the arid zone moss, *Grimmia laevigata*, carbon balance of plants was highly dependent on water availability, which was found to limit the microdistribution of the moss (Alpert and Oechel 1985). Longton (1981) speculated that, because of the extreme environmental conditions in continental Antarctica, availability of water in the physical environment was the factor most likely to determine the distribution of plant species. In the results presented here, desiccation tolerance corresponds well with the observed distribution of the three moss species studied. *Grimmia antarctici* is most common in the wetter sites, while in extremely dry sites only *C. purpureus* is found (Selkirk and Seppelt 1987). In this study, *B. pseudotriquetrum* showed a very high degree of plasticity in its response to desiccation, having a similar response to *G. antarctici* in the wet site and a similar response to *C. purpureus* at the dry site. Recent studies by Lewis Smith (1999) of relative distribution of bryophytes in Victoria Land, Antarctica have confirmed that *B. pseudotriquetrum* occupies sites with intermediate water availability whilst *C. purpureus* is found in the driest sites (*G. antarctici* was not included in this study).

Both morphological and biochemical adaptations could be important in the desiccation tolerance observed in these species. The three moss species investigated in these experiments have different

morphologies (Seppelt and Green 1998; Lewis Smith 1999). Plants of *G. antarctici* have the longest stems (10-50 mm in length) with small leaflets orientated perpendicular from the stem. *C. purpureus* leaflets are also small but they adhere more closely to the stem, with the margins of leaflets curling inwards. In addition, *C. purpureus* plants have a layer of small, thick-walled cortical cells surrounding the stem and leaf midrib. *Bryum pseudotriquetrum* stems are broader than both *G. antarctici* and *C. purpureus*, with thick succulent leaflets that adhere closely to the stem. *Ceratodon purpureus* and *B. pseudotriquetrum* stems are typically shorter (10-20 mm) than those of *G. antarctici*. These morphological differences are likely to influence the WC at full hydration (Fig. 1) and the rate of desiccation of the different species (Figs 1 and 2). Such morphological adaptations might act to increase either the external water held within the turf or the cellular water within individual gametophytes. In temperate moss species, plasticity in plant morphology has also been suggested to allow differences in water-holding capacity and rate of desiccation in some species (Bayfield 1973, Schonbeck and Bewley 1981; Titus and Wagner 1984; Gerdol *et al.* 1996).

In addition to difference among species in their tolerance of desiccation, our results also show that desiccation tolerance is highly influenced by site. Rates of desiccation at the dry site were less than half those observed at the wet site for all species (Figs 1 and 2), and the variation is greater than that expected purely on the basis of the different gradients in water potential between the samples and air. In addition, in all three species, photosynthetic efficiency was maintained at a lower WC in the dry site compared to the wetter site (Fig. 3). If morphology of moss plants is important to the rate of desiccation, then the different drying rates would suggest that moss morphology should be different at the different sites. Other studies have shown morphological differences at the turf and leaf level in *C. purpureus* and *B. pseudotriquetrum* collected from sites of varying water availability (Fowbert 1996; Seppelt and Green 1998; Lewis Smith 1999) Extensive variations in morphology have been observed in Antarctic bryophytes and have led to difficulties in the taxonomic classification of plants. For example, wide variation in the leaf margin structure of *G. antarctici* can lead to confusion with *C. purpureus* (Seppelt and Green 1998; Lewis Smith 1999). It could be that these variations in morphology are due in large part to plant adaptation to water availability in various habitats.

Although many of the protective roles assigned to sugars and polyols seem to be most important in enabling cells to survive in the desiccated state (Crowe and Crowe 1986; Koster 1991; Oliver 1996), some may allow plants to remain metabolically active for longer during desiccation and therefore might explain differences in desiccation tolerance. The three moss species show differences in both concentration and type of soluble carbohydrates. Although simple trends which explain the variation in desiccation tolerance were not apparent, there are however intriguing differences between the three species which may underlie their relative desiccation tolerance.

In all species the concentration of soluble carbohydrates is lower in the samples collected from dry sites; however, the extent of this difference is much greater in *C. purpureus* than in *B. pseudotriquetrum* or *G. antarctici*. This confirms and expands data from Melick and Seppelt (1994) which showed that total soluble carbohydrate levels were higher in *G. antarctici* collected from wetter sites and suggests that the shorter growing season experienced by plants from drier sites, results in reduced production of carbohydrates. Carbohydrate levels measured in this study were determined only at the end of the desiccation treatment but are similar in range to those reported for freshly harvested samples of the same species by Roser *et al.* (1992), although less than those measured by Melick and Seppelt (1994). Reduced carbohydrate levels are commonly found in plants following desiccation (Popp and Smirnov 1995; Marschall *et al.* 1998) and so results presented here may underestimate the concentration present in the plants prior to desiccation.

