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Photoinhibition in the Antarctic moss *Grimmia antarctici* Card. when exposed to cycles of freezing and thawing

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ABSTRACT

Freezing and thawing of the endemic moss species *Grimmia antarctici* Card. caused photoinhibition. When snow cover was removed from moss in the field, resulting in exposure to fluctuating temperatures and light conditions, photoinhibition, measured as a reduction in the ratio of variable to maximum chlorophyll *a* fluorescence (F_v/F_m), was observed. The extent of photoinhibition was highly variable and appeared to be reversible during periods of warmer temperatures. A series of controlled laboratory studies found that the light conditions that prevail between freezing and thawing events influenced the recovery from photoinhibition observed during freezing and thawing, with low light conditions facilitating the greatest rates of recovery. After four cycles of freezing and thawing, recovery from photoinhibition in hydrated moss was achieved within 12 h of transfer to 5 °C and 15 μmol quanta m^{-2} s^{-1}. These results favour the hypothesis that photoinhibition observed during freezing represents a protective process involving the down-regulation of photosystem II when photosynthetic carbon assimilation is limited by low temperatures.

Key-words: Antarctic moss; *Grimmia antarctici* Card.; photoinhibition; freezing and thawing.

INTRODUCTION

One of the possible consequences of global climate change is an increase in climatic variability and the frequency of extreme climatic events associated with the El niño southern oscillation (Tegart, Sheldon & Hellyer 1992). Variations in the frequency of the El niño southern oscillation may influence the variability of weather patterns in Antarctica, which in turn may affect the organisms living there. A potential effect of increased variability in the climate of Antarctica is changes in the frequency of freezing and thawing events. As freezing and thawing have been observed to be damaging to plants (Larcher & Bauer 1981), increases in the frequency of freezing and thawing events may have important consequences for the mosses growing in the coastal regions of Antarctica. These mosses are generally covered in snow in winter, and become exposed to fluctuating air temperatures, and hence freezing and thawing stress, during the short summer growing season when snow melts and solar radiation levels are high.

Freezing and thawing have been observed to decrease photosynthetic rates (Rumich-Bayer & Krause 1986; Rumich-Bayer, Giersch & Krause 1987; Davey 1989; Hällgren, Lundmark & Strand 1990; Kennedy 1993). When freezing and thawing occur in the light, photoinhibition or reductions in the photosynthetic light-use efficiency in plants has also been observed (Hällgren *et al.* 1990; Ottander & Öquist 1991). Thus, populations of Antarctic moss may be susceptible to photoinhibition and reductions in photosynthesis during frequent freezing and thawing over the summer months.

This study aimed firstly to assess the past frequency of freeze–thaw events in coastal Antarctica during the summer months and then determine whether frequency of freeze–thaw events is correlated with the El niño southern oscillation. Secondly, this study aimed to investigate the influence of freeze–thaw events on the photosyntheic apparatus of the Antarctic endemic moss, *Grimmia antarctici* Card. As photoinhibition has been proposed to be a process that could potentially limit the productivity of *Grimmia antarctici* during the summer growing season (Post, Adamson & Adamson 1990), and as it has been shown to be a process that can reduce growth in other moss species (Murray, Tenhunen & Nowak 1993), this study focused on whether the exposure of moss to light during freezing and thawing caused photoinhibition. The extent of recovery from photoinhibition after freezing and thawing was also assessed. In addition, the hydration state of plant tissue exposed to freezing and thawing has also been shown to modify changes in photosynthesis caused by freezing and thawing (Davey 1989; Kennedy 1993). Thus, this study also investigated the influence of desiccation on the extent of photoinhibition during freezing and thawing.

MATERIALS AND METHODS

Analysis of climatic records

In order to test the correlation between frequency of freeze–thaw events at the Australian Antarctic station

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Casey in Wilkes Land, continental Antarctica (66°17'S 110°32'E), with changes in the frequency of the El niño southern oscillation, the number of freeze–thaw events was determined from climatic records and compared with southern oscillation index values (Nicholls 1991). The southern oscillation index is an index of the atmospheric pressure difference between Tahiti and Darwin. As –7 °C is the average freezing point of moss tissue sampled from the study area (Melick & Seppelt 1992), freeze–thaw cycles were identified as days when temperatures decreased below –7 °C and then subsequently increased. Climatic data for Casey were obtained for the years 1969–1992 from the Australian Bureau of Meteorology.

Assessing the influence of freezing and thawing

The experiments were conducted at the Australian Antarctic station Casey. The field study was conducted on colonies of *Grimmia antarctica* surrounding a melt lake close to the station. Moss used in the laboratory studies was collected from the same location.

Field study

In order to assess the effects of freeze–thaw stress in the field, snow that was still covering moss early in the summer season was removed and plant responses were assessed using chlorophyll fluorescence. Removing the snow covering exposes the plants to fluctuations in temperature and to high levels of solar radiation and low humidities (Salisbury 1984). At one site snow cover was replaced immediately after initial chlorophyll fluorescence measurements were made, while at the other sites snow was replaced after a number of days of exposure in order to monitor recovery after exposure. Chlorophyll fluorescence was measured at 1300 h local time on the day of exposure and on subsequent days. Climatic data during the experiment were obtained from the meteorological observatory at Casey. Daily photon flux densities were recorded using quantum sensors attached to an electronic data collecting device (Li-1000, Li-Cor, Lincoln, NE, USA).

