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Hardening by Partial Dehydration and ABA Increase Desiccation Tolerance in the Cyanobacterial Lichen *Peltigera polydactylon*

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• *Background and Aims* The ability of partial dehydration and abscisic acid pretreatments to increase desiccation tolerance in the cyanobacterial lichen *Peltigera polydactylon* was tested.
• *Methods* Net photosynthesis and respiration were measured using infrared gas analysis during a drying and rehydration cycle. At the same time, the efficiency of photosystem two was measured using chlorophyll fluorescence, and the concentrations of chlorophyll *a* were spectrophotometrically assayed. Heat production was also measured during a shorter drying and rehydration cycle using differential dark microcalorimetry.
• *Key Results* Pretreating lichens by dehydrating them to a relative water content of approx. 0·65 for 3 d, followed by storing thalli hydrated for 1 d in the light, significantly improved their ability to recover net photosynthesis during rehydration after desiccation for 15 but not 30 d. Abscisic acid pretreatment could substitute for partial dehydration. The improved rates of photosynthesis during the rehydration of pretreated material were not accompanied by preservation of photosystem two activity or chlorophyll *a* concentrations compared with untreated lichens. Partial dehydration and ABA pretreatments appeared to have little direct effect on the desiccation tolerance of the mycobiont, because the bursts of respiration and heat production that occurred during rehydration were similar in control and pretreated lichens.
• *Conclusions* Results indicate that the photobiont of *P. polydactylon* possesses inducible tolerance mechanisms that reduce desiccation-induced damage to carbon fixation, and will therefore improve the supply of carbohydrates to the whole thallus following stress. In this lichen, ABA is involved in signal transduction pathways that increase tolerance of the photobiont.

Key words: Lichens, desiccation tolerance, abscisic acid, photosynthesis, chlorophyll, PSII activity, respiration, heat production.

INTRODUCTION

Most lichens are desiccation tolerant, and can survive in an air-dried state for long or short periods even at relative water contents (RWC) below 10 % (Kershaw, 1985). Work on desiccation-tolerant higher plants and cryptogams suggests that desiccation is harmful for many reasons (Black and Pritchard, 2002). These include damage to the cytoskeleton as a result of the large changes in cell volume that accompany desiccation. Water removal can damage membranes, increase ionic strength, change pH, crystallize solutes and denature proteins. Apparently, desiccation tolerance is achieved not by any one simple adaptive feature, but by a complex interplay of many mechanisms (Black and Pritchard, 2002). Little information is available on desiccation-tolerance mechanisms in lichens, but these include maintaining effective concentrations of enzymic and non-enzymic antioxidants, and in lichens with chlorophycean photobionts, an efficient xanthophyll cycle to prevent reactive oxygen species (ROS) formation in the photosystems (Mayaba and Beckett, 2001; Zorn *et al*., 2001; Kranner, 2002; Kranner *et al*., 2003).

Oliver *et al*. (1998) divided desiccation-tolerant plants into two groups. In the first group, plants will only survive if drying is slow enough to induce mechanisms that will 'protect' the plants during desiccation or facilitate recovery during rehydration. Plants in the second group possess constitutive tolerance mechanisms, and can tolerate even rapid drying. It is often assumed that lichens rely mainly on constitutive mechanisms, as they frequently grow in highly stressful environments where desiccation may be sudden and severe. However, the disadvantage of constitutive mechanisms is that they are present even when not needed, and at these times divert energy away from growth and reproduction. It was therefore hypothesized that selection may have favoured inducible tolerance mechanisms in environments that are usually moist, and in which lichens are predictably (and probably slowly) desiccated. The latter conditions typify the habitat occupied by cyanobacterial species of *Peltigera* growing in the Afromontane forests of South Africa. These forests are moist for much of the year, but experience a regular winter drought. The aim of the work presented here was to test for inducible tolerance in a species from this genus, *P. polydactylon*, common in this and other similar habitats.

