ANGLIA RUSKIN UNIVERSITY

THE EFFECT OF ENHANCED ULTRAVIOLET-B RADIATION ON THE PHOTOSYNTHETIC METABOLISM OF TERRESTRIAL ANTARCTIC PLANTS

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A Thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

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NOTATION

Abbreviations frequently used in the thesis:

A/c_i,	net CO_2 assimilation rate vs intercellular CO_2 concentration.
A_{max},	maximal net assimilation rate under saturating CO_2 concentration and PPFD.
BIOTAS,	biological investigations of terrestrial Antarctic systems.
F_{vm},	maximum dark-adapted variable fluorescence ratio $(F_m - F_o) / F_m$.
LCP,	light compensation point (net assimilation = 0).
PAR,	photosynthetically active radiation.
PAS,	plant action spectrum as derived by Caldwell (1971).
PPFD,	photosynthetically active photon flux.
PSII,	photosystem II.
qN,	non-photochemical quenching; 1- $(F'_m - F_o)/ (F_m - F_o)$.
qP,	photochemical quenching; $(F'_m - F_o)/(F'_m - F_o)$.
Rubisco,	ribulose-1,5-bisphosphate carboxylase-oxygenase.
UV,	ultraviolet radiation.
UV-A,	ultraviolet radiation from 320 to 400 nm.
UV-B,	ultraviolet radiation from 280 to 320 nm.
$V_{c,max}$,	maximum carboxylation velocity of Rubisco.
vs,	versus.

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ANGLIA RUSKIN UNIVERSITY

ABSTRACT

FACULTY OF SCIENCE AND TECHNOLOGY

DOCTOR OF PHILOSOPHY

THE EFFECT OF ENHANCED ULTRAVIOLET-B RADIATION ON THE PHOTOSYNTHETIC METABOLISM OF TERRESTRIAL ANTARCTIC PLANTS

By ANDREW EDWIN SMITH

October 2009

The potential effect of increased UV-B radiation on photosynthetic activity and related processes in a range of terrestrial Antarctic plant species was investigated; from a chlorophyte alga to a vascular plant (*Deschampsia antarctica*). The relative contribution of the UV-B waveband to photosystem II (PSII) damage was used to construct an action spectrum for those species found to be sensitive to UV-B exposure.

Investigation involved non-invasive measurements of photosynthesis using chlorophyll-a fluorescence emission coupled with polarographic measurement of oxygen and infra-red gas analysis of carbon dioxide. Compounds associated with UV-B protection were extracted, and analysed using high performance liquid chromatography.

Different species exhibited differing sensitivity to UV-B exposure. Reduction in the efficiency of the light independent stage of photosynthesis and decrease in leaf length were found in *D. antarctica*. A decline in the potential activity of PSII (dark-adapted chlorophyll-a fluorescence) was found in some cryptogams, but no concurrent decrease in gas-exchange parameters. Shorter wavelengths of UV-B were shown to be more effective in depression of PSII efficiency. The first action spectrum for terrestrial Antarctic plants would predict a higher weighted UV-B exposure under ambient ozone, but would expect less damage under stratospheric ozone depletion than the commonly used plant action spectrum. Some of the plants investigated contained increased amounts of UV absorbing flavonoids following UV-B exposure.

The morphological changes found in *D. antarctica* should lower photosynthetic productivity, but are dependent on developmental stage. The action spectrum produced herein would still forecast increased damage to PSII due to early spring ozone depletion, but not as great as previously predicted. Moreover, current levels of UV-B radiation may be more damaging to some species than previously thought based on other plant action spectra.

CHAPTER 1

INTRODUCTION

1.1 Ozone

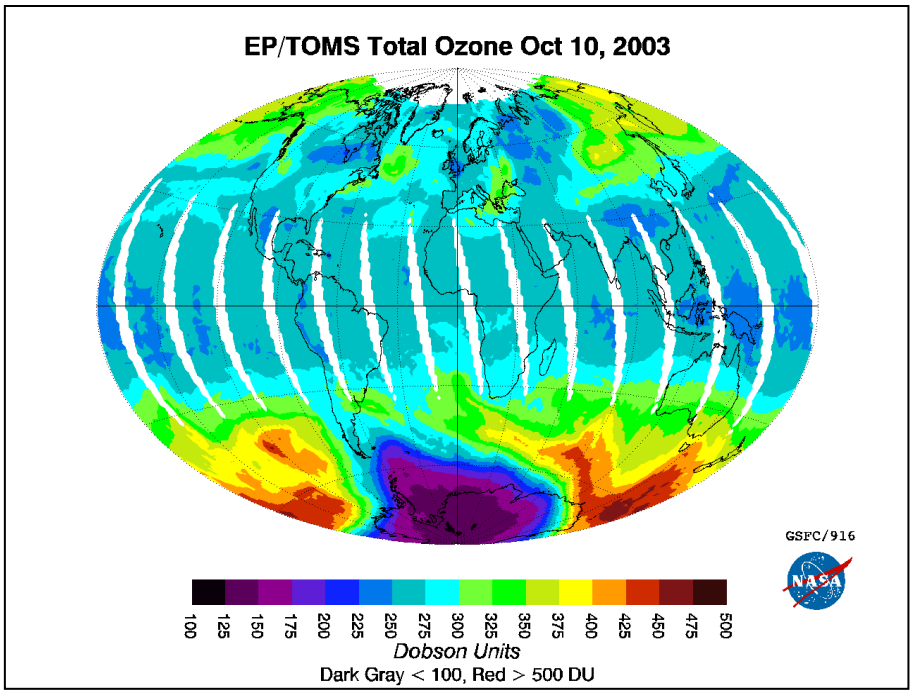
1.1.1 Ozone, ozone depletion and UV radiation

The evolution of oxygenic photosynthesis has led to a build up of oxygen in the atmosphere; this biotic oxygen source was the primary supply available for ozone formation (Kasting 1993). Ozone is formed by the absorption of wavelengths < 240 nm that split molecular oxygen, with the resultant atomic oxygen recombining with another oxygen molecule. Ozone starts to absorb electromagnetic radiation strongly from < 320 nm, and may itself be broken down by this absorption. This produces a dynamic equilibrium where ozone is constantly broken down and re-synthesised.

Ozone is distributed to a greater or lesser extent throughout the vertical column of the atmosphere, however it tends to be concentrated (ca. 90%) in one specific region, the stratosphere. The stratosphere rises from ca. 20 km to 50 km, and is characterised by an increase in temperature (-55 to -3^{o}C) with increasing altitude. Since the stratosphere contains only 9% of the total atmosphere, ozone is effectively concentrated in this section. Ozone present in the lower layer, the troposphere, is largely of anthropogenic origin (or from downward movement from the stratosphere) and is a minor component of the (unpolluted) total ozone column (Madronich 1993).

The total ozone column can be measured using differential absorption of selected UV wavelengths. The commonly used notation for the total ozone column is the Dobson unit. A Dobson unit (DU) is defined as the height the ozone would occupy in mm cm^{-2} at standard atmospheric pressure, and 0^{o}C. Accordingly, a value of 300 DU would be the equivalent to a height of 3 mm cm^{-2} of ozone. The total ozone column reading can vary depending upon seasonal and regional fluctuations. It also tends to be thinnest at the equator and rises with increasing latitude (Figure 1.1).

Ozone chemistry (formation and destruction) is complex; Madronich (1993) lists the most important reactions from both stratosphere and troposphere. Ozone formation is continually balanced with its destruction by reactive species; especially NO_x, HO_x and Cl_x. These active species are not themselves rapidly broken down, but participate in catalytic cycles; so that a single active species may destroy thousands of ozone molecules before being destroyed itself. Ozone depleting chemicals, particularly those



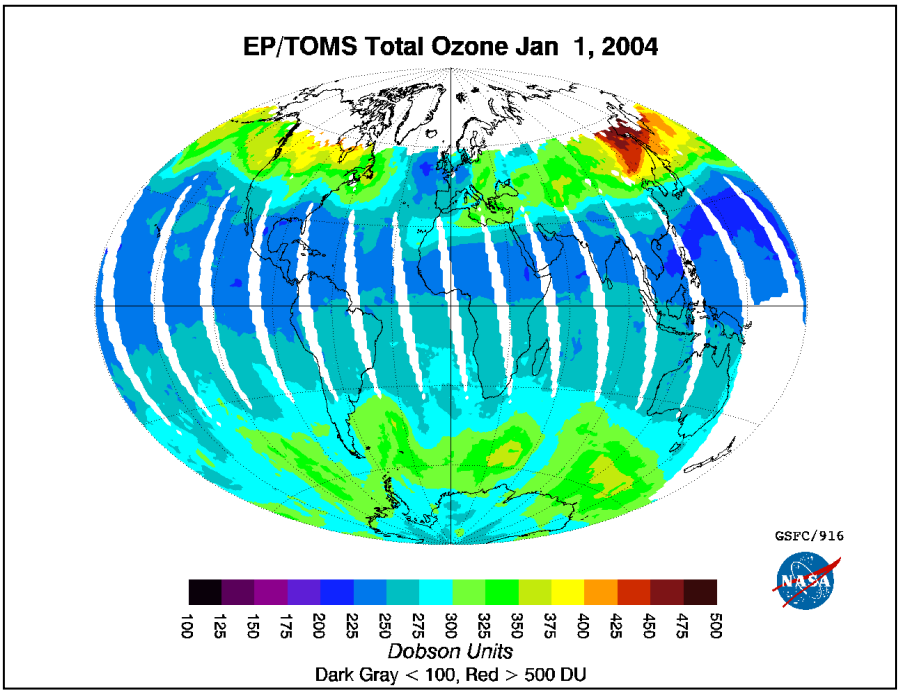


Figure 1.1 Global ozone concentration during the Austral spring (October, top) and the summer (January, bottom). Data from the NASA Total Ozone Mapping Spectrometer (TOMS).

chlorofluorocarbons (CFCs) listed by the Montreal Protocol (1987) (and subsequent additions), are readily dissociated by UV-C in the stratosphere, thus providing a reservoir of active species.

It had been suggested since the 1970s that there may be an anthropogenic effect upon the stratospheric ozone layer, on account of an increase in jet airliner traffic (NO_x) (Climate Impact Assessment Program CIAP 1971, summarised by Grobecker *et al.*. 1974). This effect was postulated to occur in the upper stratosphere at ca. 40 km, and was expected over tropical latitudes; depleting an already thin layer. Moreover, production of CFCs (Cl_x) for industrial and domestic purposes was also expected to adversely affect stratospheric ozone levels (Molina and Rowland 1974). The discovery of early spring ozone loss over West Antarctica (Farman *et al.*. 1985) confirmed this, but the location; Antarctica, and the vertical distribution; about 20 km, were not expected. Figure 1.2 shows the location and extent of ozone depletion during 1987 at Halley Bay (76^oS). Although other losses have subsequently been identified (e.g. Müller *et al.* 1997), the Austral spring depletion is the most dramatic with almost complete loss in the lower stratosphere. This loss can leave a total column mean of less than 150 DU (with readings of under 66% normal column mean called ozone 'hole' events); less than half of what would normally be expected at that time of year. Figures 1.1, 1.2 and 1.3 illustrate the loss of ozone in the lower stratosphere during the Austral spring. Although recovery does take place later in the season, the uncertainty of the duration of this loss is a major point of interest, with work reporting losses starting earlier in the winter and lasting longer, at least towards some of the outer fringes of the 'hole' (SORG 1999).