Previous studies of carbohydrate content in freshly harvested bryophyte samples from this region (Roser *et al.* 1992; Chapman *et al.* 1994; Melick and Seppelt 1994) reported that sucrose, fructose and glucose were the main soluble carbohydrates. Stachyose was not apparent in their samples although a very small quantity of trehalose and an unidentified oligosaccharide (thought to be a trisaccharide sucrose-fructose moiety) were detected in both *B. pseudotriquetrum* and *G. antarctici* (Chapman *et al.* 1994). To our knowledge this is the first report of stachyose in moss; previous studies have in fact reported that sucrose is the only free sugar available for cellular protection in desiccation tolerant mosses (Oliver 1996). The presence of stachyose is particularly significant since this and other RFOs are believed to be important in desiccation tolerance of seeds (Koster 1991; Sun and Leopold 1997; Obendorf *et al.* 1998), and cold hardiness in various higher plants (Bachmann *et al.* 1994; Castonguay and Nadeau 1998; Imanishi *et al.* 1998). In higher plants the RFOs are thought to be important both as cryoprotectant molecules, and as carbohydrate storage molecules under low temperature conditions which inhibit starch formation (Bachmann *et al.* 1994). In mosses, increases in carbohydrates associated with frost tolerance have also been observed but have mainly involved increases in sucrose content (Rutten and Santarius 1992). Recently, stachyose and other RFOs have been measured in the leaves of the resurrection plant *Boea hygroskopica* providing a strong indication that these compounds are important in desiccation tolerance of vegetative as well as reproductive cells (Albini *et al.* 1999). Stachyose could be important in both freezing and desiccation tolerance in these moss species. Evidence to support a role in desiccation tolerance comes from the fact that stachyose is one of the few carbohydrates maintained at high levels in dry site *B. pseudotriquetrum*.

Polyols are compatible solutes, believed to be important in cryoprotection of thylakoid membranes. As anti-oxidative systems they are also known to be important in maintaining desiccation tolerance in moss species (Popp and Smirnov 1995; Stewart and Lee 1972; Seel *et al.* 1992c). Both roles maybe particularly relevant in plants exposed to freezing temperatures and light during the polar, summer nights, and especially in moss from wetter sites, where freeze/thaw events are likely while moss is

hydrated. No polyols were detected in these bryophytes in previous studies (Roser *et al.* 1992; Chapman *et al.* 1994). Therefore the presence of small quantities of polyols in these desiccated samples is particularly interesting. Although the polyols make up a relatively small proportion of the soluble carbohydrates they contribute a larger proportion of the soluble carbohydrate pool in *C. purpureus* (7.5%) compared to *B. pseudotriquetrum* (5%) and *G. antarctici* (3.7%) taken from the wet sites. However, *myo*-inositol is the only polyol found in the dry site samples (and only in *B. pseudotriquetrum* and *G. antarctici*).

In interpreting differences in rates of drying among species in this experiment it is important to note that moss was desiccated in small plugs under laboratory conditions. Desiccation dynamics in the field may be substantially altered by the morphology of the whole turf or cushion. Most previous studies have involved creation of artificial plugs by threading individual gametophytes through mesh. Given the intertwined nature of the moss used in this study, desiccation in small turves was deemed to be more appropriate and should provide a better representation of natural desiccation (Lee and Stewart 1971, Alpert and Oechel 1987; Oliver 1996). These experiments were also performed at low light. High light levels have been shown to be extremely detrimental during desiccation in shade plants such as resurrection ferns (Muslin and Homann 1992) and mosses (Seel *et al.* 1992*b*). However, in the latter study Seel *et al.* (1992*b*) found that these additional photoinhibitory effects were far less pronounced in moss from high light environments. Since the moss used in this study grows under a wide range of incident light we expect that it would not be sensitive to photoinhibition. However, the possibility that there are differences among the species in their recovery from desiccation under high light is being investigated.

In conclusion, this study has shown that the three dominant moss species found at Casey show significant differences in the response of their photosynthetic functioning during desiccation. Additionally, all three species showed plasticity in their response to desiccation that was related to the moisture availability of the site from which they grew. Our results indicate that if the Casey region continues to dry as a result of climate change, we would expect to see not only reductions in the extent of the moss community but also changes in community composition. *Grimmia antarctici* is likely to become more limited in distribution as *C. purpureus* and *B. pseudotriquetrum* increase their relative abundance in drying areas.

Acknowledgements

This research was supported by grant funding and logistic support from the Australian Antarctic Division and an Australian Postgraduate Award to JW. We would like to thank all members of the 49th-51st ANARE who helped us with various aspects of this work in Antarctica. We are also grateful to Dr Gerhard Hertenberger for technical assistance with the carbohydrate analysis and to the anonymous reviewers for their helpful comments.

