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Photoprotective Pigment as an Adaptive Strategy in the Antarctic Moss *Ceratodon purpureus*

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Summary. Variation in leaf pigmentation from green to ginger is observed for *Ceratodon purpureus* (Hedw.) Brid. in Antarctica. Electron microscopy of ginger and green leaves reveals less thylakoid stacking, a response to greater light exposure, in the ginger leaves. In extremely exposed sites *C. purpureus* has low chlorophyll *a/b* ratios which correlate with decreased 77K chlorophyll fluorescence, indicating damage to chlorophyll *a*. Pigment analysis of ginger moss shows that even when the chlorophyll *a/b* ratio has not decreased the pigment composition differs from green moss. The increase in anthocyanin and decrease in chlorophyll concentrations largely account for the visual change from green to ginger. The ratio of total carotenoid to chlorophyll varies from 0.35 in green moss to 0.55 in the ginger moss, with violaxanthin increased preferentially. Since these changes in pigmentation are consistent with photoprotection and they are linked to light dependent variations in chloroplast structure, it appears that photoprotective pigments are a useful adaptation for the bright Antarctic environment.

Introduction

Ceratodon purpureus (Hedw.) Brid. is a cosmopolitan moss which grows abundantly in the vicinity of Casey Station, Wilkes Land, East Antarctica (lat 66° 17'S, long 110° 32'E). In Antarctica its distribution and appearance are influenced by various environmental factors such as low temperature, freezing, dryness and an extreme light regime. In the field the colour of *C. purpureus* varies from bright green to ginger. It is a sun tolerant moss and its chloroplasts contain only small stacks of thylakoids (Valanne 1977; Aro and Valanne 1979; Valanne and Aro 1982). The chlorophyll *a/b* ratio is low as is typical for mosses (Rastorfer 1970, 1972; Martin 1980; Aro 1982; Martin and Churchill 1982; Valanne 1984; Kershaw and Webber 1986). Such low ratios reflect large amounts of light-harvesting chlorophyll protein (LHCP) (Aro 1982). Many higher plants exposed to bright light are able to reduce the amount of LHCP which results in an increase in the chlorophyll *a/b* ratio (reviewed by Anderson 1986; Anderson and Osmond 1987). Unlike higher plants, mosses appear unable to regulate LHCP, yet they are able to grow in Antarctica fully exposed to sunlight. Apart from regulating chlorophyll complexes, plants can use photoprotective pigments such as carotenoids (Sieferman-Harms 1987). The variation in *C. purpureus* leaf pigmentation observed in the field at sites with varying light exposures suggested that the pigments may be important for photoprotection. The aim of this study was to analyse the pigment composition, photosystem II fluorescence and chloroplast structure of the ginger and green leaves of *C. purpureus*.

Materials and Methods

Sampling

C. purpureus was collected in January 1988 from Robinsons Ridge, Wilkes Land, East Antarctica (lat 66° 22'S, long 110° 36'E). During the growing season the maximum mean monthly temperature of 2.6° occurs in January with the maximum of 6.5h mean daily sunshine (Australian Meteorological Bureau data 1967-1988). Light intensity on clear days can reach 1800 μE m^{-2}s^{-1}

Typically green *C. purpureus* was collected from shaded sites below rocks, while ginger turf was found nearby exposed to full sunshine (> 1m from rocks). However *C. purpureus* also grows in a convoluted ridge and trough form often with *Rhinodina olivaecobrunnea* (Dodge and Baker) encrusting the ridge tops. The trough sampled was between ridges 12cm apart and was 10cm deep. The green moss at the base of the trough was exposed to full sunlight for only short periods of the day. The sides of the ridge experience dryer conditions and longer exposure to light and the ginger pigmentation increases up the slope. Samples were analysed on return to Casey (spectrophotometry, high pressure liquid chromatography). If stored, samples were kept frozen at −20°C and when required defrosted slowly in the dark.