Under field conditions, partial dehydration often precedes desiccation stress, and therefore the effects of partial dehydration on desiccation tolerance were tested in *Peltigera polydactylon*. Much evidence exists that in poikilohydric

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plants ABA plays an important role in desiccation tolerance (Hartung *et al*., 1997), and therefore the effect of an ABA pretreatment on desiccation tolerance in this lichen was tested. Desiccation tolerance was assessed by measuring the effects of desiccation followed by rehydration on photobiont metabolism [net photosynthesis, photosystem two (PSII) activity and the concentrations of chlorophyll *a* (chl*a*)] and mycobiont metabolism (respiration and heat production). Results presented here show that partial dehydration and ABA pretreatment increase the tolerance of carbon fixation to desiccation stress, which will reduce the effects of stress on the whole lichen thallus.

MATERIALS AND METHODS

Lichen material

Peltigera polydactylon (Necker) Hoffm. was collected from moss-covered boulders under a tree canopy in the Olandweni Valley, Cathedral Peak area, Drakensberg Mountains, KwaZulu-Natal Province, South Africa (28°45′ S, 29°10′ E). Once collected, lichens were cleaned in deionized distilled water then stored for 10 d at 15 °C, and a photosynthetic photon fluence density (PPFD) of 75 μmol photons m^{-2} s^{-1} under continuous fluorescent light. PPFDs were measured here and elsewhere over the waveband 400–700 nm using the light meter in the Parkinson leaf chamber of an Analytical Development Corporation (ADC, Hoddeston, UK) Mark III portable infra-red gas analyser. These storage conditions were assumed to remove most of the effects of stress present when lichens were collected, without being long enough to cause the harmful effects that can occur following prolonged moist storage. For each experiment, discs were cut, pooled, and each replicate derived from discs randomly sampled from this pool. Thallus water contents were calculated as RWC, estimated as (fresh mass − dry mass)/(turgid mass − dry mass), and measured as described in Beckett (2002).

Measurement of photosynthesis and respiration

Net photosynthesis and respiration were measured at 25 °C and relative humidity of 50 % using an ADC Mark III portable infrared gas analyser with a barrel-shaped Parkinson leaf chamber, modified with a water-cooled jacket. The flow rate through the leaf chamber was 120 mL min^{-1} and the PPFD was 350 μmol photons m^{-2} s^{-1}. Preliminary experiments showed that net photosynthesis saturated at approx. 150 μmol photons m^{-2} s^{-1} and no photoinhibition occurred until at least 700 μmol photons m^{-2} s^{-1}. Optimum rates of net photosynthesis occurred in lightly blotted hydrated material, i.e. no 'oversaturation' or suppression of photosynthesis at high RWCs occurred in the material. Equilibrating fully hydrated samples for 10 min gave steady-state rates of gas exchange without causing enough water loss to reduce photosynthesis. Respiration measurements were made in the same way, except lichens were maintained in the dark. Each treatment comprised five replicates of four 1-cm discs (approx. 40 mg fresh mass).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using an FMS 2 modulated fluorometer (Hansatech Instruments, King's Lynn, UK). Lichens were clamped in standard Hansatech leaf-clips with the probe withdrawn slightly from the clip to allow sampling of a larger than normal thallus area. Each replicate comprised four 1-cm thallus discs. To take a measurement, each replicate was pretreated at a PPFD of 10 μmol photons m^{-2} s^{-1} for 10 min as recommended by Campbell *et al*. (1998), placed in a leaf clip, and F_o and F_m recorded using a 0·8 s flash of light at approx. 1500 μmol photons m^{-2} s^{-1}. The actinic light at a PPFD of 33 μmol photons m^{-2} s^{-1} was then switched on, and F and F'_m measured after 5 min. The fluorescence parameters were calculated following Schreiber and Bilger (1993), Schreiber *et al*. (1995) and Campbell *et al*. (1998); $F_v/F_m = (F_m - F_o)/F_m$ and $\Phi PSII=(F'_m - F)/F'_m$). Non-photochemical quenching was estimated as the Stern–Volmer quotient ($F_m/F'_m - 1$). Each treatment comprised five replicates.