References

- Albini, F. M., Murelli, M., Finza, P. V., Ferrarotti, M., Cantoni, B. Puliga, S. and Vazzana, C. (1999)** Galactinol in the leaves of the resurrection plant *Boea hygrosopica*. *Phytochemistry* **51**, 499-505.
- Alpert, P. and Oechel, W. C. (1985)** Carbon balance limits the distribution of *Grimmia laevigata*, a desiccation-tolerant plant. *Ecology* **66**, 660-669.
- Alpert, P. and Oechel, W. C. (1987)** Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting microdistributions. *American Journal of Botany* **74**, 1787-1796.
- Bachmann, M., Matile, P. and Keller, F. (1994)** Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga reptans* L. *Plant Physiology* **105**, 1335-1345.
- Bayfield, N. G. (1973)** Notes on water relations of *Polytrichum commune* Hedw. *Journal of Bryology* **7**, 607-617.
- Björkman, O. and Demmig, B. (1987)** Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta* **170**, 489-504.
- Castonguay, Y. and Nadeau, P. (1998)** Enzymatic control of soluble carbohydrate accumulation in cold-acclimated crowns of alfalfa. *Crop Science* **38**, 1183-1189.
- Chapman, B. E., Roser, D. J. and Seppelt, R. D. (1994)** ¹³C NMR analysis of Antarctic cryptogram extracts. *Antarctic Science* **6**, 295-305
- Crowe, J. H. and Crowe, L. M. (1986)** Stabilization of membranes in anhydrobiotic organisms. In 'Membranes, metabolism and dry organisms.' (Ed. C. Leopold.) pp188-230. (Cornell University Press, Ithaca, USA).
- Davey, M. C. (1997)** Effects of short-term dehydration and rehydration on photosynthesis and respiration by Antarctic bryophytes. *Environmental and Experimental Botany* **37**, 187-198.
- Fowbert, J. A. (1996)** An experimental study of growth in relation to morphology and shoot water content in maritime Antarctic mosses. *New Phytologist* **133**, 363-373.
- Gerdol, R., Bonora, A., Gualandri, R., Pancaldi, S. (1996)** CO₂ exchange, photosynthetic pigment composition, and cell ultrastructure of Sphagnum mosses during dehydration and subsequent rehydration. *Canadian Journal of Botany* **74**, 726-734.
- Imanishi, H. T., Suzuki, T., Masuda, K. and Harada, T. (1998)** Accumulation of raffinose and stachyose in shoot apices of *Lonicera caerulea* L. during cold acclimation. *Scientia Horticulturae* **72**, 255-263.
- Koster, K. L. (1991)** Glass formation and desiccation tolerance in seeds. *Plant Physiology* **96**, 302-304.
- Lee, J. A. Stewart and G. R., (1971)** Desiccation injury in mosses. I Intra-specific differences in the effect of moisture stress on photosynthesis. *New Phytologist* **70**, 1061-1068.
- Lewis Smith, R. I. (1988)** Classification and ordination of cryptogamic communities in Wilkes Land, continental Antarctica. *Vegetatio* **76**, 155-166.
- Lewis Smith, R. I. (1999)** Biological and environmental characteristics of three cosmopolitan mosses dominant in continental Antarctica. *Journal of Vegetation Science* **10**, 231-242
- Loescher, W. H. (1987)** Physiology and metabolism of sugar alcohols in higher plants. *Physiologia Plantarum* **70**, 553-557.
- Longton, R. E. (1981)** Interpopulation variation in morphology and physiology in the cosmopolitan moss *Bryum argenteum* Hedw. *Journal of Bryology* **11**, 501-520.

- Lovelock, C. E., Jebb M. and Osmond, C. B. (1994)** Photoinhibition and recovery in tropical plant species: response to disturbance. *Oecologia* **97**, 297-307.
- Lovelock, C. E., Osmond, C. B. and Seppelt, R. D. (1995)** Photoinhibition in the Antarctic moss *Grimmia antarctici* Card. when exposed to cycles of freezing and thawing. *Plant, Cell and Environment* **18**, 1395-1402.
- Marschall, M., Proctor, M. C. F. and Smirnov, N. (1998)** Carbohydrate composition and invertase activity of the leafy liverwort *Porella platyphylla*. *New Phytologist* **138**, 343-353.
- Melick, D. R. and Seppelt, R. D. (1994)** Seasonal investigation of soluble carbohydrates and pigment levels in Antarctic bryophytes and lichens. *The Bryologist* **97**, 13-19.
- Melick, D. R., Hovendon, M. J., Seppelt, R. D. (1994)** Phytogeography of bryophyte and lichen vegetation in the Windmill Islands, Wilkes Land, continental Antarctica. *Vegetatio* **111**, 71-87.
- Melick, D. R. and Seppelt, R. D. (1997)** Vegetation patterns in relation to climatic and endogenous changes in Wilkes Land, continental Antarctica. *Journal of Ecology*, **85**, 43-56.
- Morgan, V. I., Goodwin, I. D., Etheridge, D. M. and Wookey, C. W. (1991)** Evidence from Antarctic ice cores for recent increases in snow accumulation. *Nature* **354**, 58-60.
- Muslin, E. H., Homann, P. H. (1992)** Light as a hazard for the desiccation-resistant 'resurrection' fern *Polypodium polypodioides* L. *Plant, Cell and Environment* **15**, 81-89.
- Obendorf, R. L., Dickerman, A. M., Pflum, T. M., Kacalanos, M. A. and Smith, M. E. (1998)** Drying rate alters soluble carbohydrates, desiccation tolerance, and subsequent seedling growth of soybean (*Glycine max* L. Merrill) zygotic embryos during in vitro maturation. *Plant Science* **132**, 1-12.
- Oliver, M. J., Mishler, B. D. and Quisenberry, J. E. (1993)** Comparative measures of desiccation-tolerance in the *Tortula ruralis* complex. I variation in damage control and repair *American Journal of Botany* **80**, 127-136.
- Oliver, M. J. (1996)** Desiccation tolerance in vegetative plant cells. *Physiologia Plantarum* **97**, 779-787.
- Popp, M., Lied, W., Meyer, A. J., Richter, A., Schiller, P. and Schwitte, H. (1996)** Sample preservation for determination of organic compounds: microwave versus freeze-drying. *Journal of Experimental Botany*, **47**, 1469-1473.
- Popp, M. and Smirnov, N. (1995)** Polyol accumulation and metabolism during water deficit. In 'Environment and Plant metabolism- flexibility and acclimation'. (Ed. N Smirnov.) pp199-215 (Bios Scientific Publishers, Oxford, UK.)
- Richter, A., Thonke, B. and Popp, M. (1990)** ID-1-O-methyl-muco-inositol in *Viscum album* and members of the Rhizophoraceae. *Phytochemistry*, **29**, 1785-1786.
- Roser, D. J., Melick, D. R., Ling, H. U. and Seppelt, R. D. (1992)** Polyol and sugar content of terrestrial plants from continental Antarctica. *Antarctic Science* **4**, 413-420.
- Rutten, D. and Santarius, K. A. (1992)** Relationship between frost tolerance and sugar concentration of various bryophytes in summer and winter. *Oecologia* **91**, 260-265.
- Schonbeck, M. W., Bewley, J. D. (1981)** Responses of the moss *Tortula ruralis* to desiccation treatments. II Variation in desiccation tolerance. *Canadian Journal of Botany* **59**: 2702-2712.
- Seel, W. E., Baker, N. R., Lee, J. A. (1992a)** Analysis of the decrease in photosynthesis on desiccation of mosses from xeric and hydric environments. *Physiologia Plantarum* **86**, 451-458.
- Seel, W. E., Hendry, G. A. F., Lee, J. A. (1992b)** The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. *Journal of Experimental Botany* **43**, 1023-1030.

