Acta Biologica Hungarica 61 (Suppl.), pp. 120–129 (2010)
DOI: 10.1556/ABiol.61.2010.Suppl.12

PHOTOSYNTHETIC RESPONSES, CARBOHYDRATE COMPOSITION AND INVERTASE ACTIVITY IN FRUCTAN ACCUMULATING BRYOPHYTES (*PORELLA PLATYPHYLLA* AND *SPHAGNUM FLEXUOSUM*) UNDER DIFFERENT ENVIRONMENTAL CONDITIONS (CARBOHYDRATE TREATMENTS, DARK STARVATION, LOW TEMPERATURE, DESICCATION)

Marianna Marschall*

Department of Plant Physiology, Eszterházy Károly College, H-3301 Eger, Hungary

(Received: August 9, 2010; accepted: October 10, 2010)

Photosynthetic responses, the non-structural carbohydrate pool and its alterations, and the acid invertase activity under different environmental conditions in the fructan synthesising *Porella platyphylla* and *Sphagnum flexuosum* are discussed. Sucrose and fructan are the major soluble carbohydrates in both species. TLC showed that fructans form a homologous series of increasing DP in a similar manner to fructans in Angiosperms and belong to the inulin type. Exogenous sugars (glucose, fructose, sucrose) applied in light and dark resulted in down-regulation of photosynthetic activity, but a long period (1 week) of dark starvation did not cause a significant decrease in the photosynthetic capacity. Light and exogenous sugars increased soluble carbohydrate content due to fructan-accumulation. Dark starvation, desiccation and low temperature did not influence significantly the amount of the total soluble carbohydrates, indicating the existence of a well-buffered carbohydrate pool, although changes in the ratio of fructans of different molecular weight can be detected. Alterations in the activity of acid invertase correlated well with the changes of the amount of the main soluble carbohydrates, showing the role of the enzyme in general carbohydrate and fructan metabolism.

Keywords: Fructans – photosynthetic responses – *Porella platyphylla* – soluble carbohydrates – *Sphagnum flexuosum*

INTRODUCTION

Fructans are polyfructose molecules containing glucose. They are derived from sucrose. Three fructan trisaccharides (monomer) are known. In 1-kestose forming inulins fructose is linked to carbon 1 of the fructose moiety of sucrose, in 6-kestose forming levans fructose is linked to carbon 6 of the fructose moiety of sucrose with glycosidic linkage. In neokestose-based polymers fructose moieties occur only at the termini because the glucose is contained within. Inulins may achieve a degree of polymerization (DP) of over 100, but usually do not exceed 30–35 in most plant parts [5]. Levans in higher plants can be larger than inulins, with DP up to 260 and 314, and levans of bacteria in dental plaque are highly branched and have DP in the

* E-mail: marschal@ektf.hu

Table 1
The basic reactions of fructan metabolism

Fructan synthesis	GF + GF	SST →	GFF + G
	$GFF_n + GFF_m$	FFT →	$GFF_{n+1} + GFF_{m-1} \mid n, m \geq 1$
Fructan hydrolysis	GFF_n	FEH →	$GFF_{n-1} + F \mid n \geq 1$
Sucrose hydrolysis	GF	INV →	G + F

Abbreviations: G: glucose, F: fructose, GF: sucrose, GFF: trisaccharide fructan monomer, GFF_n, GFF_m: fructan polymers, SST: sucrose:sucrose fructosyl transferase, FFT: fructan:fructan fructosyl transferase, FEH: fructan exohydrolase, INV: invertase.

thousands. Inulins and levans of higher plants may also form branches at some of the free hydroxyl groups on the fructose units, but the degree of branching is not well understood. Neokestose-based polymers seem less common in higher plants than inulins and levans, and they have DP≤30 [5].

The enzimology of fructan synthesis and hydrolysis (Table 1) in higher plants is an area of intensive study and speculation. Parallel with these studies, the numbers of investigations on invertase in Angiosperms are multiplied. Invertases (β-fructofuranosidases) can exhibit SST activity at very high sucrose concentration. Instead of water, sucrose accepts the fructosyl moiety resulting in the formation of one of the three isomers of the trisaccharide kestose. It is unlikely that this reaction is important *in vivo*. Alternatively, SST exhibits invertase activity at low sucrose concentration [4].