*Determination of chl*a

Chl*a* content was determined by grinding 50 mg fresh mass lichen material in liquid nitrogen followed by extraction in 5 mL $CaCO_3$-saturated dimethylsulfoxide. Samples were incubated in a water bath at 60 °C for 40 min, centrifuged at 1500 *g* for 6 min, and chl*a* measured spectrophotometrically using equations in Palmqvist and Sundberg (2002). Each treatment comprised five replicates.

Heat production measurements

Heat production was measured using a differential dark microcalorimeter (LKB -2277 BioActivity Monitor, Thermometric AB, Jarfalla, Sweden) with a sensitivity of 100 μW in 3·0 cm^3 glass vessels. Each treatment comprised three or four replicates of two 6-mm discs. Each point represents an average from 400 digital measurements performed with frequency 0·1 s. Data were analysed using the 'Spline' program of Hunt and Parsons (1974).

Pretreatments

Control material comprised lichens stored fully hydrated at a PPFD of 75 μmol photons m^{-2} s^{-1} of continuous fluorescent light at 15 °C. The other pretreatments were partial dehydration and treatment with ABA as described by Beckett (1999). Briefly, for the partial dehydration pretreatment, initially fully hydrated lichens were stored at 100 % relative humidity (stored on dry filter paper suspended above distilled water in a desiccator) as above for 3 d. After 3 d of storage this way, lichens reached a RWC of approx. 0·65. They were then fully hydrated by storage on wet filter paper for 1 d at a PPFD of 75 μmol photons m^{-2} s^{-1} of continuous fluorescent light at 15 °C. In the ABA treatment, ABA (± *cis-trans*; Sigma, St Louis, MO, USA) was dissolved in a drop of 1 M NaOH, and the pH of the resulting solution adjusted to 5·6 with HCl. Lichens

were pretreated by gently shaking them in 100 μM ABA for 1 h, and then storing them hydrated as above for 3 d.

Desiccation and rehydration cycle

After pretreatment, all lichens were placed in 2 × 5 cm specimen bottles in a desiccator over silica gel at 15 °C and a PPFD of 75 μmol photons m^{-2} s^{-1} under continuous fluorescent light. After 1 d the RWC reached approx. 0·02 and did not decline further. After 15 and 30 d the lichens were suddenly rehydrated by addition of 10 mL distilled water. Photosynthesis and respiration were measured in lichens at the start of the experiment, after application of pretreatments, after desiccation, then at intervals during the 8 h following rehydration. Chlorophyll fluorescence parameters and the concentrations chl*a* were measured in control material and material treated with ABA at these same times. Microcalorimetric measurements were made in untreated and ABA-treated material, following desiccation over silica gel for 2·5 h, after which time the RWC of the lichens had declined to approx. 0·05, and during rehydration following desiccation. Fluorescence parameters, chl*a* concentrations and microcalorimetric measurements were not determined in lichens given a partial dehydration pretreatment, because results for net photosynthesis and respiration showed that partial dehydration had almost the same effect as ABA pretreatment. Lichens were normally rehydrated under dim laboratory lighting (aprox. 5 μmol photons m^{-2} s^{-1}). However, in the experiments that measured respiration and heat production, lichens were rehydrated in complete darkness. For measurement of net photosynthesis, during the 10 min while actual readings of photosynthesis were taking place, lichens were exposed to 350 μmol photons m^{-2} s^{-1}. During the measurement of chlorophyll fluorescence parameters lichens were exposed to 33 μmol photons m^{-2} s^{-1} for 5 min. In the experiments in which photosynthesis, chlorophyll fluorescence and heat production were measured, the same individual replicates of material were used for each measurement throughout the entire experiment. In the experiments in which chl*a* concentrations were measured, different material was assayed at each sampling time, because of the destructive nature of the assay.

Statistical analysis

All data were statistically analysed using a two-way analysis of variance (ANOVA) with pretreatment and time as factors, and the significance of pretreatment tested using Fisher's least significant difference test (Gomez and Gomez, 1983).