Simulating freeze–thaw cycles

Samples of moss were exposed to freeze–thaw cycles by placing cylindrical cores of moss approximately 1 cm in diameter in a metal holder drilled with holes the same size as the moss cores. The metal holder was placed in a temperature-controlled water bath filled with a 60% ethylene glycol solution in a room maintained at 15 °C. The temperature of the bath was manipulated such that the moss temperature at the beginning of the experiments was 5 °C and was reduced to –12 °C over the next 6 h, then increased to 5 °C over the next 6 h. Thus, the rate of freezing and thawing was approximately 3 °C h^{-1}. This rate of temperature change was chosen as it approximated the rate of decline in air temperatures during a freeze–thaw event in the field and was similar to rates of change of temperature previously measured at other Antarctic locations (Seppelt & Ashton 1978).

Fully hydrated moss (100% relative water content) was frozen either in darkness or at a photon flux density of 350 μmol quanta m^{-2} s^{-1}. Light was provided using a slide projector suspended above the water bath. The photon flux density was attenuated to 350 μmol quanta m^{-2} s^{-1} using neutral density filters (Baltzers, Liechtenstein). After the 12 h freeze–thaw cycle, mosses were exposed to either 15 μmol quanta m^{-2} s^{-1} or 350 μmol quanta m^{-2} s^{-1} at 5 °C for the next 12 h before the next freeze–thaw cycle was commenced. Four freeze–thaw events were imposed. At the completion of the 12 h period after the fourth and final freeze–thaw event, moss from all treatments was placed under photon flux densities at 15 μmol quanta m^{-2} s^{-1}, 5 °C and 100% relative humidity for 7 d. Chlorophyll fluorescence was measured after 12 h and 7 d.

To assess the influence of tissue desiccation on response to freeze–thaw stress, moss that dried to a relative water content of 8% over 24 h in the laboratory (20 μmol quanta m^{-2} s^{-1}, 20 °C and 30% relative humidity) was exposed to 350 μmol quanta m^{-2} s^{-1} during freezing and intervening recovery periods. Chlorophyll fluorescence of the moss frozen at 8% relative water content was then compared with that of moss frozen at 100% relative water content. As the fluorescence signal of desiccated samples is very low (beyond the sensitivity of the PAM-2000), it was necessary to rehydrate desiccated samples before measurements to assess the influence of freeze–thaw stress on desiccated moss. Incubation in darkness is known to result in very small changes in chlorophyll fluorescence (Le Gouallec, Cornic & Briantais 1991). Thus, measurements of chlorophyll fluorescence after each freeze–thaw cycle were made on four samples removed from the experiment, rehydrated by spraying with water and left in the dark at 5 °C and 100% relative humidity for 24 h. A 24 h rehydration period was chosen on the basis of preliminary tests of the time required to regain predehydration F_v/F_m values.

Chlorophyll concentrations of moss before and after the four freeze–thaw cycles were determined spectrophotometrically using the methods of Porra, Thompson & Kriedmann (1989).

Chlorophyll fluorescence measurements

Photosynthetic efficiency was assessed using chlorophyll *a* fluorescence measured with a PAM-2000 chlorophyll fluorescence-measuring device controlled by DA-2000 software supplied with the device (H.Walz, Effeltrich, Germany) with the following settings: measuring light intensity, 7; damping, 5; gain, 4; saturating pulse intensity, 7; duration of saturating pulse, 0·8 s. These settings ensured an initial fluorescence (F_o) level of approximately 0·4 and a maximum fluorescence after a saturating pulse of light (F_m) of approximately 2 mv in healthy moss tissue after 10 min in darkness. The appropriateness of the saturating pulse intensity was assessed in line with the manufacturer's recommendations using the alt-M command of

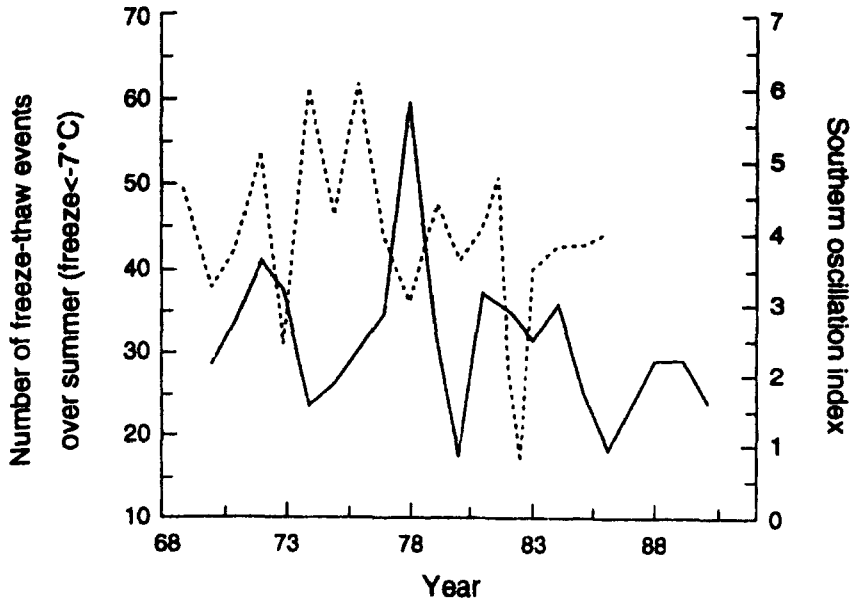

Figure 1. Variations in the number of freeze–thaw events at Casey, Wilkes Land, continental Antarctica occurring over summer (unbroken line) and the southern oscillation index averaged for the summer months (dashed line) since 1969.

the DA-2000 software. The ratio of variable to maximum fluorescence, F_v/F_m, where $F_v = F_m - F_o$ (Krause & Weis 1991) was always measured after moss had been in darkness for 10 min.