A combination of satellite and ground based ozone measurement with spectroradiometer recordings of UV-B radiation has demonstrated a correlation between a decrease in ozone and a concomitant increase in UV-B in the Antarctic (e.g. Newsham *et al.* 2002). Whilst this loss of ozone does not produce an increased overall UV-B fluence- comparable to that recorded in equatorial regions, it is a highly significant loss for an area that would normally be heavily protected by the ozone layer. Moreover, it causes a change in the relative penetration of UV-B wavelengths; the irradiance at 295 nm being over one hundred times greater during the spring compared to the late summer (Figure 1.4). Furthermore, this loss can result in the (biologically weighted) UV-B exposure during the spring being equivalent to that during the midsummer (Karentz 1991).

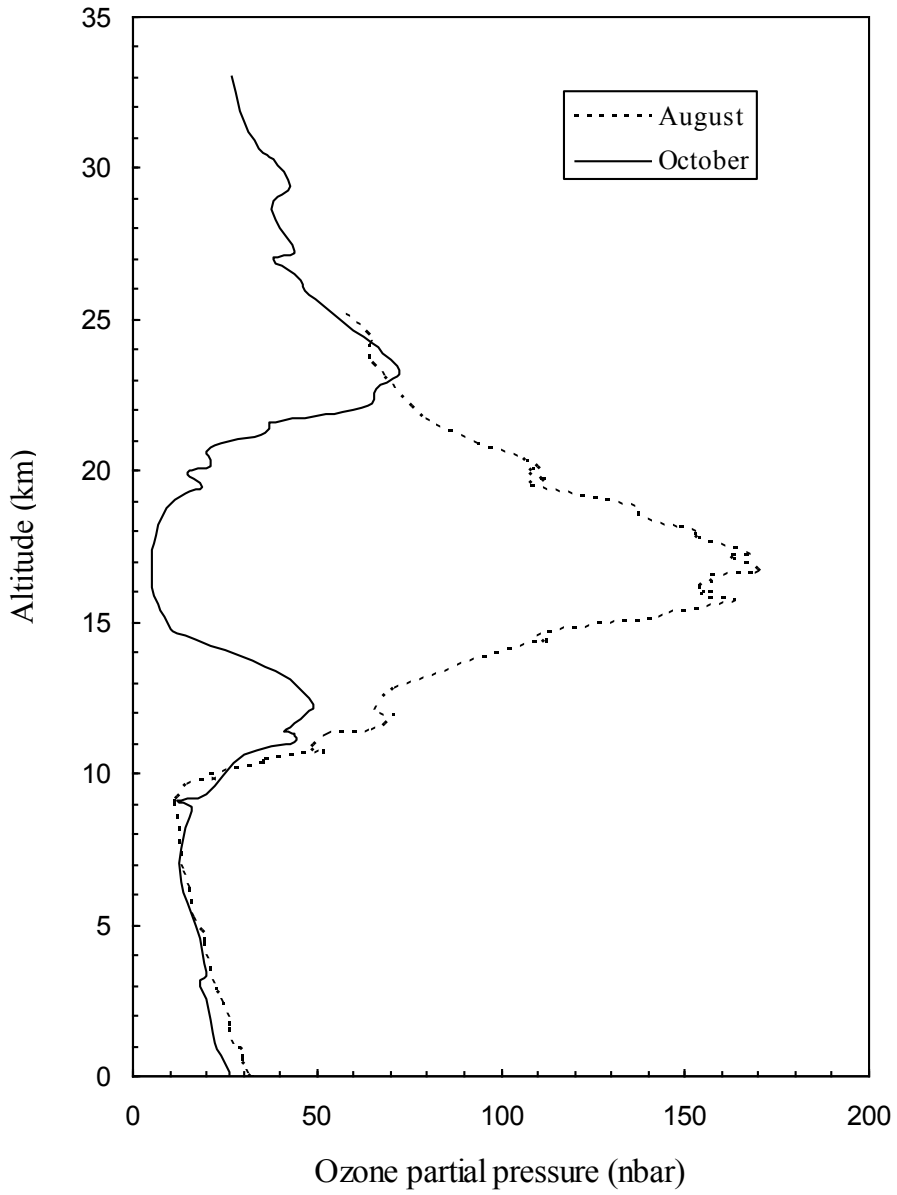
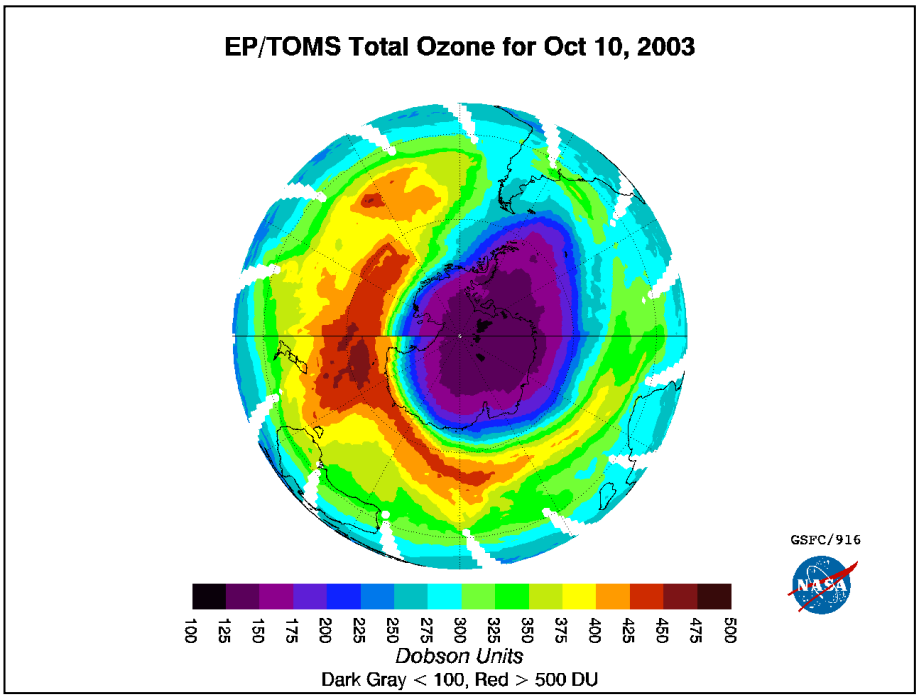


Figure 1.2 Vertical location of ozone above Halley Bay Antarctica (76^{o}S), showing a typical profile prior to ozone depletion (August) and during an October ozone 'hole' event. Data courtesy J. D. Shanklin, British Antarctic Survey.



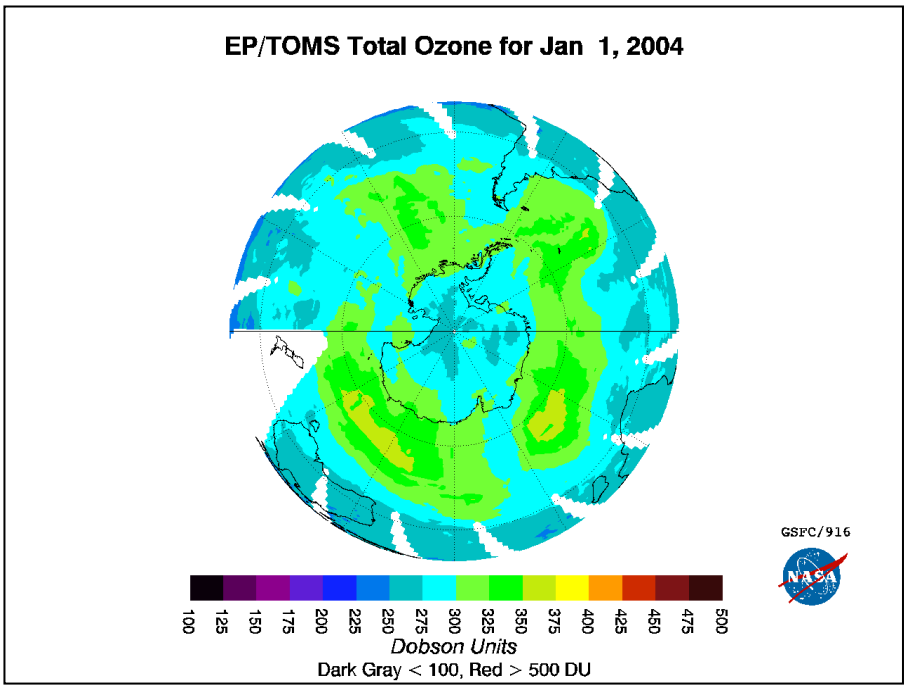


Figure 1.3 Ozone concentration over Antarctica during the Austral spring (October, top) and the summer (January, bottom). Data from the NASA Total Ozone Mapping Spectrometer (TOMS).

The physical mechanisms behind Antarctic ozone loss are a result of the unique weather systems of the continent, in particular the circumpolar vortex; which isolates the continent in the winter and enhances the formation of polar stratospheric clouds (PSCs). These PSCs are the key to a particular sequence of events. Ozone-depleting catalytic species build up on the ice crystals in these clouds, where they immediately initiate a catastrophic ozone loss (when solar rays return in the spring). Moreover, the chemistry of Antarctic ozone depletion is different to that of normal ozone breakdown because it does not involve atomic oxygen (SORG 1996). Similarly, losses may also occur over the Arctic, but the seeding of active species depends upon the presence of the PSCs, and formation of these clouds is relatively rare during the comparatively milder Arctic winter. However, work has since shown that (when these factors exist) Arctic ozone loss may be nearly as great, with losses of total ozone being as high as 30% (THESEO 2000, VINTERSOL 2003). Although ozone 'hole' formation and occurrence are a uniquely polar phenomenon, ozone depletion is considered to be occurring on a global scale, with some reports predicting an average global reduction of 3 to 4% per decade (SORG 1999). However, this may alter with time as a result of the implementation of the Montreal Protocol (1987) and its amendments (e.g. London 1990, Copenhagen 1992, and Vienna 1995), which has resulted in a decrease in the rate of increase of chlorine and bromine species being released. One estimate was for global ozone levels to return to pre-1980 levels by 2049 (WMO/ UNEP 2006), although more recent reports predict recovery up to 2080 (Tully *et al.* 2008). Nevertheless, even with full compliance to the Protocol, there will still be significant ozone depletion and ozone 'hole' events over the Antarctic in the forthcoming decades (up to 2065-WMO/ UNEP 2006). This situation is further complicated by the possible debilitating effects of 'greenhouse' gas emission, the presence of which could even increase the production of PSCs (SORG 1999).