RESULTS

The initial rates of net photosynthesis in *P. polydactylon* were approx. 4 μmol CO_2 mg^{-1} dry mass h^{-1}, and these rates were not affected by partial dehydration or ABA (Fig. 1A and C). During rehydration after desiccation for 15 d, control material initially (after 20 min) displayed a net release of CO_2, but after 2 h regained net CO_2 fixation. Photosynthesis had fully recovered after 4 h. By contrast, after the same desiccation time, lichens subjected to a partial dehydration or ABA pretreatment displayed significant net photosynthesis after rehydration for 20 min, and recovered to initial rates faster than untreated material (Fig. 1A and C). ANOVA analyses showed that the effects of these two pretreatments were highly significant ($P < 0·01$). During rehydration following desiccation for 30 d, control material again initially displayed a net release of CO_2, and photosynthesis had fully recovered after 4 h (Fig. 1B and D). The beneficial effects of partial dehydration or ABA were mostly lost after desiccation for 30 d (ANOVA analyses indicating that the effects of pretreatment were not significant; $P < 0·05$). However, compared with the controls, these lichens tended to display lower net losses of CO_2 during the early stages of rehydration. The initial respiration rates of *P. polydactylon* were slightly less than 1 μmol CO_2 mg^{-1} dry mass h^{-1}, and were not affected by partial dehydration or ABA (Fig. 1E). During rehydration following desiccation for 15 d, respiration in control material initially increased to approx. 2 μmol CO_2 mg^{-1} dry mass h^{-1}, but after 4 h decreased to control rates. Partially dehydrated or ABA-pretreated material tended to display smaller increases in respiration, to approx. 1·5 μmol CO_2 mg^{-1} dry mass h^{-1} (Fig. 1E), although ANOVA analysis indicated that rates did not differ significantly from control material. After rehydration for 4 h, the respiration rates of these lichens, like those for the controls, returned to their initial values. After desiccation for 30 d, respiration in material from all treatments initially increased to approx. 2 μmol CO_2 mg^{-1} dry mass h^{-1}, and then returned to initial values within 4 h (Fig. 1F).

During 3 d of storage following water (control) or ABA pretreatment, the maximum photochemical efficiency of photosystem II (F_v/F_m) declined slightly from approx. 0·6 to approx. 0·55 (compare points a and b in Fig. 2A). Desiccation reduced F_v/F_m to approx. 0·2 after 15 d, and to almost 0 after 30 d (Fig. 2A and B). During rehydration following desiccation for 15 d F_v/F_m recovered to approx. 0·5 after 4 h, but did not increase further by 8 h (Fig. 2A). Control and ABA-pretreated material recovered similarly. Following desiccation for 30 d, even after 8 h the recovery of F_v/F_m was only partial, to approx. 0·3, or half the initial value (Fig. 2B). ABA-pretreated material tended to recover faster than control during the first 20 min of rehydration, but after 2 h no differences existed between these treatments. The actual quantum yield of PSII (ΦPSII) was approx. 0·2 (Fig. 2C). Desiccation reduced ΦPSII to zero. During rehydration following desiccation for 15 d, ΦPSII gradually increased, recovering to initial values after 6 h (Fig. 2C). After desiccation for 30 d, ΦPSII recovered more slowly, and after 8 h had only recovered to approx. 0·15, or about three-quarters of the initial values (Fig. 2D). ANOVA analysis indicated that ABA had no significant effect on the recovery of F_v/F_m or ΦPSII during rehydration following desiccation for 15 or 30 d. NPQ was almost zero in all treatments (data not shown).

Chl*a* concentrations did not change during pretreatment (Fig. 2E). Desiccation for 15 d reduced chl*a* concentrations