Experiments were carried out on stem apices, prepared by slicing approximately 3mm from the tips of moss clumps, so that photosynthetically active leaves was collected with a minimum of damage. These apices were rinsed in distilled water and blotted to remove excess water before measuring their fresh weight. The hydration of the moss was estimated by drying samples at 70°C overnight and expressing the weight of dry material remaining as a percentage of the fresh weight, and was typically found to be 30%.

Pigment Analysis

Chlorophyll and total carotenoids were estimated by grinding 10mg fresh weight of apices in 4ml of 85% acetone. The residue was spun down and rinsed and the absorbance of the combined supernatants measured using a Hitachi (U-3200) spectrophotometer. The concentrations of chlorophylls and total carotenoids were calculated from their absorbance values using the equations of Lichtenthaler and Wellburn (1983). Anthocyanin concentrations were estimated by extracting moss apices (100mg) in 1% HCl and measuring the absorbance at 535nm (Pecket and Bassim 1974).

High pressure liquid chromatography (HPLC) of carotenoids was carried out using a Waters Associates instrument equipped with a fixed wavelength detector (436nm) using a μBondapak C_{18} reverse phase column. The carotenoids were extracted from approximately 100mg of moss apices by grinding in methanol (2ml). This extract was passed through a Waters C_{18} SepPak cartridge which retained the carotenoids, these were subsequently eluted with ethyl acetate (400μl) and filtered (Millex 0.45μm). The pigment separation method (Wright and Shearer 1984; Wright and Jeffreys 1987) used a 20 min linear gradient from 90% acetonitrile in water to ethyl acetate at a flow rate of 2 ml min^{-1}. Individual pigments were identified by their absorbance spectra in reference solvents. Fractions corresponding to each pigment were collected and the polarity of the elution solvent in each fraction increased by adding water, to approximately 10% (volume) aqueous. Each fraction was then passed through a SepPak cartridge. The increased solvent polarity results in the pigment remaining on the cartridge until it is eluted with the appropriate reference solvent: acetone for chlorophylls, ethanol for carotenoids. The absorbance spectra were then measured to identify the pigment.

Photosystem II Fluorescence

Chlorophyll fluorescence of moss apices was measured at 77K. Moss apices were placed in a holder on the base of a 1cm diameter glass rod. A light pipe attached to the other end of the glass rod carried the illumination light and returned the fluorescence to the detector. Background reflectance was subtracted. Moss apices were allowed to dark adapt at room temperature for 15 min and then rapidly frozen to 77K by lowering the base of the glass rod below the surface of liquid nitrogen in darkness. By dark adapting before freezing to 77K and using 692nm filtered light for excitation, the fluorescence of chlorophyll in photosystem II can be measured. Weak illumination ($2\mu Em^{-2} s^{-1}$) was used to obtain the initial fluorescence (F_o). Followed by 5 min exposure to brighter light ($100\mu Em^{-2} s^{-1}$) to obtain the maximum fluorescence (F_m). The variable fluorescence (F_v) is the difference between F_o and F_m. The ratio of F_v to F_m was calculated.

Chloroplast Structure

Moss apices were fixed in 3% glutaraldehyde, 0.05M phosphate pH7.2 overnight under vacuum. Samples were then rinsed in 0.05M phosphate buffer containing 1% osmium for 2h, dehydrated in acetone and embedded in Spurr's resin. Sections were stained sequentially in uranyl acetate and lead citrate.

Results

Pigment Analysis

The pigment compositions of green and ginger *C. purpureus* are shown in Table 1. Ginger turf found beyond the shade of rocks has a lower chlorophyll content, a lower chlorophyll *a/b* ratio and a higher carotenoid:chlorophyll ratio than the green moss shaded by rocks. In the convoluted ridge and trough form of growth, the moss in the base of the trough is green, becoming increasingly ginger in appearance up the sides of the ridge. Again the chlorophyll content is highest in the green moss, but the chlorophyll *a/b* ratio does not vary. As the ginger pigmentation increases, the ratio of carotenoids: chlorophyll increases, and the concentration of anthocyanins more than doubles.