- Seel, W. E., Hendry, G. A. F., Lee, J. A. (1992c)** Effects of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. *Journal of Experimental Botany* **43**, 1031-1037.
- Selkirk, P. M. and Seppelt, R. D. (1987)** Species distribution within a moss bed in Greater Antarctica. *Symposia Biologia Hungarica* **35**, 279-284.
- Seppelt, R. D and Green, T. G. A (1998)** A bryophyte flora for Southern Victoria Land, Antarctica. *New Zealand Journal of Botany* **36**, 617-635.
- Slatyer, R. O. (1967)** '*Plant-Water Relationships.*' (Academic Press: London.)
- Smirnoff, N. (1992)** The carbohydrates of bryophytes in relation to desiccation tolerance. *Journal of Bryology* **17**, 185-191.
- Smirnoff, N. and Cumbes, Q. J. (1989)** Hydroxyl radical scavenging activities of compatible solutes. *Phytochemistry* **28**, 1057-1060.
- Stewart, G. R. and Lee, J. A. (1972)** Desiccation injury in mosses. II The effect of moisture stress on enzyme levels. *New Phytologist* **71**, 461-466.
- Sun, W. G. and Leopold, A. C. (1997)** Cytoplasmic vitrification and survival of anhydrobiotic organisms. *Comparative Biochemistry and Physiology* **117A**, 327-333.
- Titus, J. E., Wagner, D. J. (1984)** Carbon balance for two *Sphagnum* mosses: water balance resolves a physiological paradox. *Ecology*, **65**, 1765-1774.
- Titus, J. E., Wagner, D. J. and Stephens, M. D. (1983)** Contrasting water relations of photosynthesis for two *Sphagnum* mosses. *Ecology*, **64**, 1109-1115.
- Tuba, Z., Csintalan, Z., Proctor, M. C. F. (1996)** Photosynthetic responses of 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 CO₂ concentration. *New Phytologist* **133**, 353-361.
- Wilson, M. E. (1990)** Morphology and photosynthetic physiology of *Grimmia antarctici* from wet and dry habitats. *Polar Biol.*, **10**, 337-341.

Figure legends

Fig. 1.

Decline in water content (g g^{-1} dry weight) as a function of time in *Grimmia antarctici*, (#) *Bryum pseudotriquetrum* (!) and *Ceratodon purpureus* (TM) collected from both wet (A) and dry sites (B). Conditions as described in Materials and Methods. Data represent the mean and SEM (Fig 1A, n=7 and Fig 1B, n=8). Note different scales.

Fig. 2.

Rate of water loss (g g^{-1} dry weight h^{-1}) as a function of time in *Grimmia antarctici*, (#) *Bryum pseudotriquetrum* (!) and *Ceratodon purpureus* (TM) collected from both wet (A) and dry sites (B). Conditions as described in Materials and Methods. Data represent the mean and SEM (Fig 2A, n=7 and Fig 2B, n=8). Note different scales.

Fig. 3.

Decline in photosynthetic efficiency, measured as the chlorophyll fluorescence parameter F_v/F_m , during desiccation of moss from wet (closed symbols) and dry (open symbols) sites. *Grimmia antarctici*, (#, £) *Bryum pseudotriquetrum* (!, j) and *Ceratodon purpureus* (@, TM). Conditions as described in Materials and methods. Data represent mean values (Wet site, n=7 and Dry site, n=8).

Fig. 4.