RESULTS

Frequency of freeze–thaw events and climate change

The number of freeze–thaw events over the summer at Casey varied widely from year to year but generally increased in years when the southern oscillation index declined (Fig. 1). This trend is most noticable before 1980. When the southern oscillation index declines (e.g. 1973, 1978 and 1983) widespread drought occurs throughout countries of the southern hemisphere. During these periods the frequency of freeze–thaw events at Casey appears to increase. In years when the southern oscillation index is higher (e.g. 1974 and 1976) there are lower numbers of freeze–thaw events in Antarctica. These results may indicate that the frequency of freeze–thaw events in Antarctica is sensitive to fluctuations in global climate.

Effects of cycles of freezing and thawing on moss

Field experiments showed that when snow cover was removed F_v/F_m was reduced, indicating that moss became photoinhibited (Fig. 2, compare control site with other sites). Over the course of the experiment it was evident that the extent of photoinhibition measured at 1300 h was correlated with both the night time minimum air temperature and the air temperature at the time of measuring F_v/F_m, but not with the photon flux density. After the initial removal of snow cover, the night time temperatures were below −7 °C. Although there was extreme photoinhibition on 18 December (site 3 had an F_v/F_m of 0·03) a strong recovery in F_v/F_m at all sites sampled was observed on the 19 December when the daytime air temperature was approximately 0 °C. After re-covering exposed moss with snow, F_v/F_m increased at a greater rate than at other sites that remained uncovered by snow (particularly in sites 1 and 2).

Variations in F_v/F_m between sites on any one day are likely to be due to differences in the microtopography between sites, which influence photon flux density absorbed by moss and moss temperature. In order to distinguish between the influence of temperature and light on photoinhibition of moss, cycles of freezing and thawing in the light and in darkness were simulated in the laboratory.

Influence of light during freezing and thawing

Freezing and thawing in the light causes larger reductions in F_v/F_m after each freeze–thaw cycle than freezing and thawing in darkness (Figs 3 & 4). Freezing in darkness resulted in a reduction of F_v/F_m from 0·77 to 0·73 (approx-

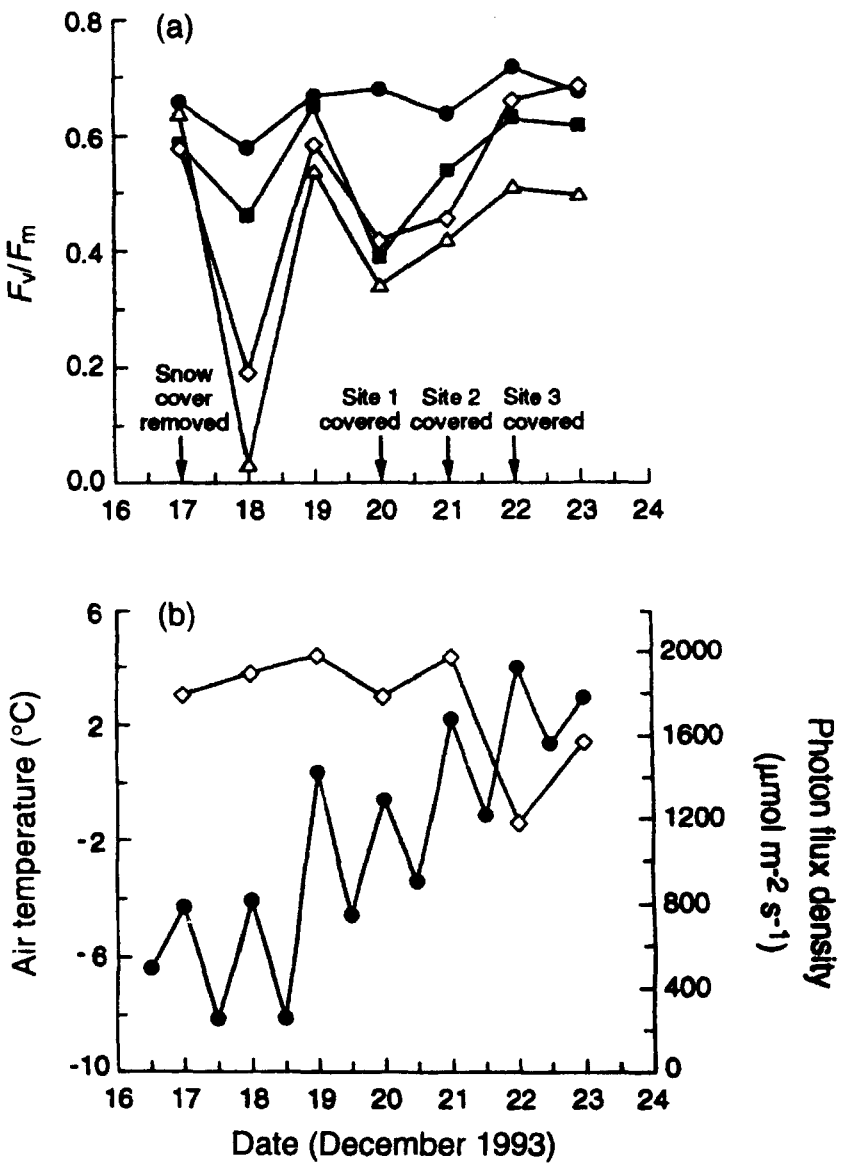

Figure 2. Changes in F_v/F_m in moss once snow had been removed at various sites around a snow drift (a). At the control site (●) snow was removed but replaced immediately. At site 1 (■) snow was replaced 3 d after removal, site 2 (◇) 4 d after removal and site 3 (▲) 5 d after initial snow removal. Variation in air temperatures (●) and photon flux densities (◇) over the 7 d in December 1993 in which the experiment was conducted (b). Measurements of F_v/F_m and photon flux densities were taken at 1300 h local time. Air temperature observations were made at 100 h and 1300 h local time and were provided by the Australian Bureau of Meteorology. F_v/F_m values represent the mean of five measurements at each site.