Since the amount of total carotenoids increased relative to chlorophyll in all ginger *C. purpureus*, the individual carotenoids were analysed using HPLC. Figure 1 shows a chromatogram of an extract of green moss in the base of the trough compared with an extract from the ginger moss higher up the side of the ridge. Table 2 shows the carotenoid levels relative to chlorophyll for comparison. The major increase in carotenoids in ginger *C. purpureus* is in violaxanthin, neoxanthin increases slightly while lutein and β-carotene decrease.

Photosystem II Fluorescence

To test the possibility that a decrease in the chlorophyll *a/b* ratio in exposed moss (Table 1) was due to damage of chlorophyll *a*, the fluorescence of photosystem II was measured. A decrease in the ratio of F_v (variable fluorescence) to F_m (maximum fluorescence) indicates damage to chlorophyll *a* in photosystem II (Krause and Weis 1984; Sivak and Walker 1985). Table 3 shows no change in the F_v/F_m ratio for the moss in the ridge and trough system where no change in the chlorophyll *a/b* ratio was observed. However in samples where the exposed moss had a

Table 1. Pigment compositions of *Ceratodon purpureus*, from different habitats. Pigments from green moss shaded by rocks ($n = 11$), and ginger moss exposed to full sunlight ($n = 6$). Variation in pigments in moss growing in a convoluted ridge and trough system where green moss occurs at the base with increasingly ginger moss higher up the sides of the ridge ($n = 3$). Means and their standard errors are shown

Sample	Chlorophyll μg chl gFW^{-1}	Chlorophyll *a/b*	Carotenoids μg gFW^{-1}	Carotenoids: chlorophyll	Anthocyanins A_{525} gFW^{-1}
Near rocks					
Shaded (green)	630±159	2.12±.06	218± 55	0.35	
Exposed (ginger)	518± 94	2.04±.13	284±114	0.55	
Ridge and trough					
Trough (green)	2236±539	2.30±.09	978±247	0.43±.01	0.76±.02
Lower ridge slope (ginger-green)	909±135	2.47±.03	444± 78	0.48±.01	1.36±.20
Upper ridge slope (ginger)	1012± 97	2.39±.05	508± 39	0.50±.01	2.00±.80

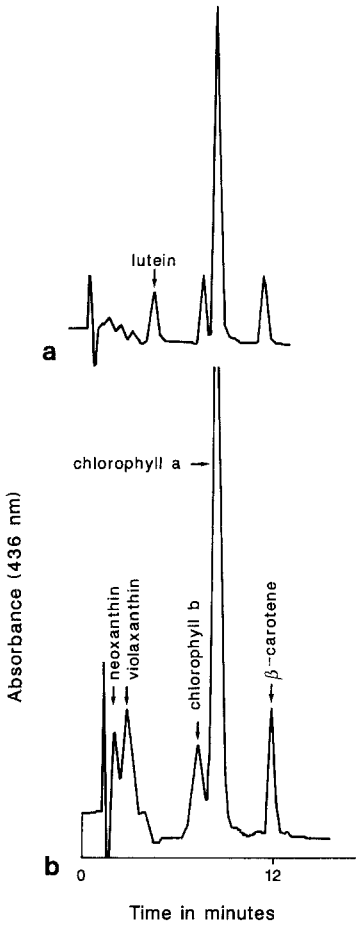

Fig. 1. HPLC separations of pigments in extracts of (**a**) green and (**b**) ginger *Ceratodon purpureus*. The most polar pigments, xanthophylls, are eluted first, followed by the chlorophylls and finally by β-carotene which elutes at 12 min