Water content (g g^{-1} dry weight) at 50% photosynthetic efficiency (F_v/F_m) for *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus* collected from wet (hatched bars) and dry sites (open bars). Conditions as described in Materials and Methods. Data represent mean values and SEM (Wet site, n=7 and Dry site, n=8).

Fig. 5.

Concentration of sucrose, stachyose, fructose, glucose, hexitol, glycerol and myo-inositol (mg g^{-1} dry weight) in desiccated samples of *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus* collected from wet (hatched bars) and dry sites (open bars). Data represent mean values and SEM (Wet site, n=7 and Dry site, n=8).

Table 1. Concentration of soluble carbohydrates in desiccated samples of *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus* collected from wet and dry sites.

Data presented as a proportion of total soluble carbohydrates (% total soluble carbohydrates \pm SEM) and total values (mg g⁻¹ dryweight \pm SEM). Unidentified soluble carbohydrates designated X1-5 were calibrated against the closest standard. Retention times for all compounds, relative to fructose are shown in column one. Data represent mean values and SEM (n=7 (wet site) and n=8 (dry site))

	<i>Bryum pseudotriquetrum</i>		<i>Grimmia antarctici</i>		<i>Ceratodon purpureus</i>	
	Dry Site	Wet Site	Dry Site	Wet Site	Dry Site	Wet Site
Identified soluble carbohydrates (%)						
Fructose	8.38 \pm 0.69	9.81 \pm 0.59	7.17 \pm 0.44	9.88 \pm 0.77	n.d.	12.73 \pm 0.58
Glucose (rtn time 1.11, 2.24 min)	3.61 \pm 0.28	5.28 \pm 0.29	4.24 \pm 0.30	5.76 \pm 0.54	7.64 \pm 0.33	8.22 \pm 0.55
Sucrose (rtn time 8.89 min)	41.80 \pm 2.80	40.25 \pm 5.94	76.08 \pm 9.21	64.78 \pm 11.53	71.73 \pm 6.49	57.47 \pm 6.17
Stachyose (rtn time 22.08 min)	20.45 \pm 1.60	11.05 \pm 1.76	n.d.	0.37 \pm 0.37	n.d.	0.65 \pm 0.65
Hexitols (rtn time 1.64 min)	n.d.	1.91 \pm 0.12	n.d.	2.50 \pm 0.06	n.d.	4.40 \pm 0.15
myo-Inositol (rtn time 3.48 min)	0.23 \pm 0.11	0.41 \pm 0.02	0.60 \pm 0.09	0.62 \pm 0.05	n.d.	n.d.
Glycerol (rtn time -7.7 min)	n.d.	1.18 \pm 0.42	n.d.	n.d.	n.d.	2.04 \pm 0.96
Unidentified soluble carbohydrates (%)						
X1 (rtn time 13.4 min)	19.28 \pm 1.50	16.39 \pm 3.10	n.d.	n.d.	n.d.	n.d.
X2 (rtn time 13.94 min)	6.25 \pm 1.39	4.75 \pm 1.35	11.91 \pm 0.93	12.13 \pm 1.67	20.63 \pm 6.10	14.50 \pm 2.74
X3 (rtn time 0.1 min)	n.d.	5.45 \pm 0.33	n.d.	1.57 \pm 1.02	n.d.	n.d.
X4 (rtn time 1.08 min)	n.d.	2.97 \pm 0.77	n.d.	1.60 \pm 1.03	n.d.	n.d.
X5 (rtn time 9.56 min)	n.d.	0.55 \pm 0.55	n.d.	0.80 \pm 0.80	n.d.	n.d.
Concentration (mg g⁻¹ dryweight \pm SEM)						
Total identified carbohydrates	21.90 \pm 1.51	40.44 \pm 4.72	26.42 \pm 2.94	33.43 \pm 5.12	5.50 \pm 0.47	20.22 \pm 1.76
Total carbohydrates	29.41 \pm 2.20	57.86 \pm 7.26	29.99 \pm 3.19	39.85 \pm 6.24	6.93 \pm 0.89	23.64 \pm 2.18