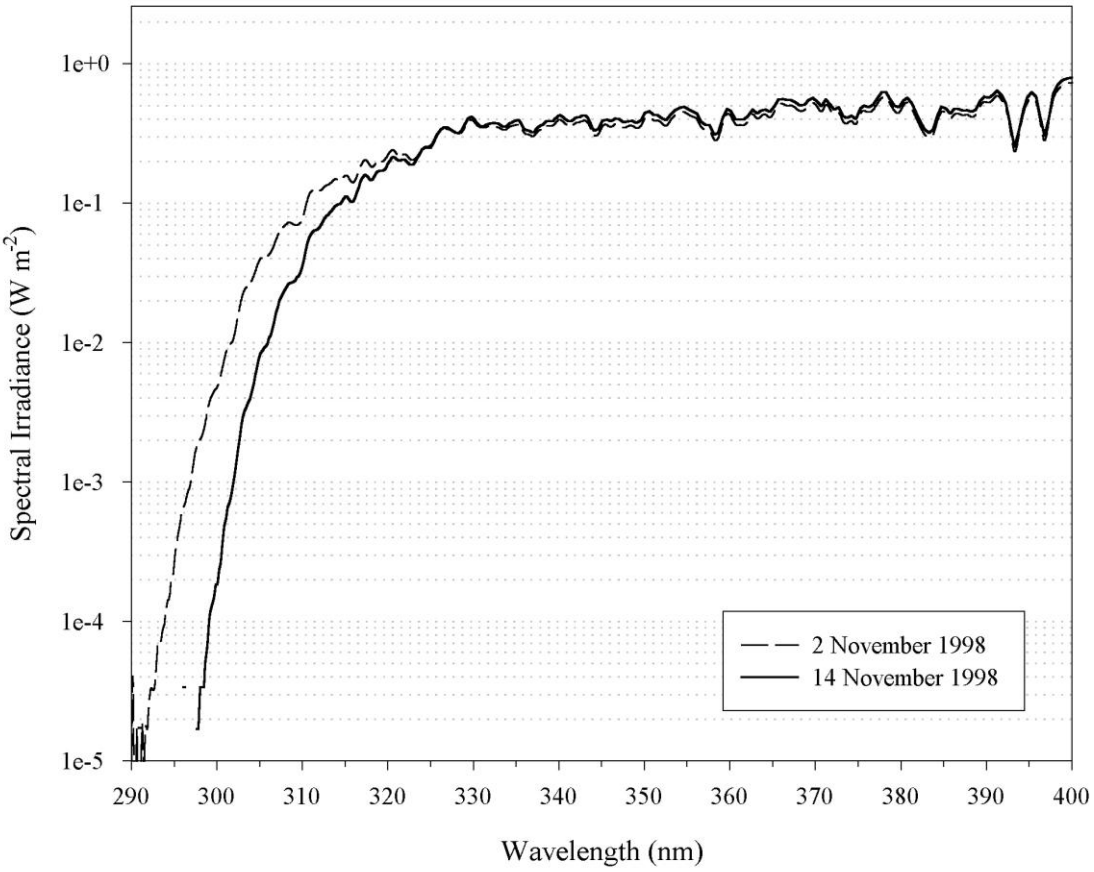
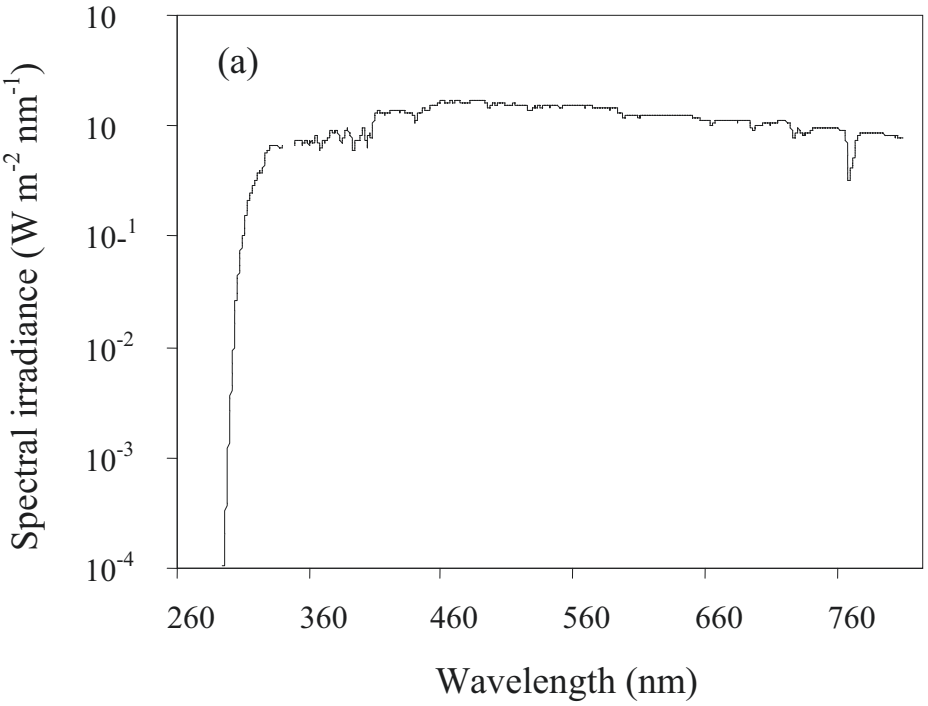


Figure 1.4 Spectroradiometer data from McMurdo station showing scans taken during ozone depletion (2 November 1998) and shortly after (14 November 1998). Total Ozone Mapping Spectrometer readings for 2 November 1998 and 14 November 1998, 170 and 299 DU respectively. Scans taken at similar solar zenith angles. UV data courtesy NSF UV Radiation Monitoring Network.

1.1.2 Consequences of ozone depletion

Much biological (at least terrestrial) evolution has probably been as a direct consequence of the production of oxygen and subsequent development of the ozone layer. This is because oxygen is an effective absorber of UV-C (< 280 nm) radiation, whilst ozone is an extremely efficient absorber of the UV-B (usually defined as 280 to 320 nm) solar radiation component. The absorbance by oxygen is a pre-requisite for land-based life as both proteins and nucleic acids absorb strongly in the UV-C waveband at ca. 280 nm and ca. 260 nm respectively.

Wavelengths shorter than photosynthetic active radiation (PAR, 400 to 700 nm) have been divided into near UV (280 to 400 nm), far UV (220 to 280 nm), and vacuum UV (10 to 220 nm). The radiation received at the Earth's surface is shown in Figure 1.5a. The transition zone between far and near UV has been termed UV-B radiation (280 to 315 nm-Coblentz 1932 cited in Caldwell and Flint 1997, and Comission Internationale de l'Eclatrage (CIE) 1974, cited in Jordan 1996). Other commonly used definitions are UV-C, 200-280 nm; and UV-A, 315-400 nm. The exact definition of UV-B has been problematic; the original CIE definition is based upon glass filters, whilst the biological effects of UV-B radiation can extend to 335 nm (Peak and van der Leun 1993, Cooley *et al.* 2000). Furthermore, as the lower definition of UV-B has been based on the shortest wavelengths detected at the Earth's surface, which in turn is dependent on the sensitivity (and duration of recording) of the equipment used to measure the radiation. Therefore, for the purposes of this study, the following definitions and terminology used by many photobiology workers are employed (e.g. Coohill 1991, Quaite *et al.* 1992). UV-A, 320-400 nm; UV-B, 280-320 nm; and UV-C, 200-280 nm, these boundaries are shown in Figure 1.5b. Moreover, the absorption by ozone of UV radiation becomes negligible above 320 nm (Figure 1.4 and 1.5a), so the 280-320 nm definition fully covers the range that would be most altered by a decrease in ozone (Setlow 1974). In contrast, UV-C wavelengths are so effectively absorbed by the atmosphere that the thinnest of ozone/ oxygen layer will suffice. Finally, only UV-B is substantially and proportionately altered by the amount of ozone present in the air column as transmission of UV-A is almost completely unaffected by passage through the ozone layer (Figure 1.4).



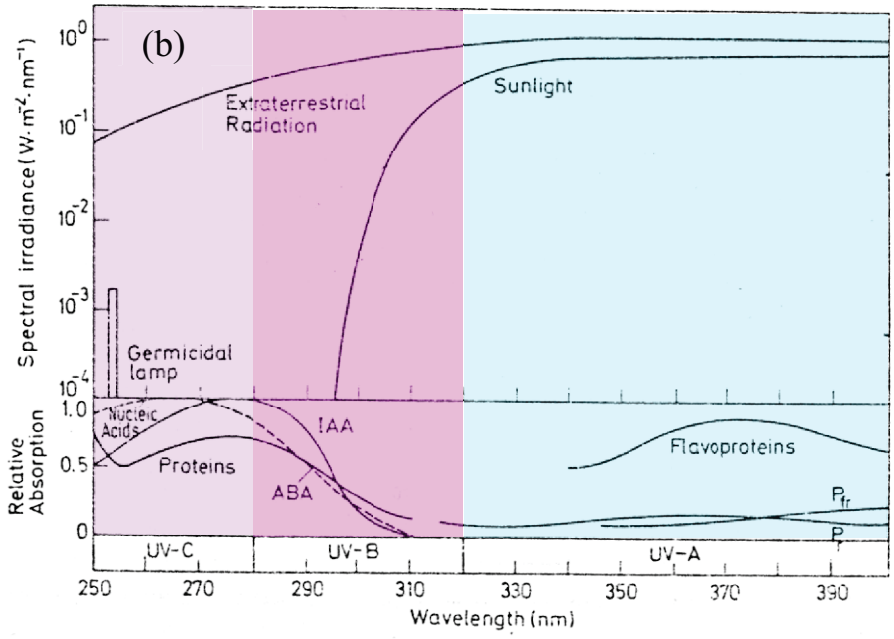


Figure 1.5 Solar spectrum and demarcation of the UV waveband.
(a) Typical solar spectrum measured up to 800 nm at ground level.
(b) UV-C 200-280 nm, UV-B 280-320 nm, UV-A 320-400 nm. ABA Abscisic acid, IAA indole acetic acid, P_{fr} P_r Phytochrome. Figure modified from Caldwell (1981).

Whilst the presence of ozone is the primary factor attenuating extra-terrestrial UV-B transmission, there are many other factors that affect solar UV-B radiation reaching the surface of the Earth (Green 1983): atmospheric scattering effects, such as Rayleigh scattering (particles with sizes less than the radiation wavelength e.g. gases) and Mie scattering (particles greater than the wavelength i.e. clouds), together with solar zenith angle and albedo effects, contribute to the combined total of direct and diffuse incident radiation (Green 1983, Madronich 1993, Grant *et al.* 2002). It is precisely because of the complex interaction of these factors that there is such difficulty in obtaining accurate estimates of incident UV-B fluence, and hence predicting future UV-B fluence under ozone depletion. Accordingly, radiative transfer models have become useful in determining potential UV-B fluence under differing ozone concentrations (e.g. Green *et al.* 1980). Nevertheless, most commonly used models lose reliability when cloud cover is taken into consideration (Madronich *et al.* 1995). However, there has been an increase in direct monitoring of UV-B using spectroradiometers. The National Science Foundation (NSF) UV monitoring network (operated by Biospherical Instruments Inc.) has been in operation in Antarctica since 1988, whilst the spectroradiometer operated by the British Antarctic Survey at Rothera has been yielding data since 1997. These data are starting to provide an historical database, which can be used to improve these and future models.

Finally, there is another important consequence of ozone depletion with respect to UV-B fluence other than overall increase in UV-B fluence and a shift to increased fluence at lower wavelengths of UV-B; namely a change in the overall ratio of UV-B:UV-A and PAR (Deckmyn *et al.* 1994, Grant *et al.* 2002). Each of these changes has the potential to damage biological systems.

1.2 UV-B research

1.2.1 Introduction

UV-B radiation is known to have many effects in biological systems because it is absorbed by carbon-carbon double bonds and conjugated molecular structures (Jagger 1967). The bond energy for a carbon-carbon double bond is 348 kJ mol $^{-1}$ and the energy in one mole of 300 nm radiation is 399 kJ, so it can be expected that radiation consisting of wavelengths of ≤ 300 nm have the potential (if fully absorbed), to cleave such bonds. Since these bonds play an integral role in DNA and protein structure, which have broad absorption bands with maxima at ca. 260 and 280 nm respectively, UV-B has the potential to have deleterious effects on both. Other examples of potential chromophores and sites of action are shown in Figure 1.5.