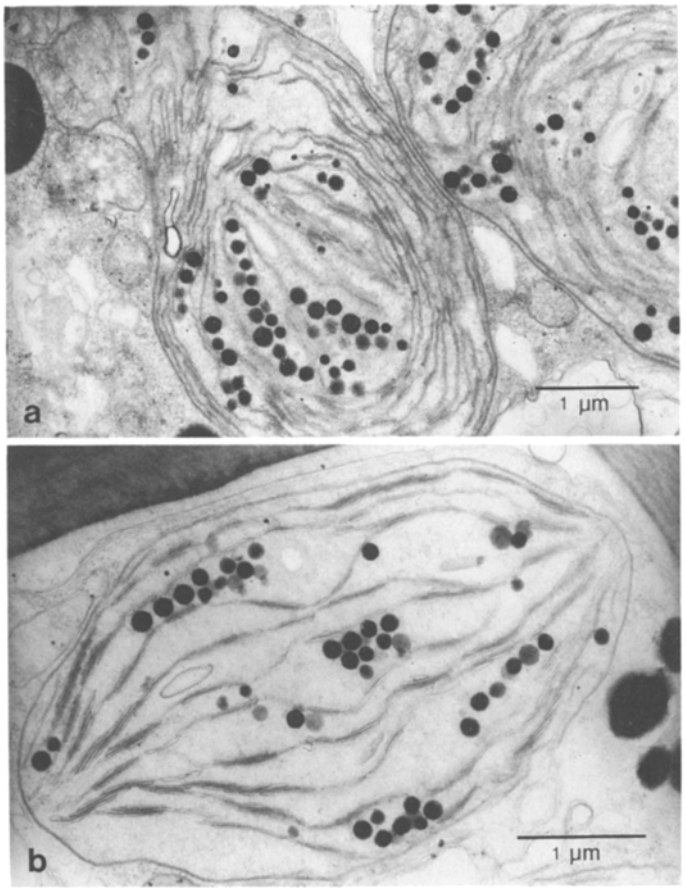

Fig. 2. Chloroplast structure of ginger leaves at the top of moss stems (**a**) and of the green leaves below (**b**). Lipid droplets are black, starch appears translucent

lower chlorophyll *a/b* ratio, some with values as low as 1.8, the fluorescence ratio was half that of green moss.

Chloroplast Structure

Ginger leaves often occur at the tips of *C. purpureus*, with green leaves lower down on the stem. The chloroplasts in the ginger leaves (Fig. 2a), were found to contain little thylakoid material relative to the stroma volume, with a large number of lipid droplets. The thylakoids are unoriented and stacks are generally only the appression of two thylakoids. In the green leaves (Fig. 2b), the chloroplasts are elongated with the thylakoids oriented axially. Most stacks are of 3 thylakoids with some consisting of up to 6 thylakoids. This suggests that the stacking of thylakoids is regulated by the light exposure, and that the green leaves are responding to low light conditions.

Table 2. Carotenoid composition of green and ginger *Ceratodon purpureus* in a ridge and trough. The carotenoids were analysed using HPLC and their individual integrated peak areas are expressed as a ratio to the total chlorophyll. Total xanthophylls (neoxanthin, violaxanthin and lutein) are expressed as a ratio to β-carotene

Sample	Carotenoid: chlorophyll				Xanthophyll: carotene
	Neo-xanthin	Viola-xanthin	Lutein	β-carotene	
Trough base (green)	0.044	0.022	0.133	0.177	2.13
Ridge side (ginger)	0.073	0.209	0.016	0.139	2.03

Table 3. The fluorescence of photosystem II in green and ginger *Ceratodon purpureus*. The ratio of variable fluorescence to maximum fluorescence is shown for moss shaded by rocks and fully exposed to sunlight, as well as for moss varying in pigmentation within a ridge and trough system

Sample	F_v/F_m	% difference
Near rocks		
Shaded (green)	0.55	—
Exposed (ginger)	0.53	4
Ridge and trough		
Trough base (green)	0.37	—
Upper side (ginger)	0.18	51

Discussion

The pigment composition of ginger leaves of *C. purpureus* is different to that of green leaves. They contain less chlorophyll, have a higher ratio of total carotenoids: chlorophyll, and a large increase in anthocyanins. The chloroplast structure suggests that the ginger leaves at the tips can shade the leaves moss below. The ginger leaves which have a decreased chlorophyll *a/b* ratio also have a decrease in the fluorescence of photosystem II.