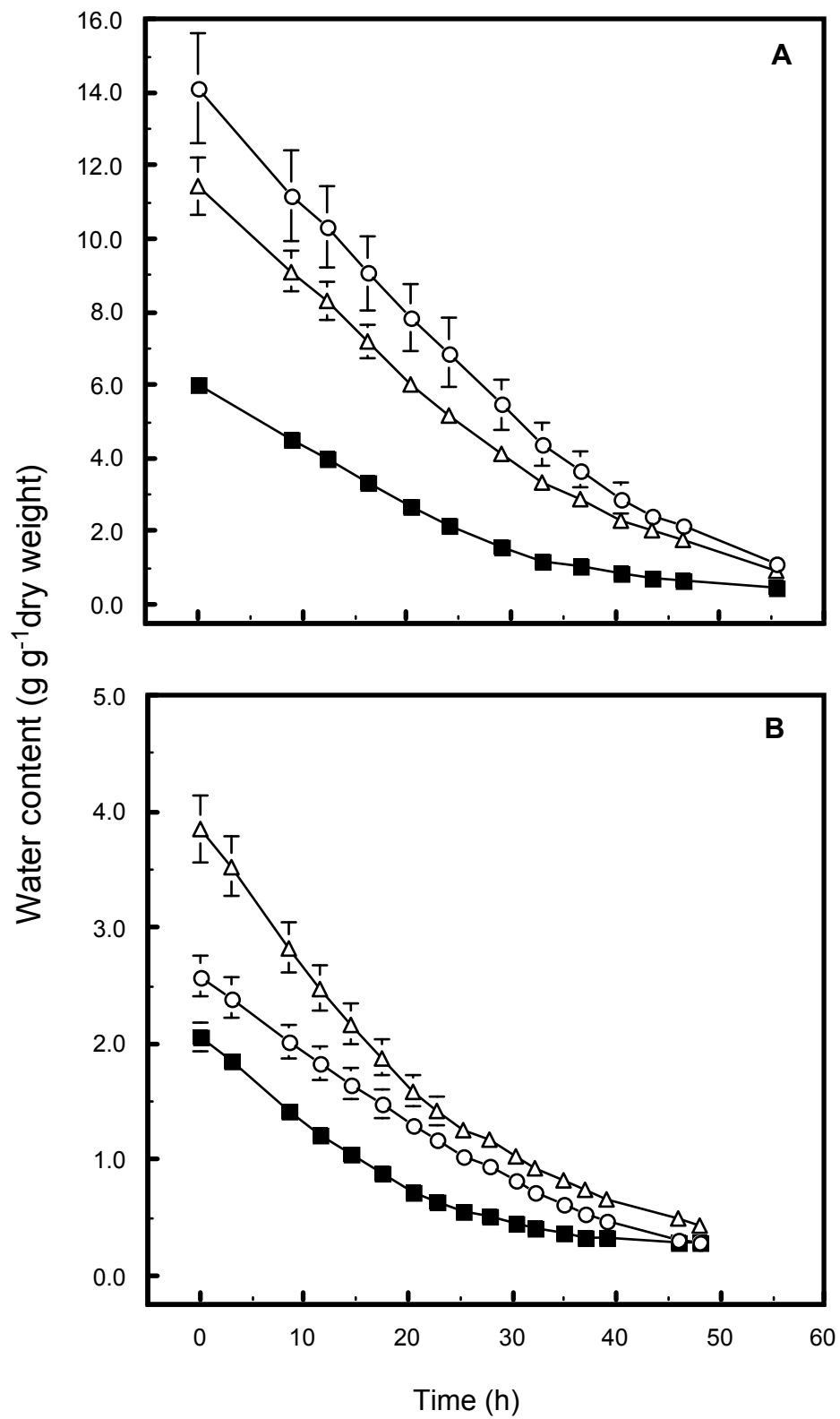


Figure 1

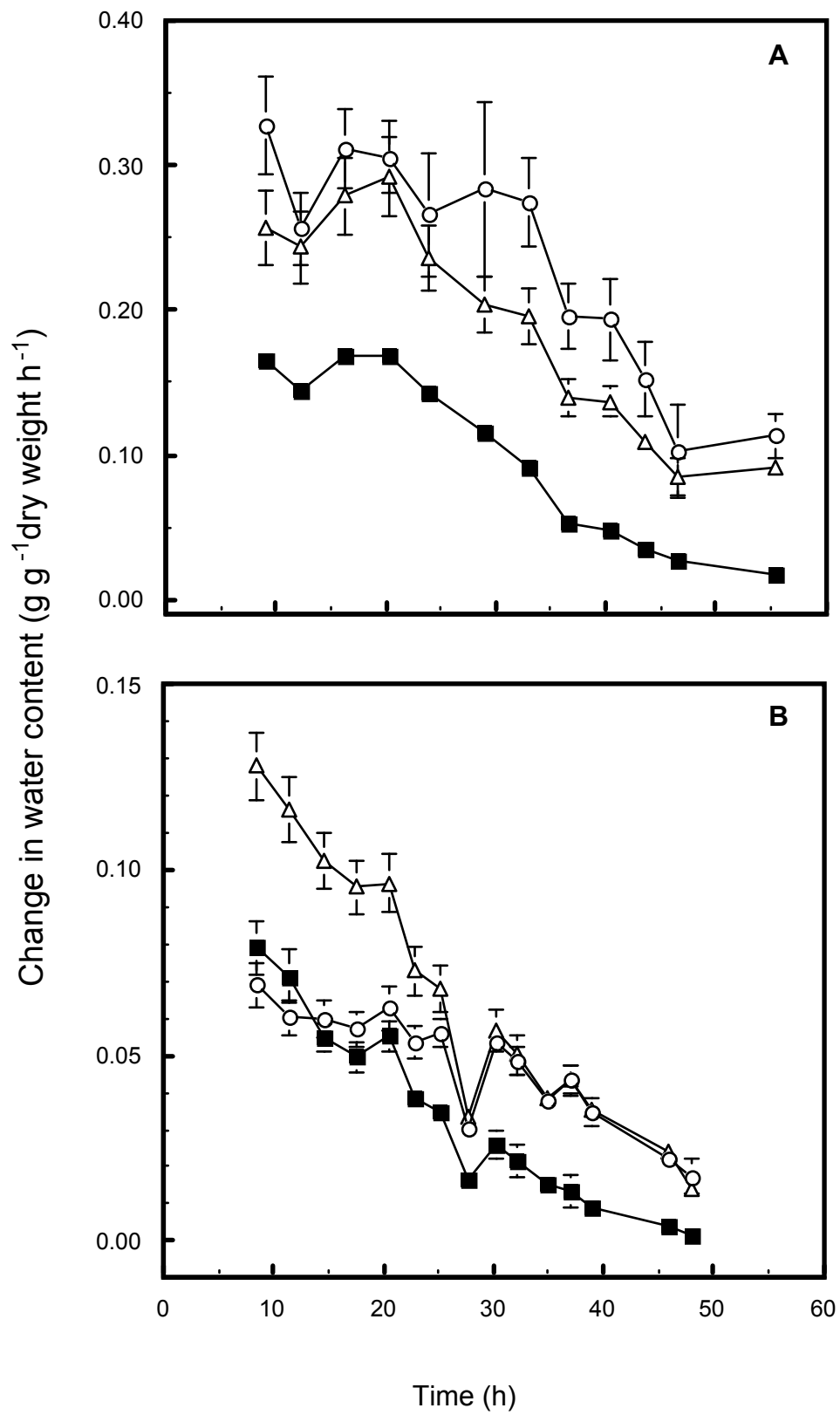