1.2.2 History and development of UV-B research on plants

Work on the biological effects of UV radiation has been carried out since, or prior to, the early 20th century. Initially this concentrated on UV-C radiation and its mutagenic effects (DNA damage). The work of Gates (1930) created an action spectrum for bacteria, which later helped to identify DNA as the agent of genetic inheritance. Other areas that have had major research inputs are the erythemal and carcinogenic effects of UV radiation in humans and animals, plus its use in phototherapy (Finsen 1901). However, one of the first reports on plants was by Schanz (1920) (cited in Caldwell 1971).

Most work up to the early 1970s on plants has been well summarised in various review articles (e.g. Caldwell 1971) and general texts (e.g. Smith 1977). These have reported a wide range of responses with effects as varied as reduced viral infection, auxin inactivation, stimulation of vegetative growth, cytological effects, and changes in mineral content. Species/ cultivar differences and wavelength dependence have also been noted (Cline and Salisbury 1966). Other than direct UV-C related DNA damage; phototropism (e.g. Mills and Schrank 1954, cited in Curry 1969), and morphological changes such as leaf curling and bronzing/ lesion formation were common observations (Caldwell 1971).

Much of this early work was performed using germicidal lamps (low pressure mercury vapour lamps) to irradiate the experimental material (summarised in Caldwell 1971). These lamps have a maximal emission at 254 nm, but there was no uniformity regarding the output; and the manufacturer's specifications often varied (Jagger 1967). Accordingly, cross comparison of early results is difficult.

Work using wavelengths centred at 254 nm is of less relevance regarding the biological effects of potential ozone depletion scenarios, since there is no effective natural UV-C penetration. Furthermore, application of equivalent energy at a particular waveband will not necessarily result in the same effect as the same amount of energy applied at a different waveband because of the specific absorption characteristics of the chromophore. As a result, UV-C effects are difficult to extrapolate to UV-B effects (Stapleton 1992). Recognising this, the CIAP recommended switching studies on plants from mainly UV-C type work to incident solar UV, i.e. primarily UV-B (Nachtwey 1975). Subsequent work that included UV-B irradiation quickly established a number of effects on plants (other than direct UV-C related DNA damage). These included morphological transformations such as curling, stunted growth and bronzing/ lesion formation on leaves and production of putative UV screening compounds, particularly those derived from the phenylpropanoid pathway (see Klein 1978, and Caldwell 1981, for reviews of work).

The last 20 years have seen an increase in work on the potential effects of UV-B radiation, reflecting concern over ozone depletion. There have been an extensive number of reviews/ synopses of this work including Stapleton (1992), Young *et al.* (1993), Teramura and Sullivan (1994), Wynn-Williams (1994), Caldwell *et al.* (1995, 2003), Jordan (1996), Lumsden (1997), Allen *et al.* (1998), Searles *et al.* (2001), Frohnmeyer and Staiger (2003), Rozema *et al.* (2005), and Björn (2007).

Work has become progressively more sophisticated, using arrays of lamps that emit in the UV-B range and/ or filters to selectively attenuate Solar UV radiation. These lamp systems have been controlled in one of three ways: a simple on/off procedure; a step-up switching system (Johanson *et al.* 1995); or a feedback system linked to UV sensors (see McLeod 1997 for review of these systems). The first two are described as 'square-wave' systems, whilst the latter is referred to as 'modulated' system. Both systems attempt to mimic the changes in received solar UV-B fluence, during both diurnal and seasonal (since UV-B fluence can change quite dramatically over these periods), using an action spectrum to calculate effective doses that are predicted to

occur under differing ozone depletion situations. This is necessary because of the lack of spectral conformity between the irradiation systems and the actual solar UV spectrum. Typical procedures involve applying the calculated enhanced exposure during the photo-period, or by maintaining a constant 'ozone depletion' by tracking the incident UV-B (i.e. a modulated system). Calculations of the expected UV-B irradiance under predicted ozone depletion are carried out by use of an appropriate radiative transfer model e.g. Green *et al.* (1980).

1.2.3 Effects of UV-B radiation on plants

Whilst entitled 'effects of UV-B radiation on plants', much of the experimental work discussed below cannot be solely ascribed to UV-B radiation, and must therefore be treated with some caution. This situation arises because of problems with UV supplementation and filtering methods (Holmes 1997). It is also probable that many responses of plants to UV-B may also be stimulated to a lesser or greater extent by UV-A and C, as there is no distinct cut-off for the wavelengths of importance as regards ozone depletion. Furthermore, exposure to UV-B radiation can elicit both UV-A and UV-C type effects (Jagger 1985). This confounding of a UV-B effect is the consequence of the tailing and merging of biological action spectra, hence it is difficult to ascribe an effect as being purely UV-B, UV-A, or UV-C mediated (Jagger 1967).

Much of the work relating to ozone depletion has been concentrated on species of economic importance, with over 200 species/ cultivars having been investigated to date, plus those associated with marine primary production. Some of this work has looked at generational effects, with studies extending over a number of years/ growing seasons (Barnes *et al.* 1988, Teramura *et al.* 1990, Gehrke 1998, Hoorens *et al.* 2004). These longer term studies have not, in the main, investigated effects such as DNA repair or possible UV-B receptors. Short-term mechanistic studies have examined sites of UV-B absorption, screens, quenchers, plus direct and indirect consequences. Numerous books and papers have been published summarising and reviewing work (particularly since Jagger 1967) including; Caldwell (1971), Worrest and Caldwell (1986), Stapleton (1992), Young *et al.* (1993), Teramura and Sullivan (1994), Wynn-Williams (1994 and papers in the same issue), Caldwell *et al.* (1995, 2003), Jordan (1996), Lumsden (1997), Rozema *et al.* (1997), Allen *et al.* (1998), Searles *et al.* 2001, Frohnmeyer and Staiger (2003), Ghetti *et al.* (2006) and references therein of the above and others listed.

One main area studied has been the effect on photosynthesis. The light-dependent stage, particularly photosystem II (PSII); and alteration to the light-independent stage, chiefly the first step involving carboxylation of ribulose bisphosphate by the enzyme ribulose bisphosphate carboxylase/ oxygenase (Rubisco) has been extensively investigated. This work has been reviewed by a number of authors e.g. Allen *et al.* (1998). Early work on the effect of UV-B radiation on photosynthetic competence identified PSII as a main site of damage (Iwanzik *et al.* 1983). Much of this work has utilised the non-invasive measurements of chlorophyll fluorescence, in particular the dark-adapted fluorescence ratio F_{vm}, which is a measure of the maximum quantum efficiency of PSII. Recent work (van Rensen *et al.* 2007) has shown that acceptor side damage to be an initial effect of UV-B exposure. UV-B-induced degradation of the PSII protein D1 has been implicated (Friso *et al.* 1994), while damage to the water-splitting complex has also been demonstrated (Renger *et al.* 1989). Other workers however, have cast into doubt PSII as the primary site of UV-B-induced damage at environmentally realistic levels of UV-B exposure (Allen *et al.* 1998). Studies on the enzyme Rubisco have shown it to be altered by UV-B exposure in both content (Vu *et al.* 1984) and activity (Jordan *et al.* 1992), with work correlating loss of carboxylation activity with a decrease in genetic expression of both sub-units of the enzyme (Keiller *et al.* 2003). Change in chlorophyll concentration is another possible effect, Robinson *et al.* (2005) noted a loss in chlorophyll in an Antarctic moss exposed to a higher level of natural UV-B. However, Niemei *et al.* (2002) reported differing responses of mosses by way of a change in chlorophyll concentration. Lastly, stomatal opening has been shown to be altered by UV-B exposure (Jansen and van den Noort 2000).

A commonly reported effect of exposure to UV-B radiation is the production of UV-B screening compounds, chiefly products of the phenylpropanoid pathway e.g. flavonoids (Cockell and Knowland 1999). The meta-analysis of 62 papers on UV-B field studies by Searles *et al*. (2001) found the parameter most altered by enhanced UV-B radiation to be an increase in UV-B-absorbing compounds. Production of flavonoid compounds would appear to be a common response in many plants; the over-production of flavonoids in *Arabidopsis* spp. was shown to have a beneficial effect on exposure to UV-B radiation (Bieza and Lois 2001).

Another potential effect of UV-B radiation is related to oxidative events within the cell, resulting from the production of reactive oxygen species (ROS) (Jordan 1996). There are a number of possible routes of oxidative damage following exposure to UV-B

radiation. Aromatic compounds such as tryptophan may, upon excitation form a triplet state, which can then lose an electron to form singlet oxygen; this is a reactive species with the potential to damage membranes. Additional molecules may also produce free radicals, which can react with any oxygen that is present. Superoxide formation can also occur; resulting in hydrogen peroxide production, which can in turn, lead to hydroxyl radical formation, probably the most potent ROS. There are a number of potential scavenging mechanisms within plants that may mop up these reactive species. Superoxide dismutase (SOD), reduced glutathione (GSH) and polyamines may all play a role in limiting ROS damage (Jordan 1996). Work by Jansen *et al.* (1996) has demonstrated an increase in SOD and glutathione reductase activity after exposure to a 3 h pulse of UV-B radiation, although the irradiation also included the (potentially more damaging) UV-C component.

The extensive work on the effects of UV-B on DNA damage and repair has primarily focused on animal models. There are two main types of damage that are thought to occur as a consequence of direct UV exposure; photoproduct (primarily of a dimeric structure) formation i.e. cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts ((6-4) photoproducts) and associated Dewar photoproducts, which are formed when the (6-4) photoproducts are exposed to UV-A radiation. Other types of DNA damage include single and double-strand breakages, and cross-linking. Both types of damage have been shown to, or are thought to, occur in plants (Sinha and Häder 2002, Taylor *et al.* 1997 and references therein). Repair of such damage is complex and varied depending upon the organism in question. The three main repair activities are photoreactivation, several types of excision-repair and recombinational repair. The latter mechanism has not been fully demonstrated and is not believed to be significant in plants (Stapleton 1992, Sinha and Häder 2002). Photoreactivation is blue light (300 to 500 nm) dependent and is mainly responsible for repair of CPDs using DNA photolyase (e.g. Pang and Hays 1991). Excision repair is mainly responsible for both CPDs and (6-4) photoproducts. There are believed to be a number of different types of excision repair, which may be species specific, but are all light independent. Pang and Hays (1991) first demonstrated the production and repair of CPDs in *Arabidopsis* spp., whilst UV-B-induced DNA damage in alfalfa seedlings has been used to construct an action spectrum (Quaite *et al.* 1992).

Morphological effects as a result of UV-B exposure have commonly been reported. Gonzalez *et al.* (1998) found a decrease in leaf area in *Pisum sativum* L. as a

result of UV-B exposure. Alteration in leaf thickness in UV-B-exposed sub-Arctic dwarf shrubs was recorded by Johanson *et al.* (1995). Shoot morphology was found to be altered in field studies on an Arctic moss (Gehrke 1999). Furthermore, changes in surface waxes were found in *Cucumis sativus* L. seedlings (Tevini and Steinmüller 1987).