Rastorfer (1970) found yellowish-brown *Bryum antarcticum* collected in the field had a chlorophyll *a/b* ratio of 1.5. After culturing under low light, the plants were dark green with a chlorophyll *a/b* ratio of 2.4. Rastorfer (1972) considered the low ratio in the field to be caused by damage due to direct solar radiation. Continuous light, at both high and low irradiation reduces the chlorophyll *a/b* ratio in *C. purpureus* (Valanne 1977). Chlorophyll *a* in reaction centres is known to be preferentially damaged by light (Oquist et al. 1987). The low chlorophyll *a/b* combined with the decreased fluorescence from photosystem II found in some ginger *C. purpureus* supports the idea that in exposed mosses with extremely low chlorophyll *a/b* ratios there has been damage to chlorophyll *a*. The reduction in total chlorophyll content observed in ginger moss is similar to the response of higher plants to bright light (Anderson 1986).

In shaded situations, *C. purpureus* is invariably bright green. The ginger form is found in sites exposed to bright sunlight. However as shown in the ridge and trough system where the differences in exposure to light intensity are not so great between the green and the ginger moss, the ginger pigmentation appears to be further increased in the presence of additional factors such as nutrient deficiencies and dryness. Ginger leaves often occur at the apices of moss stems with green leaves below. The chloroplast structure observed in the lower leaves is consistent with shading by the upper ginger leaves. The number of thylakoids in stacks increased, as well as the amount of stacked thylakoids compared to stroma thylakoids. Higher plants control the degree of stacking of thylakoids in a similar way in response to light exposure (Anderson 1986). The increase in thylakoid stacking found in the shaded green leaves suggests there is a relationship between light exposure and ginger pigmentation.

The changes in chlorophyll concentration and the ability to control thylakoid stacking suggest that the ginger *C. purpureus* is responding in a similar way to higher plants in bright light. But higher plants also have the ability to reduce the amount of light-harvesting chlorophyll protein resulting in an increased chlorophyll *a/b* ratio (Anderson 1986). Mosses have low chlorophyll *a/b* ratios and only small variations in the ratio are observed in shaded and exposed sites (Valanne 1977; Martin 1980; Martin and Churchill 1982; Martin and Warner 1984). The observation of sun and shade responses paralleling the ginger colouring suggests that the pigmentation is also a part of the response of *C. purpureus* to bright light. Analysis of the ginger leaves showed an increase in total carotenoids relative to chlorophyll, and a large increase in anthocyanins. The decrease in the ratio of xanthophylls: carotene found in the ginger moss is also found in higher plants in bright light (Anderson 1986). Carotenoids are able to act as antioxidants when the combination of light and oxygen produces free radicals (Siefermann-Harms 1987). Carotenoids also shield the secondary absorbance peaks of chlorophyll in the region 400-500nm. A major change in the carotenoids was the increase in violaxanthin relative to chlorophyll in ginger moss. A violaxanthin cycle is involved in the regulation of NADPH and the redox state of chloroplasts (Yamamoto 1979). Violaxanthin is converted to zeaxanthin in the light allowing the dissipation of excess energy from chlorophyll (Demmig et al. 1987, 1988; Demmig-Adams et al. 1989). Reconversion to violaxanthin occurs in darkness. The increase in violaxanthin in ginger *C. purpureus* indicates an increased photoprotective ability.

The ginger *C. purpureus* was found to differ in pigments, such as carotenoids and flavonoids, which are photoprotective. The ginger moss was also found to parallel some of the typical responses of higher plants to bright light, such as a decreased chlorophyll content, ratio of xanthophylls to carotene and thylakoid stacking. These observations suggest that the ginger pigmentation is closely linked with the responses of *C. purpureus* to light exposure, and can be considered as a photoprotective mechanism. It will be of interest to determine the effects of increased exposure to ultraviolet light during the Antarctic spring (Frederick and Snell 1988), as a result of stratospheric ozone depletion, on the photoprotective ability of *C. purpureus*.

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