The role of fructans as storage carbohydrates and their function in desiccation tolerance and low temperature stress is well emphasized in higher plants. Despite their physiological relevance only 15% of the whole plant kingdom and only a minor part of living organisms (Table 2) can synthesise fructans [2]. It should be interesting to answer why there are no more fructan synthesising algae, and why fructan synthesis is absent in pteridophytes and gymnosperms.

Table 2
The taxonomic distribution of the major polymeric reserve carbohydrates in biology (after 3)

Taxa	Glucose polymers	Fructose polymers
Bacteria	dextran (20 divis. parts)	fructan (levans, 6 parts)
Insects, crustacea	glycogen, trehalose	–
Vertebrates	glycogen	–
Fungi	glycogen, trehalose	? (doubtful)
Algae *(Clorophyta)*	starch (70 orders), trehalose	fructan *(Dasycladales, Cladophorales)*
Bryophytes	starch (28 orders), trehalose	fructan *(Jungermanniales, Sphagnales)*
Pteridophytes	starch, trehalose	–
Gymnosperms	starch	–
Angiosperms	starch (290 families), trehalose	fructan (17 families)

The water soluble fructans in the Angiosperms represent very important alternative storage carbohydrates, which are stored in the vacuoles. Fructans can accumulate up to 20–50% of the dry weight in different plant parts, but their content can even reach 70% of the dry weight in bulbs. The size of fructan polymers can be altered quickly; this could be an explanation for their role in osmotic adjustment. Polimerization or breakdown of fructan will alter vacuolar osmotic potential, and hence may alter turgor pressure. The DP appears to closely track changes in the external environment. Drought, high irradiance and/or low temperature favours fructan accumulation in Angiosperms, so their relevance is linked to desiccation and freezing resistance or emphasized in response to cold and dry seasons [2, 7]. Fructan content, due to the favourable properties and physiological roles of fructans, may give the most likely explanation for the global success of 15% of the Angiosperm flora which store fructan as a principal reserve carbohydrate. Fructan accumulating plants make 1/3 of the Earth vegetation, and the most successful species belong to the *Poaceae* and *Asteraceae* families. The origin and evolution of the fructan-rich Angiosperm flora occurs from the Oligocene to the mid-Miocene, and coincides with the development of the seasonal water shortage. Species rich in fructan do not accumulate more than trace amounts (~1%) of starch in their vegetative parts, which means fructan is stored largely as an alternative to, and not in addition to, starch.

Desiccation-tolerance must be a very ancient feature of terrestrial life. Tolerant species occur in all the kingdoms of life (Cyanobacteria, Protista, yeasts, fungi, nematodes, tardigrades, rotifers, terrestrial algae, bryophytes, lichens, pteridophytes, and ~350 angiosperm species, embryos in seeds, pollen and spores). This suggests that desiccation-tolerance is an ancient trait, must be present in the ancestors of all living organisms, and thus the genetic potential for desiccation-tolerance is universal [6]. This also means that the genes required for tolerance appear to be present even in desiccation intolerant species, but they are not expressed; on the other hand, with gradual drought-hardening their genetic program can be elicited within certain limits. Desiccation-tolerance in bryophytes is qualitatively different from the mechanism of flowering plants. Poikilohydric bryophytes have retained the alternative strategy of photosynthesizing and growing actively when water is available, and drying out and suspending metabolism when it is not. Their cells have two stable states, either fully turgid or desiccated, with relatively brief transitions in between. In the course of drying out and rehydrating they must pass through the levels of water stress experienced by drought-tolerant vascular plants. They only transiently face the problem of metabolizing under water stress. It is like a "drought avoidance" strategy in vascular plants. The time required to recover from desiccation increases and the degree of recovery decreases with length of desiccation; both also depend upon temperature and intensity of desiccation. Truly desiccation-tolerant "resurrection" species are the rare exception, making up less than 0.15% of vascular plants. Desiccation-tolerant Angiosperms cannot survive desiccation if water loss occurs less than 12 h, because their inducible mechanisms (protein synthesis) to establish tolerance require longer time. Desiccation-tolerant bryophytes can tolerate rapid (generally 3 h, but even 1 h) drying and recover completely upon rehydration. Recovery of respiration, photosynthesis and protein

synthesis takes place within minutes or an hour or two; recovery of the cell cycle, food transport and the cytoskeleton may take 20 h or more. Positive carbon balance is essential to survival of repeated cycles of drying and wetting; significant growth requires continuously wet periods of a few days or more. The mechanisms of desiccation-tolerance in bryophytes, including expression of LEA proteins, high content of non-reducing sugars and effective antioxidant and photo-protection, are at least partly constitutive, and employ an active rehydration-induced repair and recovery mechanism. During their recovery phase the changes in gene expression resulting from mRNA sequestration and alterations in translational controls elicited upon rehydration are also important to repair processes following rewetting [6].