UV-B radiation may also perform an informational role, via a putative chromophore in a signal transduction process mediating its effect. The photoreceptor may be a flavin or pterin similar to cryptochrome (Jenkins *et al.* 1997), though glutathione has also been implicated in an alternative role in the perception of UV-B and the related production of chalcone synthase (Loyall *et al.* 2000). Furthermore, whilst there may be distinct UV-B and UV-A/ blue light receptors, a synergistic effect has been demonstrated of the UV-A/ blue light receptor on the UV-B signal transduction pathway (Jenkins 1997).

UV-B responses that have been described from both long and short-term studies are mixed and seem to be dependent on duration and region of UV-B waveband that the plants are exposed to as well as plant species/ cultivar (Teramura 1983). The meta-analysis by Searles *et al.* (2001), concluded that UV-B-absorbing compounds were most prevalent, but morphological changes were less responsive to altered UV-B exposure while photosynthesis *per se* and photosynthetic pigment content were not thought to be effected. The high Arctic field supplementation study on a number of growth parameters in a variety of tundra plants (Rozema *et al.* 2006) found no evidence of alteration after seven years of enhanced UV-B exposure. Nevertheless, there is evidence of interaction and synergy between effects (Teramura and Sullivan 1994). Thus a UV-B-induced morphological change could ameliorate effects of other stresses (e.g. high light and drought stress, Poulson *et al.* 2002). This, in turn, can lead to other 'UV-B' effects such as alteration in CO_2 exchange and thus carbon fixation and overall productivity. Thus one primary site of action of UV-B exposure could lead to many secondary effects, thereby making it hard to elucidate the primary target of UV-B. These types of interactions may help in explaining some of the conflicting literature.

Some workers have indicated positive effects of UV-B radiation. Two potential areas of beneficial consequences are first, water stress amelioration as a result of response similarity i.e. leaf curling and stomatal effects. Second, production of flavonoids and related phenolic compounds, which may have a dual role of UV protection and resistance to pathogen attack (e.g. McCloud and Berenbaum 1994).

Nevertheless, there is also the possibility of competition for 'phenolic' resources. Teramura *et al.* (1990) reported an increase in crop yield after UV-B exposure in a 'UV-sensitive' soybean cultivar. However this increase in productivity coincided with a drought season and was prior to and subsequent to a decline in yield compared with the naturally irradiated control. Work on *Phaseolus vulgaris* by Deckmyn and Impens (1995) using different thicknesses of 'Plexiglas' to partially screen incident UV-B noted increased pod abortion with the high UV-B. This resulted in fewer, but heavier, pods and gave a larger harvest index compared to the low UV-B plants. These positive responses in economically important species may not be relevant to Antarctic species, where survival of a vegetative clonal plant rather than sexual reproduction may be more important.

Other environmental conditions can play an important role in determining any effect of UV-B, including; temperature, water availability, nutrient availability and limiting (or) excess PAR. UV-B exposure has been shown to reduce CO_2 enhanced growth in loblolly pine (Sullivan and Teramura 1994). Work on mosses and the interaction of UV-B and CO_2 produced mixed results, with the high UV-B treatment found to be less inhibitory than the ambient UV-B exposure (Sonesson *et al.* 1996). Similarly, Tosserams *et al.* (2001) found that high levels of UV-B ameliorated the effect of elevated CO_2, but the alteration was dependent on developmental stage and could not be fully resolved to UV-B exposure alone. High PAR was found to ameliorate UV-B damage (Adamse and Britz 1992). Yet while plants placed under water stress are less effected by UV-B exposure (Sullivan and Teramura 1990). Heavy metal exposure will also increase UV-B-induced damage (Dubé and Bornman 1992).

1.3 Antarctica

1.3.1 General description of Antarctica

Antarctica consists of two distinct geological regions; the older East and a younger West, separated by an ice-filled channel. The continent covers a vast area (14 x 10^6 km^2); 97% of this is permanently covered in snow and ice, with a mean thickness of nearly 2 km constituting 90% of the total freshwater present on the Earth (Walton 1984).

The Antarctic has been broadly divided into three geobotanical zones; sub-Antarctic; maritime and continental (Longton 1988). Moreover, the vegetation has been classified (in line with comparable Arctic zones) as mild, cool, cold and frigid (Longton 1988 and references therein). Macroscopic life generally exists mainly on the sub-Antarctic islands and coastal fringes that are ice-free during the summer. Further inland on the continent, lichens and associated endolithics may be found sporadically on isolated nunutaks to 80^{o}S (Longton 1988). Other generally ice-free areas where microbial communities have developed in the so-called Dry Valleys of Victoria Land.

1.3.2 Climate and environment

The Antarctic environment is harsh in many ways. On much of the ice-free land mass the soils are immature, lacking nutrients, and poorly developed. High nutrient levels are confined to small areas enriched by birds and/ or other animals. Availability of free water is also an important limiting factor for survival of Antarctic plants, particularly on continental Antarctica, where precipitation events are rare, with most liquid water coming from spring and summer ice/ snow melt (Longton 1988). Furthermore, those plants growing on the coastal fringes are subject to saline conditions.

Mean temperatures are low and probably the most important limiting factor, with the number of acceptable degree days above freezing being the most significant factor (Smith 1994). Mean monthly temperature on the milder coastal fringes can be below 0^{o}C for most months, although ice-free ground temperature can rise to 20^{o}C during the summer at Signy I., 60^{o}S (Davey 1991) and Rothera Point, 67^{o}34'S (Newsham *et al.* 2002).

Freeze-thaw cycles are another potential stress for plants (Davey *et al.* 1992, Kennedy 1993). Whilst radiative heating of dark surfaces can lead to snow-melt during the short Austral summer, snow may sublimate rather than provide free water, particularly in the Dry Valleys of Victoria Land. Liquid water formed during the summer can also lead to water-logging and pond formation above the ever-present permafrost. This water-logging can be significant as it can cause root anoxia- another potential stress. The low precipitation combined with dry katabatic winds, especially around coastal and polar plateau interfaces, make Antarctica a very arid place. Accordingly, adaptations to water conservation and/ or desiccation tolerance are vital to survival.

PAR can also be relatively high, with plants that occur within the Antarctic Circle potentially exposed to continuous PAR for weeks including reflection from snowfields, with (at times) little diurnal fluctuation in the light regime, potentially negating (dark) photoinhibitory repair mechanisms (Lovelock *et al.* 1995b). Moreover, photoinhibition in a liverwort has even been observed on cloudy days (Davey 1997), and may be an important factor in limiting productivity (Adamson *et al.* 1988). Despite low solar angle at the polar regions, reflectance from snow and ice can increase the received PAR. Thus relatively high PAR in conjunction with low temperatures can potentially favour frequent photoinhibitory events (Farage and Long 1991). However, photoinhibition may also be a way to control/ limit damage to PSII at low temperature (Lovelock *et al.* 1995b).

These multiple 'stress' factors are now potentially compounded by the addition of the early spring ozone 'hole' and concomitant increase in UV-B radiation. This stress, however slight, may change the already fragile competitive balance and thus ecosystem composition (Wynn-Williams 1994).

1.3.3 Antarctic terrestrial vegetation

The extensive Pleistocene (2 mya-18 kya) glaciation of the Antarctic, coupled with poor propagule input/ development, has resulted in an extreme paucity of macroscopic vegetation. However, many propagules deposited over time should still be viable and able to develop on meeting suitable environmental conditions (Kennedy 1996). There are very few known endemic species represented in the plant taxa, in particular bryophytes (Peat *et al.* 2007), and many early endemic species were

incorrectly identified. Most plant species would therefore appear to have been deposited from refugia (possibly on sub-Antarctic islands) after the last extensive glaciation, rather than survived in isolated pockets (Longton 1988).

Only two vascular species are represented on the Antarctic continent; the herb *Colobanthus quitensis* (Kunth) Bartl. (Antarctic Pearlwort; Caryophyllaceae) and the hair grass *Deschampsia antarctica* Desv. (Antarctic hair grass; Poaceae). Neither species is endemic, though the hair grass occurs on the Antarctic Peninsula as far south as 68°43'S (Smith and Poncet 1987).

Cryptogams are well represented in terms of species number, with around 250 recorded in the cold-Antarctic, and are found in all zones; from the mild to the frigid (Longton 1988). Mosses may be described as (1) cosmopolitan e.g. *Bryum argenteum* Hedw., *B. pseudotriquetrum* (Hedw.) Schwaegr. and *Ceratodon purpureus* (Hedw.) Brid.; (2) bipolar e.g *Sanionia uncinata* (Hedw.) Loeske, (3) of more restricted distribution e.g. *Tortula princeps* (De Not.) Mitt. (= *Syntrichia princeps* de Not.) and *Sarconeurum glaciale* (C. Muell.) Card. et Bryhn*;* or (3) endemic e.g. *Grimmia lawiana* Willis in Filson (Longton 1988). The thallose liverwort *Marchantia berteroana* Lehm. et Lindenb. occurs in the maritime Antarctic (including Signy I.) and further south along the Peninsula as far as Leonie I. The leafy liverwort *Cephaloziella exiliflora* (Tayl.) Steph. is the most southerly hepatic, being found to 75ºS around northern Victoria Land. Distribution of bryophytes is thought to be dependent on temperature and, at a local level, on water availability (Lewis-Smith 1999). Longton (1988) gives a comprehensive description of the biology of Antarctic bryophytes.

There are some representatives of the chlorophycean algae e.g. *Prasiola crispa* (Lightf.) Menegh., which occurs throughout the coastal sites of Antarctica and is particularly associated with high nitrogen environments, such as those found in close proximity to penguin/ skua nesting sites. Cyanobacteria, such as *Nostoc commune* Vauch. and *Phormidium autumnale* (Agardh) Gomont, are widely distributed, especially as thick crustose mats. Lichens are one of the predominant taxa, becoming increasingly dominant with increasing latitude.

1.3.4 Survival of Antarctic vegetation

Extremely low temperatures have undoubtedly effected vegetation composition and survival. The Antarctic growing season is necessarily short and is typified by lower temperatures compared to equivalent northerly latitudes. Such low temperatures restrict free water supply (particularly on the continent) and the more abundant vascular plants that are associated with the more benign climate of the sub-Antarctic islands often have xerophytic characteristics such as tightly rolled leaves and sunken stomata on the inner leaf surface (Larcher 1995). Such characteristics are found in Antarctic *D. antarctica*, whilst *C. quitensis* has thick, succulent-type leaves. These factors would imply that water availability (and possibly salinity in coastal locations) is a primary stress associated with the Antarctic environment. However, lack of nutrient availability and low temperatures can generate more extreme xeromorphic features than water availability itself (Stäfelt 1956 cited in Esau 1960). Bryophytes are poikilohydrous and are able to survive prolonged periods of desiccation (Longton 1988).

Antarctic photosynthetic activity (and hence productivity) is low. However, both Antarctic vascular plants appear to have broad photosynthetic maxima typically centred at, or below, 15^{o}C whilst *D. antarctica* is also capable of 30% of maximum photosynthetic assimilation rate (as measured by net CO_2 exchange) at 0^{o}C (Edwards and Smith 1988). Similarly, some Antarctic cryptogams have been shown to have optimal net assimilation rates of between 2 and 5^{o}C (Longton 1988). Recovery from dehydration of cryptogams has also been assessed, with resumption of photosynthesis depending on hydration state of the moss (Lovelock *et al.* 1995a and b, Kennedy 1993).