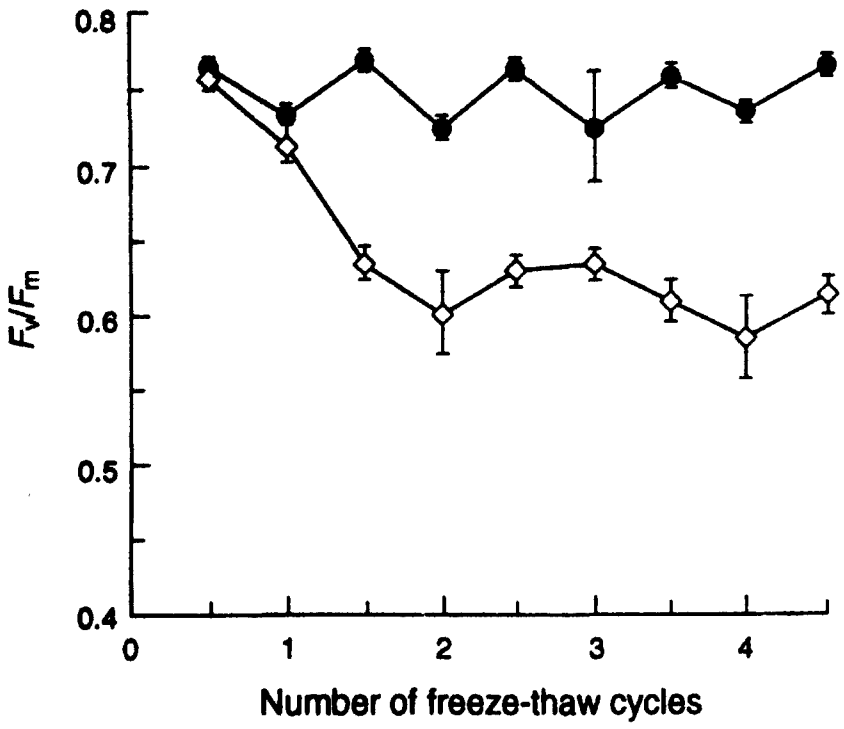

Figure 3. Changes in F_v/F_m in moss frozen in darkness during four freeze–thaw cycles. In the 12 h period between freezing and thawing moss were kept at either 15 μmol quanta m^{-2} s^{-1} (closed symbols) or 350 μmol quanta m^{-2} s^{-1} (open symbols). Values are the means of five samples. Error bars are the standard errors about the means.

imately 5%), while freezing in the light generally reduced the F_v/F_m to approximately 0·5 (30% reduction in F_v/F_m) with each freeze–thaw event (Fig. 4). Reductions in F_v/F_m over the course of the experiment were also dependent on the conditions moss experienced between freeze–thaw cycles (compare Figs 3 & 4). Exposure of moss to 350 μmol quanta m^{-2} s^{-1} between freezing and thawing resulted in a 15% reduction in F_v/F_m that was sustained over the course of the experiment (Figs 3 & 4).

F_o declined in all mosses, regardless of the light regime to which they were exposed, over the course of the four freeze–thaw cycles. Moss frozen in the dark, with periods between freeze–thaw cycles under low light conditions, showed an approximately linear decline in F_o over time (Fig. 5). Exposure to high light during the period between freezing and thawing for samples frozen in darkness accelerated the rate of F_o decline. However, the F_o values after four cycles of freezing and thawing were similar in moss frozen in the dark regardless of the light regimes that were imposed between freezing and thawing.

Moss frozen in the light also showed a general declined in F_o over the four freezing and thawing cycles (Fig 6). F_o declined to approximately 60% of its initial value after the first freeze–thaw cycle. However, F_o recovered to close to its initial value during the period between freezing and thawing. F_o of moss exposed to 350 μmol quanta m^{-2} s^{-1} during the period between freezing and thawing did not recover to the same extent as moss exposed to 15 μmol quanta m^{-2} s^{-1} between freezes. After the final 12 h period at 5 °C at either 350 or 15 μmol quanta m^{-2} s^{-1}, the change in F_o for moss frozen in the light was less than for moss frozen in darkness (compare Figs 5 & 6).

As the F_o signal is thought to arise from the light harvesting pigment protein complexes of photosystem II (PSII) (Krause & Weis 1991), total chlorophyll concentrations and chlorophyll *a* to *b* ratios were assessed to determine whether reductions in F_o could be attributed to reduced chlorophyll concentrations. Total chlorophyll concentrations and chlorophyll *a* to *b* ratios (Table 1) did not change over the four freeze–thaw events.

Recovery from freezing and thawing

After four cycles of freezing and thawing followed by 12 h of exposure to 15 μmol quanta m^{-2} s^{-1} at 5 °C, F_v/F_m of moss frozen in darkness with low light between freezing and thawing had recovered to levels similar to non-frozen controls, while those exposed to 350 μmol quanta m^{-2} s^{-1} either during freezing and thawing or between freezing and thawing had F_v/F_m values that were ≥ 90% of those of non-frozen controls (Table 2). In contrast to the recovery observed in F_v/F_m, the reductions in F_o caused by freezing and thawing were largely irreversible even after 7 d (Table 3). Moss frozen in the dark and exposed to low light conditions between freeze–thaw cycles had lower F_o values (50% of initial values) than any other treatment. The recovery of photosynthetic light use efficiency (F_v/F_m) within 12 h and the sustained reduction in F_o indicate that the variable fluorescence, F_v, was reduced in all treatments after freezing and thawing and did not recover within 12 h or 7 d.