Leafy liverworts *(Jungermanniales)* contain a diverse range of soluble carbohydrates, including sucrose, fructan and polyols such as mannitol, sorbitol and volemitol. Mosses have a simple soluble carbohydrate pool consisting of sucrose. *Sphagnum* species – as an exception amongst mosses – synthesise fructan and have a considerable amount of sucrose as well.

In both leafy liverworts and mosses, starch, glucose and fructose are present at relatively low concentration [4].

Desiccation-tolerance, return to the normal metabolism upon rehydration after a long period of desiccation, and significant photosynthetic activity under low temperature are common but not general phenomena in bryophytes.

The aim of this paper is i) to provide data about the photosynthetic responses, the non-structural carbohydrate pool and its alterations, and the acid invertase activity under different carbohydrate supply, desiccation, low temperature in two fructan synthesizing bryophytes: *Porella platyphylla* and *Sphagnum flexuosum*; ii) to obtain new information about the physiological role of soluble carbohydrates, especially fructans in bryophytes, and the role of the carbohydrate pool and its metabolism under various environmental conditions.

MATERIALS AND METHODS

Porella platyphylla (L.) Lindb. and *Sphagnum flexuosum* Doz. & Molk. were collected from two locations in Heves County in Hungary (Felsőtárkány, Nyírjes tó near Sirok). The plants were acclimatized at 100% RH, 20 °C, at a PPFD of 100 μmol m^{-2} s^{-1}, and a photoperiod of 12/12 light/dark for 3 d before use in experiments. During the course of various treatments the samples were kept at 20 °C, at a PPFD of 120 μmol m^{-2} s^{-1} with a 12-h light period unless otherwise indicated. Plants were exposed to the following treatments.

Sugar feeding in light and dark, dark starvation

Plants were placed on filter paper disks moistened with 10 mol m^{-3} glucose, fructose and sucrose solutions (distilled water was also used for controls in light and dark as

well) for 4 or 7 days both in light (120 μmol m^{-2} s^{-1} PPFD) and in dark at 5, 10 and 20 °C. Before harvest they were rinsed twice with distilled water to remove the sugars from the surface of the tissue. During the "dark starvation treatment" plants were moistened with distilled water and kept in the dark for 4 or 7 days.

Desiccation

The plant material was placed in glass desiccators under the following conditions to obtain the required water status: 100% RH (distilled water), 54% RH (saturated calcium nitrate), ca. 0% RH (silica gel). The latter two treatments resulted in slow and fast drying of the bryophytes, respectively. Rehydration was achieved by spraying the plants with a minimum amount of distilled water.

Water deficit

Water deficit was applied by exposure to polyethylene glycol 3350 (PEG 3350) solutions for 2 d. The plants were placed in Petri dishes containing 9 cm diameter filter paper discs moistened with PEG solutions to obtain the desired water potential [4].

Low temperature

Plants were exposed to 5 °C in the light (120 μmol m^{-2} s^{-1} PPFD) for 4 d.

CO_2 assimilation was measured in normal air by infrared gas analyzer in an open gas-exchange system using a temperature controlled leaf chamber. Assimilation rates (*A*) were calculated after [8]. Oxygen exchange was measured in a liquid phase oxygen electrode as in [4]. For the carbohydrate determinations tissue was extracted with 80% ethanol followed by hot water and boiling in 3% HCl for 3 h for starch. Fructans were quantified using a ketose-specific method with resorcinol, and separated by TLC on silica gel plates and detected with urea-phosphoric acid stain. Total soluble sugars were detected by the Dubois method. Glucose, fructose and sucrose were quantified by GC as in [4], or by a combined enzymatic assay (invertase-glucose-oxidase-peroxidase) of glucose release. Invertase was assayed by enzymatic assay of glucose after incubation of sucrose [4]. Data have been subjected to one way analysis of variance.

RESULTS

In *Porella platyphylla* and *Sphagnum flexuosum* sucrose and fructan are the major soluble carbohydrates. Starch was present in less than 1% of the total soluble sugars. TLC showed that fructans form a homologous series of increasing DP in a similar manner to

fructans in Angiosperms. The trisaccharide has the same mobility as 1-kestose in the *Helianthus* standard suggesting that the fructan could be of the inulin type.