Reproductive strategies tend to be severely limited in Antarctica, primarily because of the short growing season. Whilst both vascular plant species are known to flower in the Antarctic, the setting of seed is sporadic at best (Convey 1996). Furthermore, more southerly populations of these plants can extend their sexually reproductive development over two seasons (Corner 1971); similarly some moss species may also extend sporophyte development through two seasons (Longton 1988). Bryophyte sporophyte production declines with increasing latitude, being almost negligible on the continent (Longton 1988).

1.3.5 Effect of exposure to UV-B radiation on Antarctic vegetation

The work on polar species has generally been cached in terms of comparison with temperate plants. Some field studies have been carried out in the Arctic, with Björn and co-workers using fluorescent lamps to increase the UV-B exposure of a dwarf shrub ecosystem (Johanson *et al.* 1995), and laboratory studies to assess interaction with other environmental factors, e.g. CO_2 (Sonesson *et al.* 1995). A lot of work has been carried out on marine/ freshwater species (e.g. El-Sayed *et al.* 1990, Karentz 1991, Smith *et al.* 1992, Neale *et al.* 1994, Quesada *et al.* 1995, and Bischof *et al.* 1998) with weighting functions and action spectra produced for marine phytoplankton (Mitchell 1990, Boucher and Prezelin 1996). Much work (since the discovery of ozone thinning) on the terrestrial biota has been performed on cyanobacteria; see review by Vincent and Quesada (1994).

There have been a number of reviews outlining possible ozone depletion scenarios (Karentz 1991, Wynn-Williams 1994, Marchant 1997, Rozema *et al.* 2005, Newsham and Robinson 2009). The work by Karentz (1991) emphasised the scarcity of work prior to discovery of the ozone 'hole' and the lack of previous and subsequent direct recordings of UV-B fluence; the first direct measurements were only taken in 1988. The author also highlights the wavelength shift with decreasing ozone concentration, whilst noting the importance of confounding factors such as clouds, snow and ice. One consideration from this review is the need for a molecular marker that can demonstrate a change in proportion with total UV-B exposure (and known turnover time) to assess previous and future effects of ozone depletion events. Also, as the ozone 'hole' has been present since possibly the late 1970s, the author states "...any biological and subsequent ecological effects that can result have already been initiated...". This is of particular importance regarding the use of material collected for further investigation as the generational times of the biota may be relatively long. Thus plants would have already been exposed to many years of increased UV-B exposure, thus it is important to be able to use material cultivated under a known irradiation environment prior to exposure to altered UV-B fluence.

The review by Wynn-Williams (1994) suggests three potential strategies that could be adopted by terrestrial Antarctic organisms against UV-B "behavior *(sic)* (escape and avoidance), prevention (screening and quenching), and remedy (repair mechanisms).". Whilst the author supports the possible use of plastic cloches for

investigating climate change scenarios, the use of cloches for field investigations has since been questioned for a number of reasons such as adverse temperature and moisture effects (Kennedy 1995a, 1995b). UV-B quenching and/ or screening compounds appear to be an important protective strategy with putative examples for cyanobacteria being extra-cellular scytonemin and intra-cellular mycosporine-like amino-acids (MAAs), algae (MAAs), lichens (carotenoids), and mosses (flavonoids and carotenoids). Wynn-Williams also suggests that the cosmopolitan ancestry of most of the biota, in that *D. antarctica* has a high alpine distribution so will have undoubtedly experienced high UV-B fluence in its evolutionary past, such plants may still retain UV-B protective strategies. Several areas of future work were suggested including: analysis of photoprotective pigments, use of organisms as indicators of change, propagule resistance, and genetics of resistance. The lack of work on higher plants was also noted.

Marchant's brief review (1997) is more general, covering all Antarctic organisms (and effects on dissolved organic and inorganic matter). Again the production of putative UV-B protective screening compounds in terrestrial organisms is highlighted. The author concluded there was a "pressing need for a better knowledge of UV-B-induced impacts on biological systems" to be able to accurately forecast future ozone depletion scenarios.

The review of Rozema *et al.* (2005) highlighted differences between UV-B exposure and UV-B exclusion experiments. They also compared these studies with those carried out in the Arctic and noted a lack of long-term field studies and the possibility of differing evolutionary pressures causing differences in UV-B responses between species. The recent meta-analysis of UV-B effects on polar plants by Newsham and Robinson (2009) focused on data generated from field studies carried out in the Antarctic and Arctic. They also found that increases in UV-B absorbing compounds together with a loss of above-ground biomass and DNA damage were the most consistent responses. It was concluded that polar bryophytes respond to UV-B in a similar way to angiosperms. They also noted a differential response dependent on exposure protocol with lamp-based systems inducing a lesser response than screening. It was considered that this may have been as a result of using unsuitable weighting functions.

Work that has been published more recently relating to the effect of UV-B include studies on cryptogams (Newsham *et al.* 2002 & 2005, Lud *et al.* 2003, Robinson *et al.* 2005, Clarke and Robinson 2008), vascular plants (Ruhland and Day 2000, Xiong and Day 2001, van de Staaij *et al.* 2002, Ruhland *et al.* 2005) and a variety of species (Huiskes *et al.* 2001, Lud *et al.* 2001).

Newsham *et al.* (2002) found no evidence of a link between decreased ozone concentration and F_{vm} in the liverwort *Cephaloziella varians* and moss *Sanionia uncinata*, but did note an increase in carotenoids attributed to enhanced UV-B exposure. A subsequent paper (Newsham *et al.* 2005) noted an increase in anthocyanins and decrease in total chlorophyll concentration in *C. varians* with increasing UV-B exposure. Lud *et al.* (2002 & 2003), working on the moss *Sanionia uncinata,* found no evidence of alteration in photosynthesis measured by way of fluorescence as a consequence of UV-B exclusion. Similarly, Robinson *et al.* (2005) did not find any alteration in F_{vm}, but did report both increases in both carotenoids and in leaf density in UV-B-exposed samples of the moss *Grimmia antarctici*. Clarke and Robinson (2008) found that increased cell-wall bound phenolics to provide cosmopolitan moss species enhanced UV-B screening compared to an endemic moss.

Xiong and Day (2001) found a decrease in quantum yield in both *C. quitensis* and *D. antarctica*, but no change in F_{vm}, so concluded UV-B was damaging the light independent stage and not PSII photochemistry. Furthermore, an increase in methanol soluble UV-B absorbing compounds was noted in *D. antarctica*. However, Huiskes *et al.* (2001), Lud *et al.* (2001), van de Staaij *et al.* (2002) and Ruhland *et al.* (2005) all found no significant effects of UV-B exclusion or enhanced UV-B exposure on the concentration of flavonoids in *D. antarctica*.

Most other published work relating to terrestrial organisms has been on the alga *Prasiola crispa*. This work has used supplementation of UV-B with fluorescent lamps and/ or screening of selected solar wavebands. Thus, Jackson and Seppelt (1997) used 0.1 mm thick 'Mylar' and polycarbonate filters to screen out wavelengths <320 nm from natural plots of algae and noted a depression in F_{vm} in the UV-B-exposed samples compared to the screened material, and higher absorbance of a methanolic extract at 325 nm relative to the screened sample. Post and Larkum (1993) looked at seasonal fluctuations in putative screening compounds present in the alga and (in a growth cabinet study) the effect of UV-B on physiological parameters. They found an increase in absorbance at 325 nm of extracts obtained during the summer compared with the

winter samples. Cultured samples that were exposed to enhanced UV-B fluence showed no alteration in absorbance at 325 nm (based on chlorophyll content) after four weeks, but a decrease in chlorophyll content on a fresh mass basis. Maximum photosynthetic rates, and quantum efficiency were lower, whilst dark respiration and light compensation points were higher in samples exposed to UV-B radiation. However, the low PAR used, 29 W m^{-2}, could enhance the damaging effects of UV-B (Adamse and Britz 1992). Furthermore, the alga was cultured at 3^{o}C, whilst the physiological measurements were taken at 20^{o}C. Since DNA photolyase repair mechanisms are temperature dependent (Pang and Hays 1991), it would be expected that more rapid repair activity at 20^{o}C would have an effect on the physiological status of the plant. Lastly Lud *et al.* (2001) using UV-B screens found that DNA damage by way of CPD formation to be most sensitive to UV-B exposure, but found no effect on photosynthesis (net productivity or PSII efficiency).

The possible relationship of flavonoid content with temporal variation of the total ozone column in herbarium specimens of the moss *Bryum* spp. has been studied by Markham *et al.* (1990) and Ryan *et al.* (2009). Work by Post and Vesk (1992) on photoprotective pigments in *C. exiliflora* correlated carotenoids and anthocyanin-like pigments with UV-B exposure.

General conclusions from previous work and thus current understanding are still relatively limited as a result of so few data. Production of UV-B screening/ shielding compounds and/ or photo-protective compounds (carotenoids) would appear to be a primary strategy amongst some of the terrestrial Antarctic vegetation. Again this has to be viewed with caution as work on putative cryptogam screening compounds is still limited and has yet to confirm a protective role, as has been demonstrated with flavonoid production in an *Arabidopsis* mutant (Bieza and Lois 2001).

1.4 Limitations associated with work on UV-B radiation

Work on the effect of UV-B radiation on plants has specific problems that are not comparable to work on other factors such as CO_2, water and temperature. Thus both the quantity (energy) and quality (wavelength) of the UV-B radiation are important, as is the ratio with other incident wavebands (Caldwell *et al.* 1994). Investigations into the effects of exposure to UV-B radiation must be lucid in describing both the quantity and quality of the UV-B manipulation. Moreover, it is essential to provide un-weighted data, as there is much debate (e.g. Caldwell *et al.* 1986, Holmes 1997), about the correct usage and application of action spectra regarding UV effects in plants.

Plants have evolved over millions of years under the influence of light, therefore its perception, use of, and protection from excess, must have played an integral part in that evolution for them to tolerate even short term UV-B exposure. The complexity of photomorphogenic effects is highlighted by the study of phytochrome. Phytochrome is a chromoprotein in which the functional component is an open chain tetrapyrrole that can exist in two distinct photoconvertible states; Pfr (formed by red-light from 650 to 700 nm), and Pr (formed by far-red light from 700 to 730 nm). Pfr is generally recognised as the biologically active form. Phytochrome has been resolved into at least five different gene products/ forms and would therefore appear to consist of a family of proteins linked to the same, or similar, chromophore. The functions of phytochrome are multiplicit, with prominent roles in the control of germination, de-etiolation and flowering. Accordingly it has been shown to moderate and/ or control a wide variety of genes. The control and action of phytochrome has been summarised by a number of reviews (e.g. Smith and Whitelam 1990). This complexity and organisation undoubtedly developed over evolutionary time; the same is also likely to be true for any other light-receptors.

Evidence for putative UV-A/ blue light receptors (cryptochromes, Jenkins *et al.* 1997) and the blue light absorbing phototropin (Briggs and Christie 2002) would indicate similar complexity to phytochrome. There have also been a number of propositions for UV-B signal perception and transduction involving glutathione (Loyall *et al.* 2000), and jasmonic acid (Mackerness *et al.* 1999); as summarised by Frohnmeyer and Staiger (2003). This would imply that subtle differences in UV spectral irradiance may have profound effects on the response to UV-B as there is a probable synergistic effect (Jenkins *et al.* 1997).