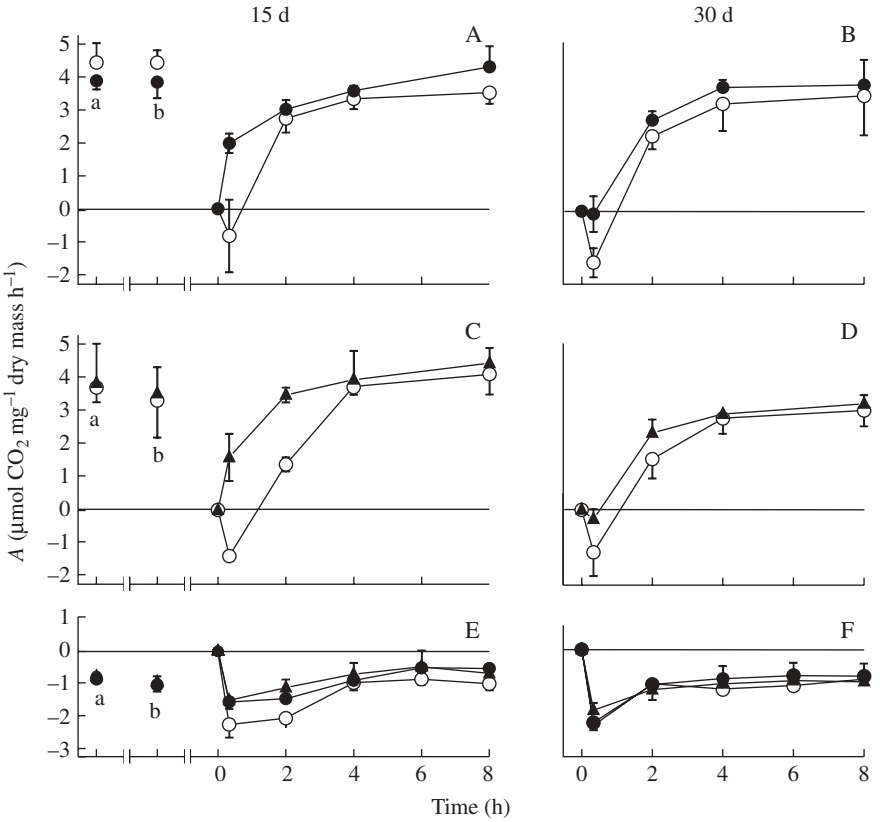

FIG. 1. The effect of desiccation for 15 and 30 d followed by rehydration on photosynthesis in *Peltigera polydactylon* pretreated with partial dehydration (A and B) or 100 μM ABA (C and D), and the effects of these two pretreatments on respiration (E and F). a, After collection from the field; b, following 3-d pretreatment or maintenance moistened with distilled water ('control'). Time 0 indicates the start of rehydration. In A and C the effects of pretreatment were significant ($P < 0{\cdot}001$ and 0·002, respectively), and in the other graphs the effects of pretreatments were not significant. Open circles, control material; closed circles, material pretreated by partial dehydration; closed triangles, ABA-pretreated material; error bars denote the standard deviation, $n = 5$.

by about one-quarter; after rehydration for 8 h they had recovered their initial values. Desiccation for 30 d reduced chl*a* concentrations by about one-third, and they did not recover during rehydration (Fig. 2F). ANOVA showed that the changes of chl*a* concentrations in response to desiccation were the same in ABA and water-treated lichens.

Control and ABA-pretreated lichens produced heat at rates of approx. 2 mW g^{-1} dry mass. ABA pretreatment induced a small increase in heat production (Fig. 3). After desiccation for approx. 2·5 h, heat production by both control and ABA-pretreated lichens dropped to almost zero. Following rehydration, heat production increased to approx. 8 mW g^{-1} dry mass and then gradually declined, although production was still approx. 6 mW g^{-1} dry mass 3 h after rehydration. No significant differences existed in heat production during rehydration between control and ABA-pretreated lichens.

DISCUSSION

Partial dehydration increases the tolerance of carbon fixation to desiccation in Peltigera

Results presented here show that reducing the RWC of the lichen *Peltigera polydactylon* to approx. 0·65 for a few days increases the tolerance of carbon fixation to desiccation, even if material is stored fully hydrated for 1 d after partial dehydration (Fig. 1). Care is therefore needed when testing the effects of desiccation on lichens, because the hydration state of a sample before measurement can influence tolerance, even if material is maintained fully hydrated before testing. The *Peltigera* used in this study was from an Afromontane forest, and in such habitats, lichens predictably desiccate during the dry winter months, allowing enough time for the lichens to induce tolerance mechanisms. It is envisaged that lichens from harsher, xeric sites will be found to have largely constitutive desiccation tolerance mechanisms, because if desiccation is rapid and irregular, insufficient time will be available to induce tolerance. However, in *P. polydactylon*, partial dehydration clearly hardens carbon fixation to the harmful effects of subsequent desiccation stress.