Exogenous glucose, fructose and sucrose treatments applied in dark for 7 days resulted in the down-regulation of the photosynthetic activity in *P. platyphylla*. Sucrose feeding in the dark caused more than 50% decrease in the photosynthetic activity of *P. platyphylla*. A longer period of dark starvation hardly influenced photosynthetic activity. Photosynthetic capacity was still retained after 7-d-darkness. Photosynthetic oxygen evolution was unaffected by glucose and fructose feeding applied for 4 days in light and by dark starvation. However, sucrose feeding in light decreased the rate of photosynthesis by 25% ($P < 0.05$). Sucrose feeding and dark decreased the rate of respiration by 40 and 29% respectively ($P < 0.05$).

In *S. flexuosum* exogenous glucose, fructose, sucrose applied in light resulted in ~50% decrease, while sugars applied in dark resulted in ~70% decrease in photosynthetic activity. Of the sugars, glucose feeding had the highest down-regulating effect on photosynthesis in *S. flexuosum*, while in *Porella* sucrose did. Seven-d-darkness caused only 36% decrease in photosynthetic activity.

In *P. platyphylla* the soluble carbohydrate composition was not greatly affected by sugar feeding applied for 4 days in light. Fructan was slightly increased by sucrose feeding after 4 days. The amount of high molecular weight (HMW) fructans was significantly increased by glucose and sucrose feeding after 7 days. Dark starvation even after a week did not cause a significant decrease in the non-structural carbohydrates. Dark starvation for 4 d caused a 70% decrease in sucrose, glucose and fructose, whereas low molecular weight (LMW) fructans were decreased by 40% ($P < 0.05$) and HMW fructans changed only slightly.

In *P. platyphylla* desiccation decreased the glucose, fructose, sucrose and total fructan content of the leaves. Glucose and fructose content increased fivefold when desiccated leaves were rehydrated for 1 h ($P < 0.05$). Sucrose and fructan content returned to pre-desiccation levels on rehydration after 1 h. During desiccation the amount of the LMW fructans decreased, and the proportion of the HMW increased. *P. platyphylla* was exposed to low water potential by PEG 3350 treatment for 2 d. Fructan concentration was increased by low water potential, the concentration doubling below –0.62 MPa. Further fructan accumulation did not occur at more negative water potentials. Other sugars were unaffected, resulting in an increase in the proportion of fructans in the soluble carbohydrate pool. The specific activity of acid invertase with the straightening of the water stress did slightly change; it showed a small increase at –4.5 MPa. Exposing *P. platyphylla* to 5 °C in the light for 4 d, total fructan increased by 65% ($P<0.05$). This increase was caused by the alteration of the LMW fructans, while HMW fructans did not change.

The proportion of the non-structural carbohydrates in the different parts of *S. flexuosum* can be characterized well. The capitulum contains 28% fructan, 71% other sugars, mainly sucrose, and 1% starch. The stem contains 13% fructan, 86% other sugars, and 1% starch. The capitulum is dominated by LMW fructans in 60%, while 79% of the stem fructans belong to the HMW fructans. The stem generally contains half of the capitulum fructan amount.

In *S. flexuosum* glucose, fructose and sucrose feeding in light resulted in a significant increase in the total soluble sugar concentration after a week ($P < 0.05$). Fructan content did increase significantly by glucose feeding ($P < 0.05$). Sucrose remained constant. Seven-d-dark starvation did not cause a significant decrease in the non-structural carbohydrates.

Acid invertase activity was significantly increased by 7-d-dark starvation in both bryophytes. The enzyme activity was decreased by glucose and fructose feeding in darkness, and it was increased by glucose in light ($P < 0.05$).

DISCUSSION

Two fructan synthesising bryophytes were investigated. Sucrose and fructan are the major soluble carbohydrates in *Porella platyphylla* and *Sphagnum flexuosum*. TLC showed that fructans form a homologous series of increasing DP in a similar manner to fructans in Angiosperms and belong to the inulin type.