A significant problem associated with UV-B (and photobiological) work is the use of filters and/ or lamps to simulate a predicted ozone depletion event. As ozone depletion will only substantially modify wavelengths between 290-320 nm (Caldwell and Flint 1997), any lamp/ filter array that alters fluence outside this range will not accurately simulate the actual consequence of ozone depletion (Figure 1.4). Moreover, it will not reflect the relative ratio of solar radiation in this region. The use of filters that cut-off radiation at ca. 320 nm is arbitrary, reflecting the physical definition of the UV-B upper limit, and thus does not allow for the biological characteristics of UV absorption i.e. the absorption of a particular chromophore can cover a large spectral range which could include and extend beyond this boundary (Jagger 1967). Furthermore, as the various lamps that are used do not quantitatively and qualitatively match the solar spectrum, the actual spectral irradiance will differ from the ozone-depleted spectrum; even if total integrated UV-B radiation is of similar quantity. Accordingly, for preliminary screening and observations of potential UV-B effects, it may be preferable to exclude all UV-B (and UV-A) radiation from a first control, and then to discretely screen off, or enhance, selected portions of the UV-waveband.

To calculate incident UV-B fluence as a consequence of ozone depletion models have used radiation transfer models (RTMs) e.g. Green *et al.* (1980). Likewise, the relative efficiency of those wavelengths on biological systems has been determined using biological weighting functions (BWFs) e.g. that for DNA damage (Setlow 1974). These have been both been combined to ascertain estimates of future UV-B exposure, and to overcome difficulties with matching lamp output to solar UV-B exposure. RTMs have been of use in predicting future ozone loss and of UV exposure prior to implementation of the UV spectroradiometer network. Whilst direct measurement of UV-B has only been carried out relatively recently with high quality instrumentation, ozone data has been collected over a longer period. The use of satellites e.g. total ozone mapping spectrometer (TOMS) has improved the amount of datasets relevant to the Antarctic. The model of Green *et al.* (1980) has been widely used, but there have been caveats; Madronich (1993) noted that there were problems when used outside of its fitted characteristics, with cloud cover being a primary concern. Moreover, Fiscus *et al.* (1994) working on soybean, suggested that it may be underestimating UV-B dose, and thus leading to over-exposure of plants to UV-B for the ozone depletion scenario stated. However, as the number of reliable datasets increases from the spectroradiometer

network then RTMs should become more accurate in calculating historical and future UV-B fluence.

BWFs used to weight the UV fluence delivered to the experimental material in both field manipulations and laboratory studies are needed for a number of reasons. As previously mentioned, solar spectrum is not matched by the lamps that are used to expose plants to UV-B radiation. Furthermore, the amount of UV-B received decreases as the wavelength decreases, whilst biological damage increases as wavelength decreases (Madronich *et al.* 1995). BWFs may then be used to assess the potential damage to a biological system under a given ozone depletion scenario. Such work gives rise to the radiation amplification factor (RAF) (Madronich *et al.* 1995). The application of a BWF is only valid if the weighting function (action spectra) has been rigorously constructed, and is the appropriate function for the species in question. Accordingly, there may not be a generally applicable response, such as the naked DNA spectrum (Setlow 1974), available for multi-cellular and pigmented plants as a result of the complexity of the factors involved in the transmission of UV-B through the plant to potential target sites. A commonly used weighting function, the plant action spectrum (PAS, Caldwell 1971), is a composite spectrum produced mainly from monochromatic studies. Although widely used, this spectrum has limitations; the data for the spectrum stops at 313 nm, as a result of lamp limitation rather than the action spectrum itself, see also criticism from the author (Caldwell and Flint 1997). There have been more recently published action spectra (e.g. Flint and Caldwell 2003a, Yao *et al.* 2006), which may, in time, become more widely used; in particular that of Flint and Caldwell (2003a) as this has also been evaluated in field experiments (Flint and Caldwell 2003b, Flint *et al.* 2004b).

Apart from the choice of action spectrum to use for weighting UV-B exposure, there is also the problem of choice of normalisation wavelength. To gain a like-for-like comparison of UV-B exposure carried out using different UV emitting lamps and/ or filter combinations, it is important to provide details on the calculation of dosage e.g. action spectrum used, duration etc. However, action spectra are not commonly used as absolute values, but converted to similar scale by normalising to unity at a particular wavelength. This has been done at the most effective (damaging) wavelength (Caldwell 1971, Rundel 1983). However, other wavelengths have been used, causing confusion in the literature. Rupert (1982) suggested using 300 nm as the standard normalisation wavelength for ease of cross-comparison and this is now widely used in the reporting of

UV-B studies. However, the use of normalisation of action spectra does cause problems with interpretation of different action spectra (Sutherland *et al.* 1994).

Errors may easily be generated from a number of sources; use of inappropriate RTM, or one that under/ over estimates a given scenario, use of BWF that either has not been adequately constructed, or is not applicable to the plants being studied. Work by Flint *et al.* (2004a) found that varying height of UV-B emitting lamps caused problems. Finally, the target of the UV-B radiation may absorb outside the physical boundary of UV-B waveband. Any of these factors could lead to inappropriate exposures (Caldwell and Flint 1997).

1.5 Fieldwork versus laboratory studies

It is not possible to fully replicate Antarctic conditions in a controlled environmental chamber. There are technical problems in attaining low temperature, together with high irradiance, albedo and associated freeze-thaw cycles, which characterise the natural environment. It is possible however, to investigate subtle effects of UV-B exposure, and of selected UV-B wavebands via growth cabinets. Nevertheless, the conditions in the growth chambers are dissimilar to those experienced in the field. This is of significance as amelioration of UV-B damage is observed when there is simultaneous exposure to UV-A (Quesada *et al.* 1995) and high PAR (Adamse and Britz 1992). Hence the lack of response to enhanced UV-B exposure in a growth cabinet experiment would imply that there would also be no alteration in the field.

Technical limitations of cabinets and experimental space, together with restricted availability of material, meant that for the series of experiments described in this thesis, it would not be possible to have a complete range of UV controls, i.e. a randomised block design for replication, which would require more than two growth cabinets. It was also considered desirable that the methods employed would, as far as practicable, be compatible with the current methods employed by terrestrial Antarctic field researchers (BIOTAS Manual of Methods, Wynn-Williams 1992).

Although not accurately reproducing the Antarctic environment, growth cabinet work carried out under low PAR should enhance any UV-B effect. Also, the ratio of UV-B: UV-A: PAR can be approximated to values found in the Antarctic. Hence repair mechanisms, if present, should be operative. Small scale growth chamber studies can also help to identify potentially susceptible species that may be worth further field investigation, or monitoring as long term indicator species.

1.6 Experimental aims and objectives

The overall aims of this study were to investigate the effects of exposure to increased UV-B radiation on the photosynthetic performance of Antarctic plants, and possible adaptive strategies to that altered fluence. This would help to develop a better understanding on the possible consequences of early spring ozone depletion on the survival and development of terrestrial Antarctic ecosystems.

To maintain parity with the current field work being carried out by the British Antarctic Survey (BAS), initial studies were done using the cloche materials already deployed in the field i.e. UV-excluding and UV-transmitting 'Perspex'. This method can be criticised, e.g. for not allowing substantial UV-A penetration. However, the alternative of using 'Mylar' (and equivalent) controls also presents problems. Since the cut-off for 'Mylar' excludes most UV-B present in the field but not UV-A, this will alter the relative amounts of UV-A, UV-B and PAR, and thus is still not an ideal control procedure. Furthermore use of cellulose acetate has also been questioned as the material itself may alter plant growth (Krizek and Mirecki 2003).

The proposed work was to initially investigate effects of exposure to the UV-B waveband; to identify potentially susceptible species, then to focus on those discrete wavelengths that are most altered by ozone depletion. This is important as not all of the UV-B waveband is significantly changed due to ozone loss. It should then be possible to ascertain if any effect should occur under field conditions, and hence be relevant to the survival and productivity of that species.

It is well established that exposure to high (PAR) irradiance can reduce UV-B damage (Adamse and Britz 1992), so if no effect is apparent during the growth cabinet study, then it will be likely that there will be negligible effect in the Antarctic. Similarly, UV-A exposure can counteract UV-B damage (Quesada *et al.* 1995), so there should be a greater chance of UV-B-induced damage when using the BIOTAS cloche materials.

The objectives were to:

1. Study the effect of short term UV-B exposure on photosynthetic performance in cryptogams (using non-invasive methods of chlorophyll-a fluorescence and gas exchange), and identify sensitive species. In relation to this, investigate possible UV-B screening/ protective compounds that may be produced as a result of this short-term exposure (Chapter 3).

2. Study, in more detail, the photosynthetic performance of the hair grass *Deschampsia antarctica* and whether other changes in development/ morphology become apparent (Chapter 4).

3. Ascertain the wavelength dependence of any effects of UV-B exposure and construct effectiveness (action/ response) spectra (Chapter 5).

4. Predict, from the above objectives, potential consequences (on those sensitive species) of ozone depletion and possible reasons for differing protective mechanisms among the plants studied (Chapter 5).

5. Assess the physiological research equipment for use on cryptogams in a remote field setting. This arose from a fieldwork opportunity that presented itself during the course of the study (Appendix 2).

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

The basic premise for this thesis was to elucidate the effects of UV-B exposure on the physiology of photosynthesis. The techniques utilised may be used for basic screening for potential UV-B radiation sensitivity. One of the most important procedures used the rapid non-invasive technique of F_{vm} determination (Bolhàr-Nordenkampf and Öquist 1993). Other non-invasive techniques were used to further elucidate potential damage sites and/ or effects of UV-B radiation. This study also used Infra red gas analysis of CO_2 and H_2O exchange (Long and Hällgren 1993), combination with fluorescence quenching and polarographic measurement of oxygen exchange following the procedures initiated by Walker and co-workers (summarised in Walker 1988). UV-B protective/ screening compounds, especially flavonoids, have often been implicated as part of a UV-B response (Cockell and Knowland 1999) and were thus also investigated because these compounds protect the photosynthetic apparatus.

Morphological changes that have been noted as a consequence of UV-B exposure (e.g. Ruhland and Day 2000) could impact photosynthesis. The potential effects on morphology included leaf surface observations via scanning electron microscopy. Furthermore, as enhanced UV-B exposure can lead to an alteration in carbohydrate resource allocation (Gwynn-Jones 2001), the soluble carbohydrate content of the hair grass was also studied. These techniques were only used to complement the core physiology.

Some published studies have managed to manipulate UV and microclimate in the Antarctic (e.g. Day *et al.* 2001), the best alternative, growth cabinets, will not replicate Antarctic light and temperature environment, resulting in compromises. The UV-A: UV-B ratio was kept similar to that recorded in the Antarctic as this had been shown to effect possible UV-B response (Quesada *et al.* 1995). Temperatures were maintained as low as was practicable in growth cabinets, but these matched those attained during the author's fieldwork at Edmonson Point (Appendix 2).

Once any UV-B effect has been noted, it is then important to find out if that effect is potentially occurring be as a result of exposure to wavelengths < 320 nm, which will be most altered as a result of ozone thinning. Resolving the potential damage into those wavebands that would be most affected by ozone depletion was carried out using two principal methods: progressively filtering out shorter wavelengths using long pass filters, or, selectively enhancing wavebands using interference filters with or without a background light environment. This so-called action spectra work has not been carried out on any terrestrial Antarctic species prior to this study. The former was carried out in the growth cabinet, and the latter using a xenon lamp as a high UV output light source. Fluorescence measurements were chosen for this aspect of the work as they are rapid and non-invasive, allowing for long term repeated exposure to UV-B radiation.