ABA can substitute for partial dehydration

Exogenous application of ABA is as effective at inducing tolerance as partial dehydration (Fig. 1). Presumably, for lichens growing in the field, as for higher plants (Bray, 1997), mild water stress induces ABA biosynthesis, which in turn activates signal transduction pathways that

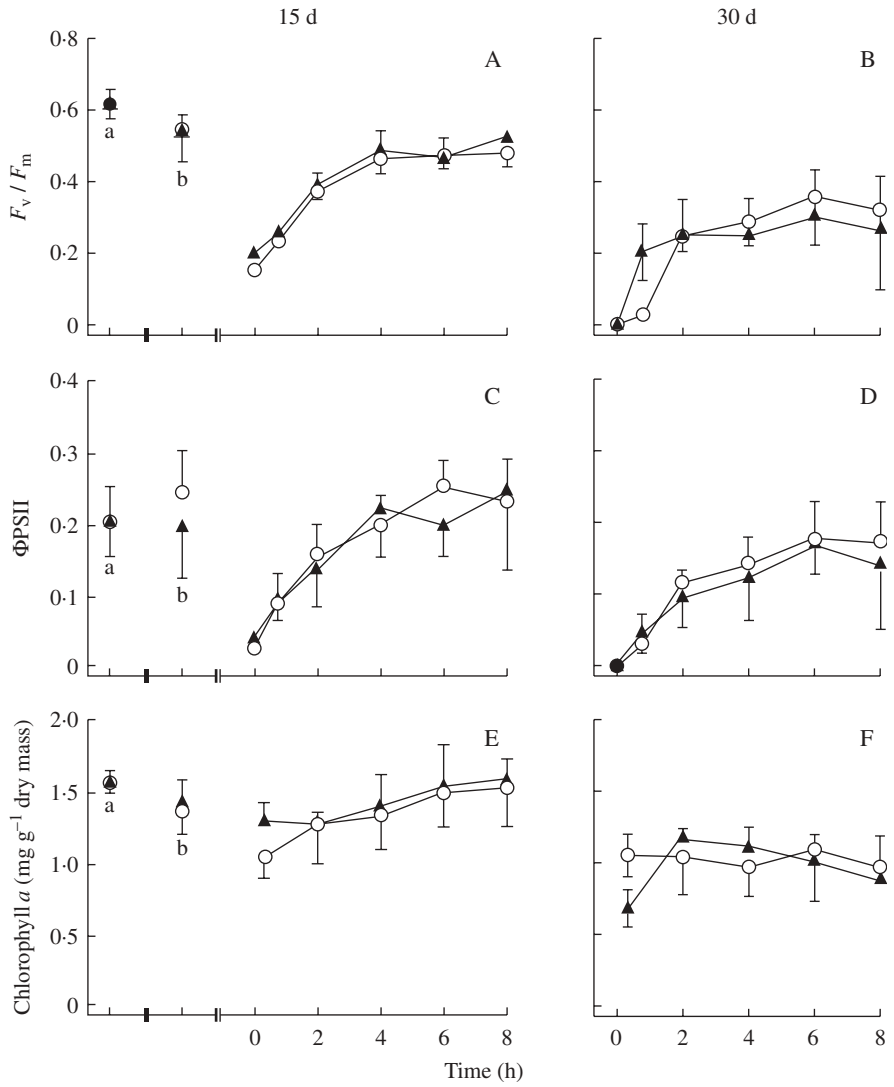

FIG. 2. The effect of ABA on the maximum quantum efficiency (F_v/F_m; A and B), the actual quantum efficiency (ΦPSII; C and D) of PSII, and chl*a* concentration (E and F) in *Peltigera polydactylon* before and after desiccation for 15 and 30 d followed by rehydration. a, After collection from field; b, following 3-d pretreatment. Time 0 indicates the start of rehydration. Two-way analysis of variance showed that in all graphs the effects of ABA were not significant. Open circles, control material; closed triangles, ABA-pretreated material; error bars denote the standard deviation, $n = 5$.