Figure 2

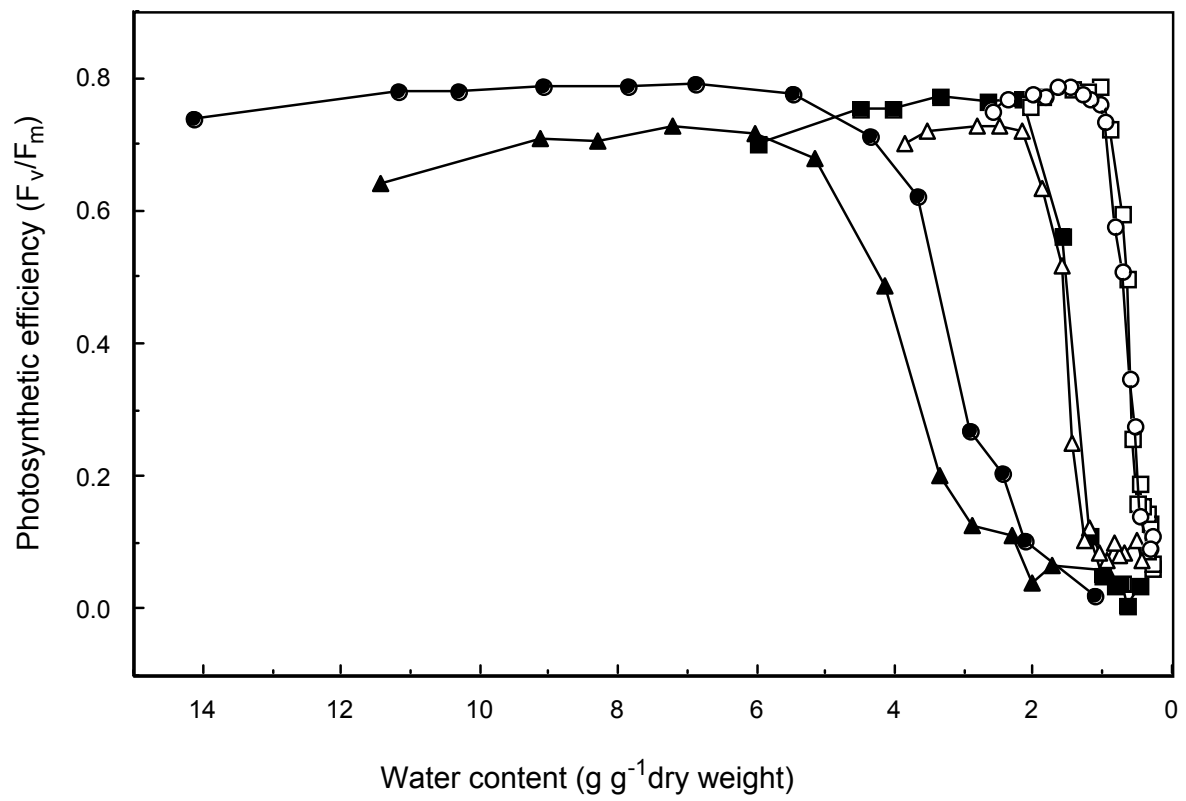


Figure 3