Exogenous sugars supplied for 7 d either in light or dark caused depression of photosynthetic activity in both bryophytes. Sucrose feeding in the dark caused more than 50% decrease in the photosynthetic activity of *P. platyphylla*, while sucrose feeding in light decreased photosynthesis by 25%. In *S. flexuosum* exogenous glucose, fructose, sucrose applied in light resulted in ~50% decrease, while sugars applied in dark resulted in ~70% decrease in photosynthetic activity. Sucrose feeding in *P. platyphylla*, glucose feeding in *S. flexuosum* had the most relevant down-regulating effect on photosynthetic activity. Photosynthetic capacity was still retained after 7-d-darkness. This contrasts with the loss of photosynthetic capacity and degreening in higher plants exposed to prolonged dark. Sucrose feeding and dark storage decreased respiration rate by 40 and 29%, respectively. These responses of photosynthesis and respiration presumably contribute to conservation of resources when photosynthesis is prevented (by, for example, temporary burial under dead leaves) but allows rapid resumption of photosynthesis when the plant is illuminated.

Light and exogenous sugars increased soluble carbohydrate content due to fructan-accumulation in both bryophytes. In *P. platyphylla* the fructan-accumulation was caused by the HMW fructans. Dark starvation, desiccation and low temperature did not influence significantly the amount of the total soluble carbohydrates, indicating the existence of a well-buffered carbohydrate pool dominated by a large amount of sucrose. Fructan is generally conserved at the expense of sucrose. Total fructan content hardly changes, although changes in the ratio of fructans of different molecular weights can be detected. Overall, the conservation of the soluble carbohydrates in *P. platyphylla* and *S. flexuosum* contrast with the response of higher plants, where soluble sugars fall to very low levels after shorter exposure to the dark. This could be related to the very small proportion of non-photosynthetic sink tissue in bryophytes.

In contrast to liverworts, mosses have very little or no acid invertase activity [4]. In *P. platyphylla* high acid invertase activity was found [4], while *S. flexuosum* showed 10% of the "Porella enzyme" activity. The acid invertase activity in *S. flexu-*

osum was found sixfold higher (0.63 ± 0.02 μmol glucose min^{-1} g^{-1} d. w.) than in "real mosses".

What is the relevance of high acid invertase activity in a plant which can synthesise fructan? Enzymes catalyzing sucrose hydrolysis, fructan hydrolysis and kestose synthesis cannot be easily distinguished in crude extracts [4]. Although the "Porella enzyme" has properties typical of acid invertase [4], its possible role in fructan metabolism cannot at present be ruled out. In *P. platyphylla* and *S. flexuosum* high sucrose concentration coexists with acid invertase. In higher plants a negative correlation between sucrose concentration and soluble acid invertase activity has been noted. It is clear that either sucrose and invertase are in different cells or subcellular compartments or the invertase usually has low activity *in vivo*. Neutral red staining revealed a large central vacuole in *P. platyphylla* which would allow acid invertase and sucrose to occupy separate compartments [4]. The vacuole could be the site of acid invertase and fructan while the sucrose could be compartmented in the cytosol.

Acid invertase activity was significantly increased by 7-d-dark starvation in both bryophytes. The enzyme activity was decreased by glucose and fructose feeding in darkness, and it was increased by glucose in light. This increase can be related to the possible role of the enzyme in fructan synthesis, and is largely in line with the accumulation of the LMW fructans. The slow rate of sucrose mobilisation in the dark suggests that only a small invertase capacity would be required if this was the sole function of the enzyme. Remarkable enzyme activity was found in the dark.

The soluble carbohydrates did not show significant responses to changes in the environment indicating that they are well-buffered and available to act as desiccation protectants. During the desiccation of *P. platyphylla*, similarly to the Angiosperms, fructan accumulation occurred. The proportion of HMW fructan did increase upon desiccation, suggesting the polymerization, while the amount of LMW fructan decreased.

Low temperature at light caused fructan-accumulation in both bryophytes as in Angiosperms. This accumulation is due to the increase of the LMW fructans, while alteration in the HMW fructans was not detected. Exogenous sugars in light, as in Angiosperms were described, resulted in fructan-accumulation in the examined species. Generally, fructan-accumulation is enhanced by treatments which reduce growth and hence there are lower requirements for assimilates of the rest of the plant, and current photosynthate diverted into fructan.

Dark starvation induced a limited fructan breakdown in both bryophytes, which affected LMW fructans only.