increase tolerance. Only very limited information is available on the role of ABA in lichens, but Dietz and Hartung (1998) showed that ABA occurs in both the mycobiont and the photobiont of a range of lichens, including cyanobacterial species. Dietz and Hartung (1999) later used chlorophyll fluorescence to show that ABA pretreatment does not increase the desiccation tolerance of PSII in *Xanthoria parietina*, *Hypogymnia physodes* or *Peltigera praetextata*. While the present results on PSII activity agree with those of Dietz and Hartung (1999) (Fig. 2C and D), they also show that ABA can protect net photosynthesis (Fig. 1). Apart from measuring different parameters, Dietz and Hartung (1999) desiccated their lichens for 3 months after ABA pretreatment. The present results show that the beneficial effects of ABA pretreatment become less with time, decreasing considerably from 15 to 30 d of desiccation (Fig. 1). The difference in the duration of desiccation may explain why Dietz and Hartung found no beneficial effect of ABA pretreatment, while in the present study ABA hardened lichens to desiccation.

Compared with the little information available on ABA in lichens, more is known about its occurrence and function in free-living cyanobacteria and fungi. In cyanobacteria, ABA application can affect heterocyst frequency, Ca^{2+} transport and nitrogenase activity (Huddart *et al*., 1986; Marsálek and Simek, 1992; Pandey *et al*., 1996). Close and Lammers (1993) showed that while ABA is present in the cyanobacterium *Anabaena*, dehydration does not promote its synthesis, and exogenous ABA applications do not stimulate the synthesis of dehydrin proteins. Dehydrin proteins are often believed to be involved in desiccation tolerance in seeds and poikilohydric angiosperms (Black and

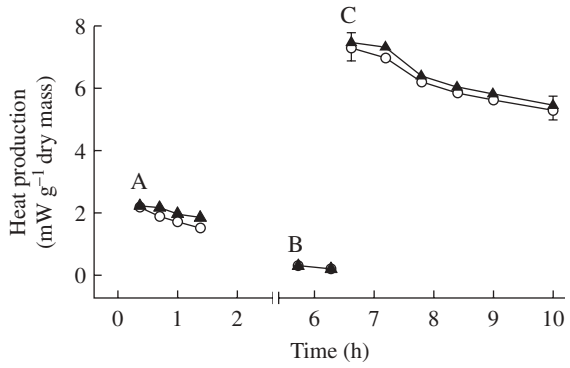

FIG. 3. The effect of ABA pretreatment on heat production by *Peltigera polydactylon* before desiccation, while desiccated and during rehydration. (A) Following 3-d pretreatment with ABA or distilled water (control); (B) after desiccation for 3 h to a water content of approx. 0·1; (C) after rehydration. Open circles, control material; closed triangles, ABA-pretreated material. Points represent selected fitted values with 95 % confidence limits, calculated using the 'Spline' program of Hunt and Parsons (1974), $n = 3$ (control) or 4 (ABA-pretreated).

Pritchard, 2002). However, it would be unwise to conclude that ABA has no role in desiccation tolerance in cyanobacteria based on the single report of Close and Lammers (1993) and, moreover, the photobiont of *P. polydactylon* is *Nostoc* rather than *Anabaena*. Some fungi contain ABA (Dörffling *et al*., 1984), although it appears to be synthesized by a different pathway to higher plants (Hirai *et al*., 2000). The widespread occurrence of ABA in free-living cyanobacteria and fungi is consistent with the present finding that this hormone may have an important role in desiccation tolerance in *Peltigera*.

Desiccation tolerance of the photobiont rather than the mycobiont is increased

In *P. polydactylon*, partial dehydration and ABA treatment probably improve the desiccation tolerance of the photobiont more than that of the mycobiont. While these pretreatments significantly increased the recovery of net photosynthesis during rehydration following desiccation, they had little significant effect on respiration and heat production, parameters that mostly indicate fungal metabolism (Figs 1E and F and 3). By volume, the fungus comprises 90 % of a lichen thallus (Collins and Farrar, 1978), and it seems likely that respiration and heat production largely originate from the mycobiont. The small effect of partial dehydration and ABA pretreatments on respiration also indicates that the improved recovery of net photosynthesis during rehydration is genuine, and cannot be explained simply by a reduction in the respiratory burst. However, recovery of respiration and heat production following desiccation are probably rather insensitive measures of fungal tolerance. In future studies more sensitive assays for fungal metabolism, e.g. rates of eukaryotic protein synthesis, will be used to test whether hardening can increase the desiccation tolerance of the mycobiont.