Influence of desiccation during freezing and thawing

The fluorescence signal of desiccated samples is very low (beyond the sensitivity of the PAM-2000). Therefore

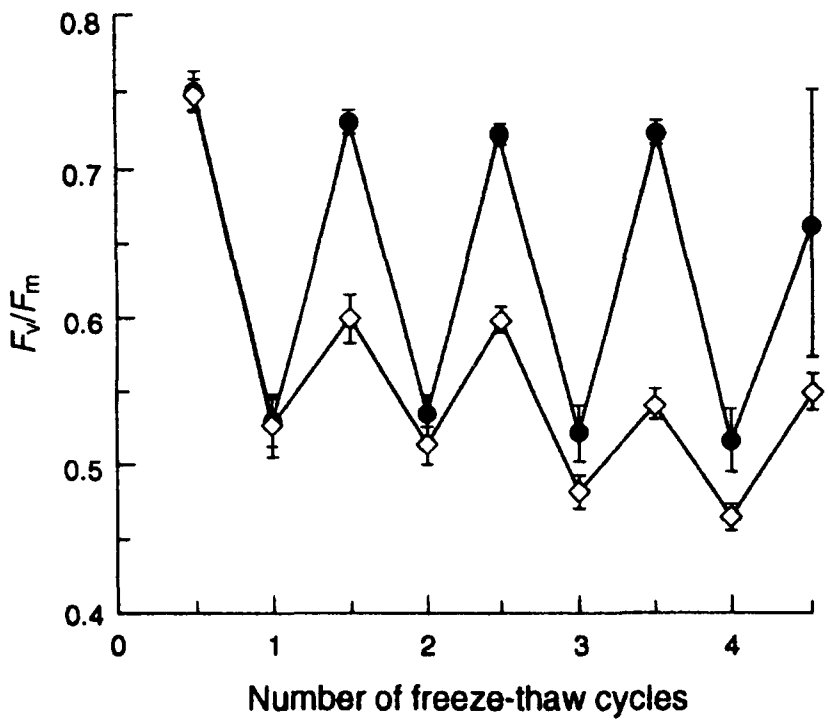

Figure 4. Changes in F_v/F_m in moss frozen at 350 μmol quanta m^{-2} s^{-1} during four freeze–thaw cycles. In the 12 h period between freezing and thawing moss were either kept at 15 μmol quanta m^{-2} s^{-1} (closed symbols) or 350 μmol quanta m^{-2} s^{-1} (open symbols). Values are the means of five samples. Error bars are the standard errors about the means.

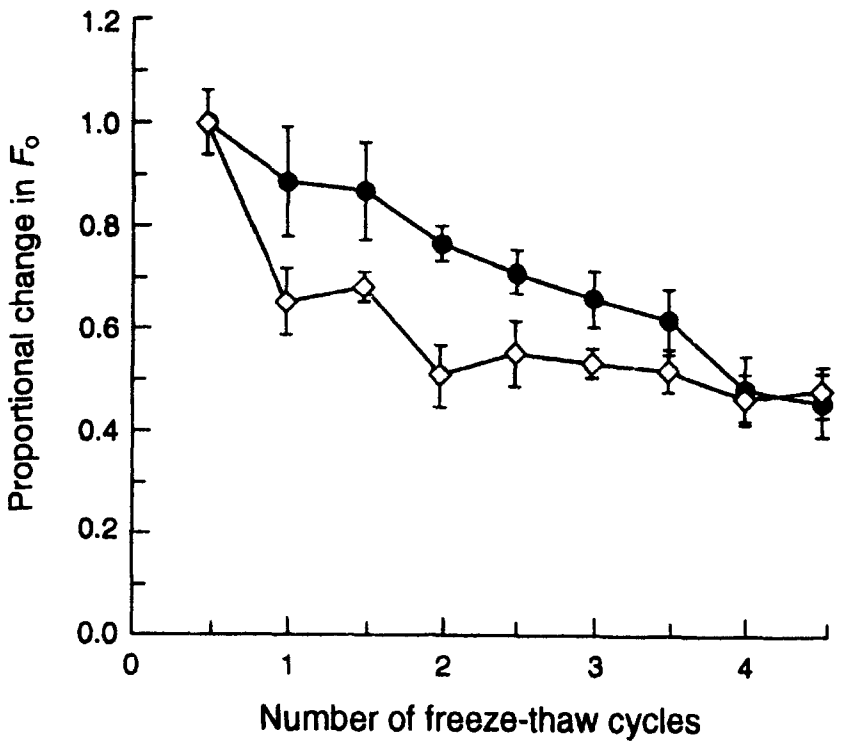

Figure 5. Proportional changes in F_o of moss frozen in darkness during four freeze–thaw cycles. In the 12 h period between freezing and thawing mosses were kept at either 15 μmol quanta m^{-2} s^{-1} (closed symbols) or 350 μmol quanta m^{-2} s^{-1} (open symbols). Values are the means of five samples. Error bars are the standard errors about the means.

desiccated samples were rehydrated before measurements to assess the influence of freeze–thaw stress. Desiccated moss exposed to four cycles of freezing and thawing and then rehydrated in the dark for 24 h before chlorophyll fluorescence measurements exhibited very little change in F_v/F_m compared to similarly treated moss that was fully hydrated (Fig. 7). In addition, F_o values of desiccated moss were similar to the initial values.