Fructan content in Angiosperms is generally 20–50% of the dry weight, while in *P. platyphylla* and *S. flexuosum* it is only 1–3%. Fructans accumulate in vacuoles in Angiosperm cells. Fructans are predominantly stored in underground plant parts in very high concentrations. The vacuolar localisation has not been proved yet in the examined bryophytes, although a large acidic vacuole was detected in *P. platyphylla* [4]. Neither of the bryophytes have sink tissue under the ground. Fructan-accumulation usually occurs in Angiosperm taxa which have the strategy of desiccation-tolerance in their photosynthetic tissue, or they can tolerate different levels of water stress. The

desiccation-tolerant leafy liverwort, *P. platyphylla* has a constitutive mechanism of desiccation-tolerance, which includes some inducible elements. The fructan-accumulating *S. flexuosum* living in a constantly moist environment is not considered to be desiccation-tolerant, although hummock forming *Sphagna* have a special morphological adaptation [1], which provides relatively constant water content for the chlorophyllous cells by an increased water holding capacity in hyalocysts and the cell walls. Plants synthesising water-soluble fructans can rapidly mobilize their storage carbohydrates, and can easily respond to the unfavourable changes of the environment by quick alteration of the degree of polymerization of the fructans. The possibility of the rapid change of the size of the fructan polymers can be the reason for their suggested role in osmotic adjustment in Angiosperms. It is likely that total fructan amount making up 1–3% of the dry weight in bryophytes is insufficient to function in the osmoregulation, therefore fructan in these species can have other roles. Fructan could act in a similar manner to sucrose; may stabilize macromolecules during desiccation, help in maintaining the glassy state of the cytoplasm, prevent sucrose crystallization, act in scavenging ROS. Vacuolar localization of fructans in Angiosperms is contradictory to the fulfilment of their cytoplasmic functions. However, cellular localization of fructans in bryophytes has not been confirmed yet. Fructan content seems to be advantageous evolutionally in seasonally dry or cold environment [2]. The genetic origin and the distribution of the fructan-rich Angiosperm flora from the Oligocene to the mid-Miocene coincide with the development of the seasonal water shortage. The high and relatively constant level of osmotically active sugars, mainly sucrose, is an important constitutive component of the mechanism of desiccation-tolerance in bryophytes. The significance of fructans in plants living in a constantly moist environment, such as *Sphagna*, can hardly be explained. It may be related to the strong effect of another seasonally influencing environmental factor, such as low temperature. Because of the known examples of fructan-synthesising algae and bryophytes, the first occurrence of fructans in the plant kingdom can be dated much earlier. According to our present knowledge a Silurian or a late-Devonian origin could be suggested. A number of the features of carbohydrate metabolism in *P. platyphylla* and *S. flexuosum* invite further investigation and could illuminate topics of current interest in higher plants.

The origin and the physiological role of fructan synthesis in the two bryophyte orders (*Jungermanniales*, *Sphagnales*) cannot be as easily explained as in Angiosperms and further research is needed. This strongly suggests that the ability to synthesise fructans in bryophytes and Angiosperms arose on separate occasions.

REFERENCES

1. Hajek, T., Beckett, R. P. (2008) Effect of water content components on desiccation and recovery in *Sphagnum* mosses. *Ann. Botany 101,* 165–173.
2. Hendry, G. A. F. (1993) Evolutionary origins and natural functions of fructans – a climatological, biogeographic and mechanistic appraisal. *New Phytologist 123,* 3–14.

3. Hendry, G. A. F. (1996) Fructan and the ecology and the evolution of the *Compositae*. In: Hind, D. J. N. (ed.) *Proceedings of the International Compositae Conference, Kew, 1996. Vol. 2. Biology & Utilization* (vol. eds Caligari, P. D. S. Hind, D. J. N.). Royal Botanic Gardens, Kew, pp. 121–128.
4. Marschall, M., Proctor, M. C. F., Smirnoff, N. (1998) Carbohydrate composition and invertase activity of the leafy liverwort *Porella platyphylla*. *New Phytologist 138,* 343–353.
5. Nelson, C. J., Spollen, W. G. (1987) Fructans. *Physiol. Plantarum 71,* 512–516.
6. Proctor, M. C. F., Oliver, M. J., Wood, A. J., Alpert, P., Lloyd, R. Stark, L. R., Cleavitt, N. L., Mishler, B. D. (2007) Desiccation-tolerance in bryophytes: a review. *The Bryologist 110,* 595–621.
7. Thomas, H., James, A. R. (1999) Partitioning of sugars in *Lolium perenne* (perennial ryegrass) during drought and on rewatering. *New Phytologist 142,* 295–305.
8. von Caemmerer, S., Farquhar, G. D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta 153,* 376–387.