The mechanisms involved in the improved rates of recovery of net photosynthesis during rehydration following desiccation in the lichens receiving partial dehydration or ABA pretreatments remain unclear. These treatments do not appear to preserve electron transport (Fig. 2A–D) or promote chlorophyll retention during desiccation (Fig. 2E and F). The moderate chlorophyll losses that occur during desiccation were probably the result of photo-oxidation of chlorophyll, as lichens were desiccated at a PPFD of 75 μmol photons m^{-2} s^{-1} (Proctor and Tuba, 2002). It would appear unlikely that hardening treatments could influence the rate of chlorophyll photooxidation. Kranner *et al*. (2003) also reported some chlorophyll loss in *Peltigera* during desiccation for several weeks, followed by recovery within hours during rehydation. The ability of *Peltigera* to relatively rapidly re-synthesize chlorophyll merits further investigation. As discussed in the Introduction, differences in the desiccation tolerance of lichen species from contrasting habitats have been suggested to be related to an ability to maintain effective concentrations of enzymic and non-enzymic antioxidants during stress (Mayaba and Beckett, 2001; Kranner, 2002; Kranner *et al*., 2003). In future work, it is the intention to test if hardening treatments can maintain the efficiency of antioxidant systems in *Peltigera* during desiccation and rehdyration cycles.

Significance of the burst of heat production during rehydration

Rehydration following desiccation induced a large, sustained increase in heat production in *Peltigera* (Fig. 3). During the first few minutes of rehydration, some of the heat produced will be generated by the dissolving of intracellular ions and rehydration of proteins. This heat is not reflected in Fig. 3 because, to rehydrate the lichens, the sample chambers from the microcalorimeter had to be temporarily removed, and measurements could not be resumed for 15 min. However, such physico-chemical heat production will only be short lived, and cannot explain the continued high rate of heat production observed.

Production of heat following stress in fungi and other organisms is generally considered to result from the controlled uncoupling of respiration in mitochondria. Uncoupling can occur, firstly, via the alternative oxidase that dissipates the redox potential, and, secondly, via uncoupling proteins that dissipate the proton motive force (for reviews, see Jarmuszkiewcz, 2001; Hourton-Cabassa *et al*., 2004). While stress disrupts normal mitochondrial function and increases superoxide production (Møller, 2001), uncoupling electron flow from phosphorylation protects cells by reducing the formation of harmful ROS (Skulachev, 1998). In lichens, possession of efficient alternative oxidase and protein uncoupling systems may be an essential component of desiccation tolerance, because they will reduce mitochondrial superoxide synthesis during rehydration following desiccation. Surprisingly, the data presented here appear to be the first to describe heat production by lichens. While ABA pretreatment does not affect heat production (Fig. 3), microcalorimetry may be a useful tool for future studies on desiccation tolerance.

Conclusions

Although constitutive desiccation tolerance mechanisms are probably essential in lichens growing habitats in which they experience sudden and severe stress, these mechanisms are present even when not needed, and at these times divert energy away from growth and reproduction. In less harsh habitats, in which desiccation is preceded by a period of more moderate stress, selection may have favoured inducible tolerance mechanisms. In *P. polydactylon*, the physiology of hardened and non-hardened lichens can readily be compared. When studying desiccation tolerance, this may be a more useful approach than comparing the physiology of dissimilar species, differing in tolerance, because it can be difficult to know which metabolic components contribute to tolerance. Future work needs to focus on precisely how partial dehydration and ABA pretreatments protect net photosynthesis from desiccation. Improved net photosynthesis during rehydration will directly benefit the photobiont, but because the mycobiont depends upon the photobiont for a supply of carbohydrates, hardening will benefit the whole lichen thallus.

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