DISCUSSION

The El niño southern oscillation is a global climate fluctuation that occurs approximately every 3–5 years (Bigg 1990). The southern oscillation causes a reduced pressure gradient across the Pacific Ocean, resulting in drought in many continents and an increase in the frequency of hurricanes in the Pacific (Bigg 1990). The analysis presented here shows that the frequency of freeze–thaw cycles in Antarctica seems to move out of phase with the southern oscillation index (Fig. 1). Thus, in El niño years when the southern oscillation index is low, the frequency of freeze–thaw events in Antarctica would be expected to increase. The relationship between the southern oscillation index and the frequency of freeze–thaw events indicates that anthropogenic-induced changes in climate that alter the frequency or intensity of the El niño southern oscillation may influence the frequency of freeze–thaw events in the Antarctic.

In the field study described here, removal of snow, which protects moss from fluctuations in light intensities and temperature (Salisbury 1984), resulted in photoinhibition (Fig. 2). In addition, controlled laboratory studies showed that, while F_v/F_m was reduced by 5% after freezing and thawing in the dark, and by 15% when exposed to 350 μmol quanta m^{-2} s^{-1} for 12 h at 5 °C between freezing and thawing, it was reduced by 30% when moss was exposed to the same light level during freezing and thawing. Thus, light during freezing and thawing increases

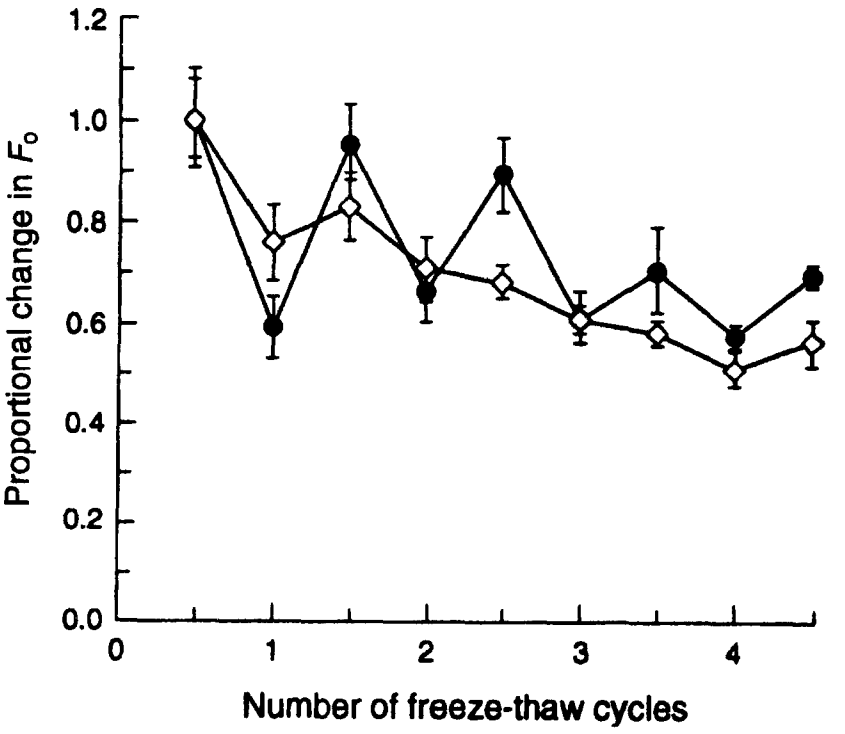

Figure 6. Proportional changes in F_o of moss frozen at 350 μmol quanta m^{-2} s^{-1} during four freeze–thaw cycles. In the 12 h period between freezing and thawing mosses were kept at either 15 μmol quanta m^{-2} s^{-1} (closed symbols) or 350 μmol quanta m^{-2} s^{-1} (open symbols). Values are the means of five samples. Error bars are the standard errors about the means.

Table 1. Chlorophyll concentrations in mmol g^{-1} dry weight of moss and chlorophyll *a/b* ratios before and after four cycles of freezing and thawing. Moss was frozen in darkness (D freeze) or in the light (L freeze) with the intervening 12 h between freezing and thawing at 350 μmol quanta m^{-2} s^{-1} (HL recov.) or 15 μmol quanta m^{-2} s^{-1} (LL recov.). Values are means of five samples ± standard errors

	D freeze, LL recov.		D freeze, HL recov.		L freeze, LL recov.		L freeze, HL recov.	
	Chl conc.	Chl *a/b*	Chl conc.	Chl *a/b*	Chl conc.	Chl *a/b*	Chl conc.	Chl *a/b*
Before four cycles of freezing and thawing	2·42 ± 0·40	2·39 ± 0·09	4·28 ± 0·18	2·41 ± 0·03	3·91 ± 0·67	2·35 ± 0·05		
After four cycles of freezing and thawing	2·11 ± 0·24	2·40 ± 0·02	4·31 ± 0·47	2·48 ± 0·04	4·05 ± 0·25	2·31 ± 0·05	4·60 ± 0·42	2·35 ± 0·03

Table 2. Long term recovery of F_v/F_m after four freeze–thaw events in moss frozen in darkness (D freeze) or in the light (L freeze) with the intervening 12 h between freezing and thawing at 350 μmol quanta m^{-2} s^{-1} (HL recov.) or 15 μmol quanta m^{-2} s^{-1} (LL recov.). Values are the means of five samples ± standard error