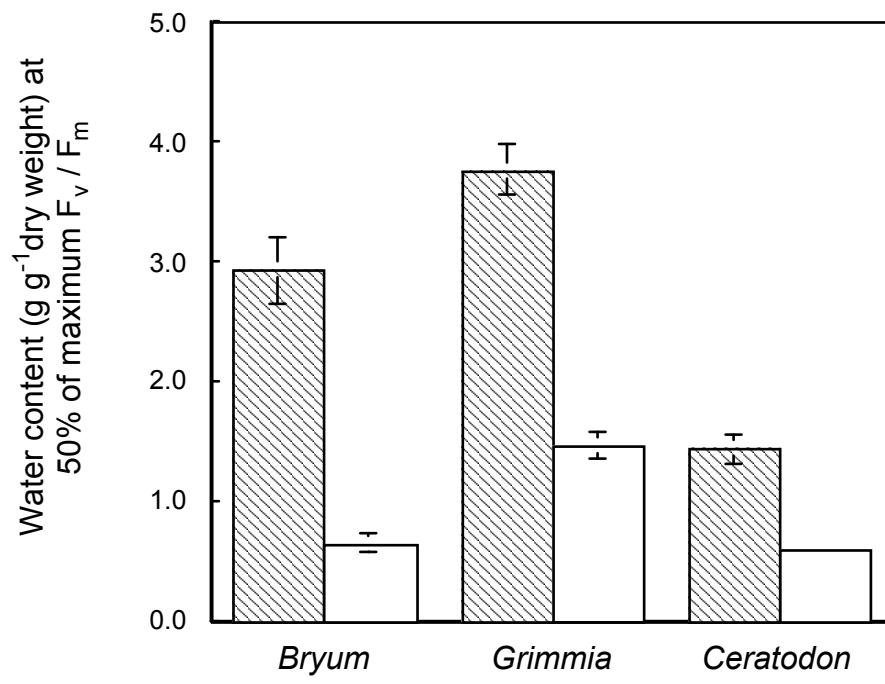


Figure 4

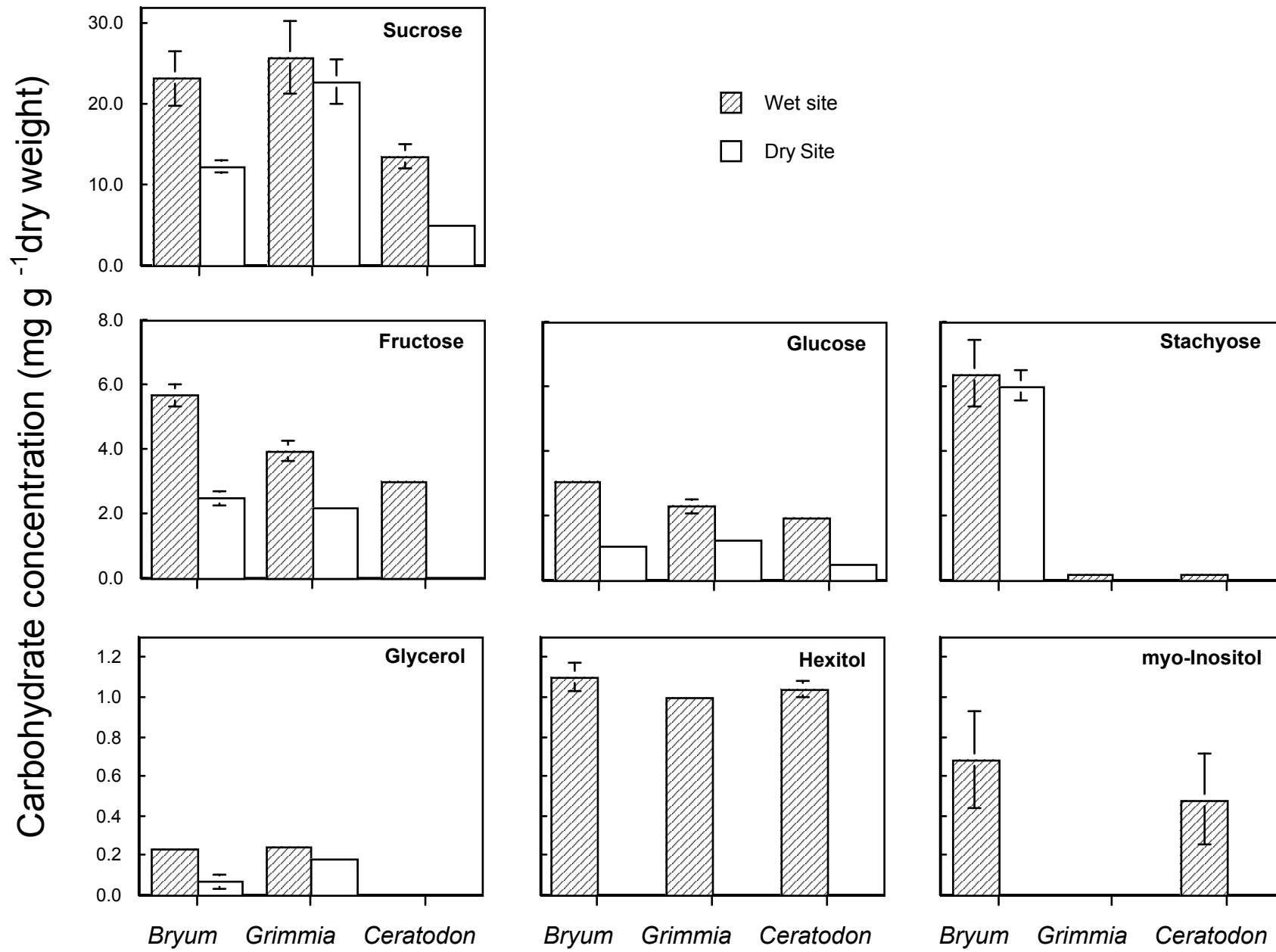


Figure 5