	Control (no freeze)	D freeze, LL recov.	D freeze, HL recov.	L freeze, LL recov.	L freeze, HL recov.
Recovery in 12 h	0·777 ± 0·007	0·759 ± 0·005	0·612 ± 0·011	0·660 ± 0·088	0·548 ± 0·012
Recovery after 12 h low light	0·778 ± 0·004	0·764 ± 0·005	0·736 ± 0·004	0·743 ± 0·004	0·718 ± 0·004
Recovery after 7 d low light	0·784 ± 0·003	0·774 ± 0·005	0·770 ± 0·003	0·768 ± 0·001	0·768 ± 0·003

Table 3. Long term recovery of F_o expressed as a proportion of the intial F_o after four freeze–thaw events in moss frozen in darkness (D freeze) or in the light (L freeze) with the intervening 12 h between freezing and thawing at 350 μmol quanta m^{-2} s^{-1} (HL recov.) or 15 μmol quanta m^{-2} s^{-1} (LL recov.). Values are the means of five samples ± standard error.

	Control (no freeze)	D freeze LL recov.	D freeze HL recov.	L freeze LL recov.	L freeze, HL recov.
Recovery in 12 h	0·858 ± 0·089	0·450 ± 0·059	0·475 ± 0·048	0·694 ± 0·014	0·709 ± 0·047
Recovery after 12 h low light	0·892 ± 0·078	0·472 ± 0·102	0·558 ± 0·063	0·623 ± 0·052	0·637 ± 0·019
Recovery after 7 d low light	1·014 ± 0·057	0·503 ± 0·029	0·647 ± 0·021	0·644 ± 0·05	0·658 ± 0·014

depressions in photosynthetic light-use efficiency in moss. Light in conjunction with freezing temperatures has also been shown to cause photoinhibition in pine (Hällgren *et al.* 1990; Ottander & Öquist 1991).

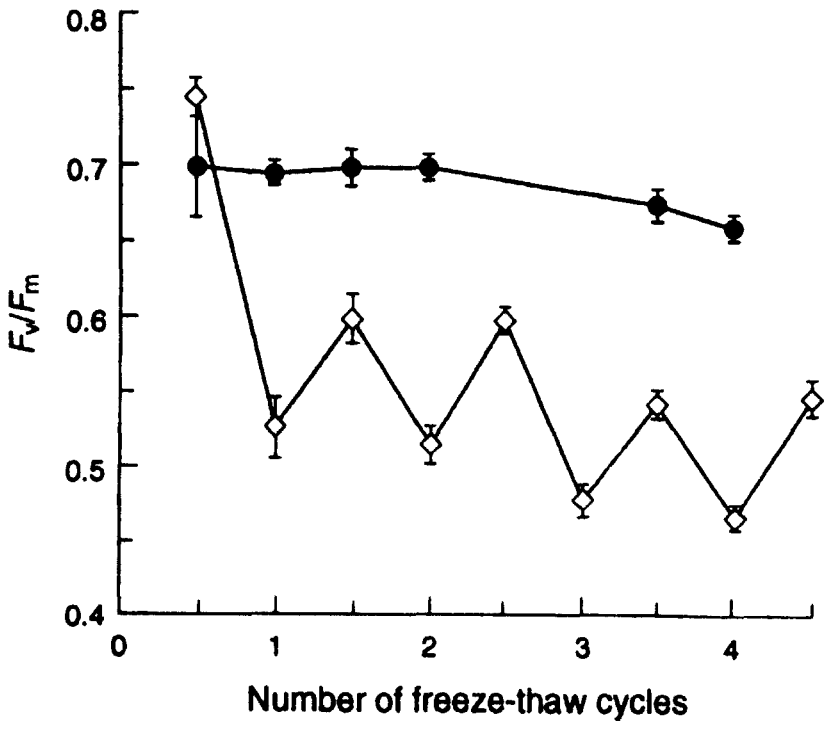

Figure 7. Changes in F_v/F_m in moss frozen at 350 μmol quanta m^{-2} s^{-1} for four freeze–thaw cycles. Moss was either at 100% relative water content (open symbols) or 8% relative water content (closed symbols). Samples frozen and thawed at 8% relative water content were rehydrated before measurements of F_v/F_m were taken. In the 12 h period between freezing and thawing moss were kept at 350 μmol quanta m^{-2} s^{-1}. Values are the means of 5 samples for moss at 100% relative water content and three of four samples for those frozen at 8% relative water content. Error bars are the standard errors about the means.

Despite extreme photoinhibition in the field experiment the day after snow removal (the 18 December), there was substantial recovery from photoinhibition on the next, warmer, day. Similarly, reductions in F_v/F_m in the laboratory experiments were reversible, although the rate and level of recovery achieved was dependent on the photon flux density to which they were exposed. Similar abilities to recover F_v/F_m after freezing and thawing have been found by Rütten & Santarius (1992) in the moss *Plagiomnium*, by Aro & Karunen (1988) in the moss *Ceratodon purpureus* and by Ottander & Öquist (1991) in Scots pine. Furthermore Rütten & Santarius (1992) and Ottander & Öquist (1991) also found that recovery of F_v/F_m was highly correlated with recovery of photosynthetic oxygen evolution, indicating that there was little damage to the photosynthetic apparatus. Furthermore, recovery from photoinhibition at cold temperatures in cold acclimated cereals has been shown to be only partially dependent on protein synthesis (Hurry & Huner 1992).

It has been proposed that in cold acclimated plants photoinhibition may not represent damage to the photosynthetic apparatus but possibly reflects a mechanism to control the flow of energy through PSII, leading to protection of PSII from photooxidative damage when photosynthetic carbon assimilation is limited by cold temperatures (Hurry & Huner 1992; Huner *et al.* 1993; Öquist, Hurry & Huner 1993). The recovery from photoinhibition observed in this study gives further support to the idea that photoinhibition represents a down-regulation of PSII centres that protects PSII when photosynthetic carbon metabolism is limited by cold temperatures, rather than representing irreversible damage to PSII.

The tolerance of freeze–thaw events exhibited by *Grimmia* in the experiments described here contrasts with

he experiments of Kennedy (1993). Kennedy (1993) found that freezing and thawing the Antarctic moss *Polytrichum alpestre* resulted in large reductions in carbon assimilation that were particularly severe after the first freezing and thawing event in a cycle of five freeze–thaw events. The influence of freezing and thawing on photosynthesis is dependent on the rate of freezing, the time spent at the minimum temperature and the minimum temperature to which tissue is frozen (Burke *et al.* 1976). In Kennedy's experiments, moss was frozen at 0·8 °C min^{-1}, which is a faster rate than used in these experiments (3 °C h^{-1}) and may have resulted in the irreversible decreases in photosynthetic rates after freezing and thawing observed in his study (Kennedy 1993).

In contrast to the recovery of F_v/F_m (Table 2) after mosses were exposed to cycles of freezing and thawing, F_o, and therefore also F_v, underwent a sustained reduction over the four freezing and thawing cycles that was not reversible within 7 d of the last freeze–thaw event (Table 3). Reductions in F_o and F_v were most severe in those samples frozen in darkness. Rütten & Santarius (1992) also observed that F_o was reduced when *Plagiomnium* moss species were frozen and thawed. The reduction in F_o in their experiments was attributed to loss of chlorophyll from the thylakoid membranes. Chlorophyll loss after freezing and thawing was not observed in these experiments (Table 1). The chlorophyll *a/b* ratio was similar to that observed in other moss species (Rincon 1993), and was not altered in response to freezing and thawing, indicating little change in the degree of thylakoid membrane stacking (Anderson 1986). Fluoresence arises mainly from the light harvesting complexes of photosystem II (LHCII) (Krause & Weis 1991). Therefore the sustained reductions in fluorescence may represent changes in the arrangement of LHCIIs within the thylakoid membrane such that the number of electrons reaching PSII, as indicated by F_v, is reduced. Detachment of LHCIIs from PSII and subsequent attachment to PSI (i.e. a state 1 to state 2 transition) could explain the observed concurrent reductions in F_o and F_v (Krause & Weis 1991). Trissl & Wilhelm (1993) have suggested that state 1 to state 2 transitions occur more commonly among lower plants. Therefore it could be possible that state 1 to state 2 transitions occur in moss in response to freezing and thawing and may contribute to protection from photoxidative damage during freezing in the light by reducing the size of the light harvesting antenna of PSII.

Desiccation before freezing and thawing resulted in insensitivity of F_v/F_m to freezing and thawing in the light. A similar insensitivity to freezing and thawing in desiccated tissue was also found in the Antarctic moss *Polytrichum alpestre* (Kennedy 1993), and in algae (Davey 1989). Desiccation has been shown to result in the disconnection of LHCII from the PSII reaction centres in the green algae associated with desiccation tolerant lichens (Bilger *et al.* 1989) and in a loss of fluorescence signal in the desiccation-tolerant ferns (Muslin & Homann 1992; Eickmeier, Casper & Osmond 1993) and mosses (Seel *et al.* 1992). Thus, during desiccation, light energy absorbed by the LHCII cannot transfer energy to PSII. As a consequence, PSII cannot be over-reduced and is thus protected from potential photooxidative damage. Therefore, desiccation of moss before freeze–thaw events may help to ameliorate the influence of freezing and thawing, particularly in the light.

The complete recovery of photosynthetic efficiency of PSII in Antarctic moss after exposure to freeze–thaw stress observed in these experiments indicates that these mosses are able to tolerate cycles of freezing and thawing and that photoinhibition during freezing and thawing is possibly a protective process involving the down-regulation of PSII when carbon assimilation is limited by low temperatures (Hurry & Huner 1992; Huner *et al.* 1993; Oquist et al. 1993). Therefore, increases in the frequency of freeze–thaw events that may occur during global climate change are unlikely to cause damage to the photosynthetic apparatus of *Grimmia antarctici*, particularly if conditions favour desiccation before freezing and thawing. Similarly, Nicholls' (1991) proposed that Australian native vegetation was adapted to changes in climate due to the El niño southern oscillation, most notably through tolerance of drought and fire.

Although freeze–thaw events do reduce rates of photosynthetic carbon assimilation owing to low-temperature limitations on the enzymes of the photosynthetic carbon reduction cycle (Rütten & Santarius 1992), there was no evidence in this study of any additional damage to the photosynthetic apparatus caused by freezing and thawing in darkness or in the light. Thus, if the frequency of freeze–thaw events increases as a result of anthropogenic climate change it is unlikely that freezing and thawing events will have any additional influence on moss above that imposed by reductions in the time available for photosynthetic carbon fixation. Reductions in the time in which environmental conditions are favourable for photosynthesis may reduce growth and the ability to build up carbon stores necessary for basal metabolism during winter and season transition periods (Sagisaka *et al.* 1991). Other environmental changes potentially associated with global climate change that may influence moss growth and distributions in the future are changes in snowfall and ice accumulation patterns (Smith 1990). These will alter the availability and distribution of surface water during the summer months and the length of the summer growing season.

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