2.2 Set up of enhancements

2.2.1 UV sources

a. Growth cabinets

A Fisons 600H (humidifier) 'Fi-totron'™ cabinet was used for most of the UV enhancement work. The cabinet was modified in the following ways:

1. Sheet window glass at the base of the light-box was replaced with 4 mm 'Sanalux'™ glass (Deutsche Spezialglas), which has the same optical properties as Schott filter WG 305, and has a broader cut-off than solarised cellulose di-acetate. The transmission spectrum of the 'Sanalux'™ glass is shown in Figure 2.1. An added advantage is that the transmission properties of the glass do not deteriorate with prolonged solar UV exposure (Holmes 2002). The primary function of the glass was to filter out the UV-C output of the UV emitting fluorescent lamps (see below), as this is never part of the incident solar fluence reaching the Earth's surface.

2. Four of the 12 white fluorescent lamps were replaced with two 313 nm and two 353 nm peak wavelength emission lamps (Q-Panel Co). Ballasts for the lamps were also changed to Magnetick rapid start ballasts (supplied by Q-Panel Co). These new lamps were also individually switchable. The transmission spectrum of the modified cabinet is shown in Figure 2.2. The individual peak maxima of the lamps under the 'Sanalux'™ shifted to ca. 323 nm and 351 nm respectively. The lamps were arranged in the following order; WWBAWWTWWABWW (W, white; B, 313 nm; A, 353 nm; T, tungsten). The experimental shelf was located approximately 45 cm below the lamp unit base, a position that was determined empirically to give uniform visible and UV light distribution.

3. The 313-nm lamps were wrapped in three strips of aluminium foil each 15 cm long along the ends and centre of the lamp. This reduced both the overall UV-B fluence and helped to equalise the UV-B fluence within the cabinet.

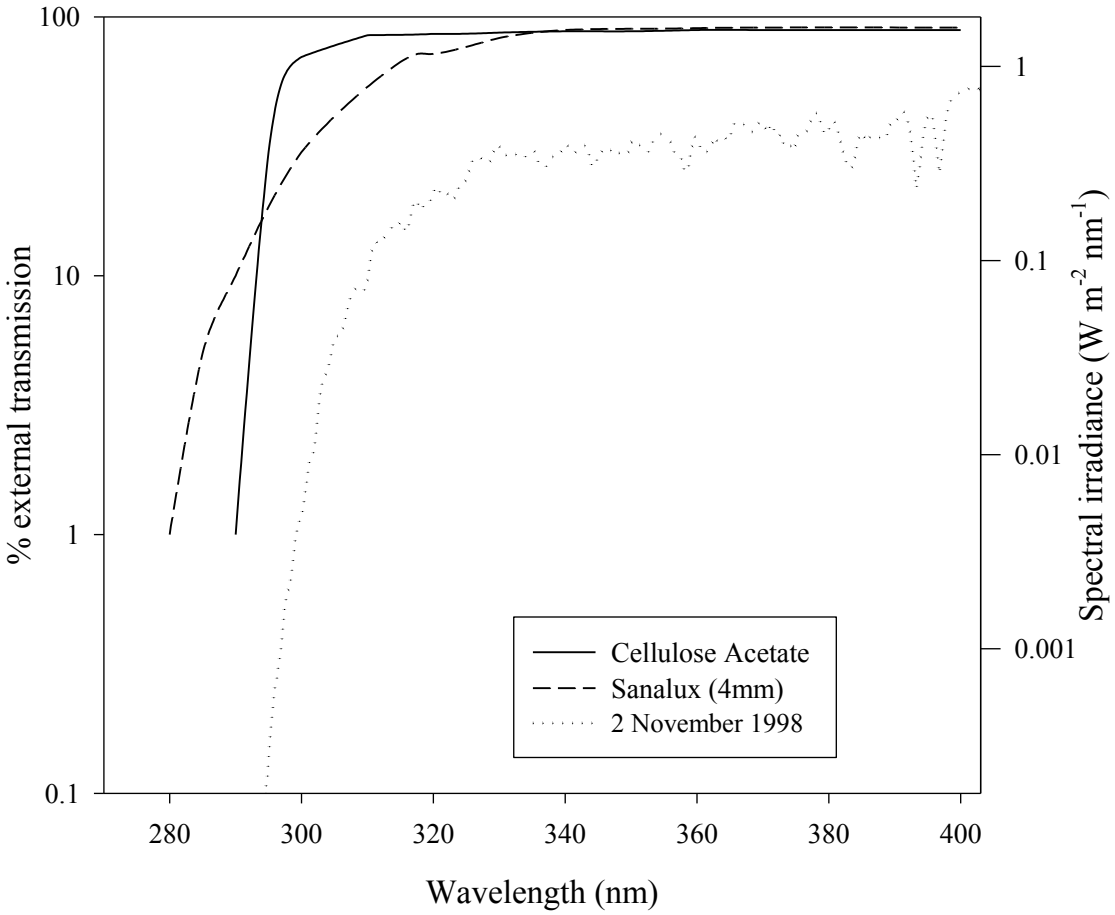


Figure 2.1 Spectral quality of UV-transparent 'Sanalux'™ glass used in the modified Fi-totron™ growth cabinet.
The properties of cellulose acetate, and a solar scan taken at McMurdo station, Antarctica during spring ozone depletion (170 DU, 2 November 1998) are also shown.
Antarctic data from NSF Spectroradiometer network.

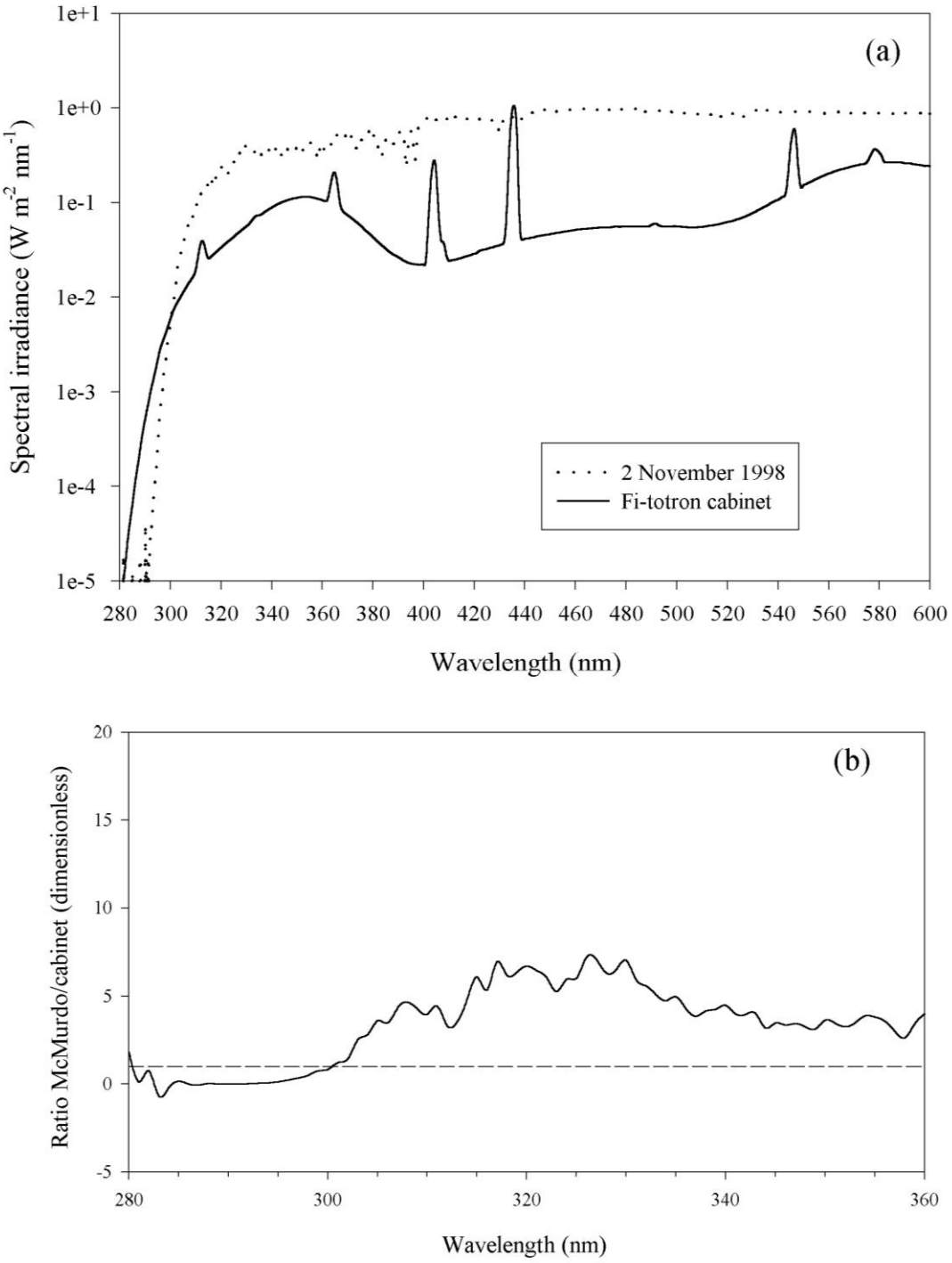


Figure 2.2 Spectral characteristics measured in the modified Fi-totron^{TM} growth cabinet in comparison with a solar scan taken at McMurdo station, Antarctica during spring ozone depletion (170 DU 2 November 1998).

(a) Spectral scan of growth cabinet and McMurdo.

(b) Ratio plot of same data (McMurdo/ cabinet) up to 360 nm with reference line (y = 1). See text for full explanation.

b Xenon arc lamp

A 1000 W high pressure, water-cooled xenon arc-lamp (Muller, Moosinning, Germany) was used to study action/ effectiveness spectra. The lamp was equipped with a dichroic filter to reduce heat load. The resulting output had a typical spectrum consistent with that of a xenon source (M. G. Holmes *pers. comm.* July 1997).

2.2.2 UV filters

To selectively exclude different wavebands of UV radiation, both within the growth cabinets and in the field; plastic filters were used and constructed into 'cloches'. The cloches were 100 mm high and fitted over standard (300 mm x 150 mm x 50 mm) plant trays. The materials used were 'OX/02', a plastic with 50% transmission at 272 nm; and 'VE' (both from ICI), which has 50% transmission at 415 nm and approaching 100% transmission at 430 nm. Transmission spectra of the materials are shown in Figure 2.3. The cloches used were two distinct shapes; standard BIOTAS design wedge-shaped cloche for fieldwork (Wynn-Williams 1992), and a cuboid for use in the growth cabinet. Another plastic, 'VA', (also from ICI), was obtainable in the UK, but only 'VE' and 'OX/02' material were available in the field studies so, to be consistent, subsequent UK-based work was carried out using the 'VE' and 'OX/02' plastics. Some of the transmittance data for 'VA' are shown for comparison.

2.2.2.1 Long pass filters

To obtain a greater discrimination between UV wavebands and improved resolution of potential UV effects, long-pass filters (2 mm or 3 mm depth, and either 5 cm x 5 cm or 5 cm diameter) were used (WG range, Schott, Germany). The transmission properties, transmission under cabinet lamps and PAS-weighted transmission of the filters are shown in Table 2.1, Figures 2.4 and 2.5 respectively. The differential irradiance (Figure 2.4) shows the relative UV enhancement of each filter up to 360 nm allowing for some separation of the UV waveband. However, when weighted to the PAS using the irradiance recorded in the growth cabinet, the most distinct filters were WG 305 and WG 320 respectively (Figure 2.5).

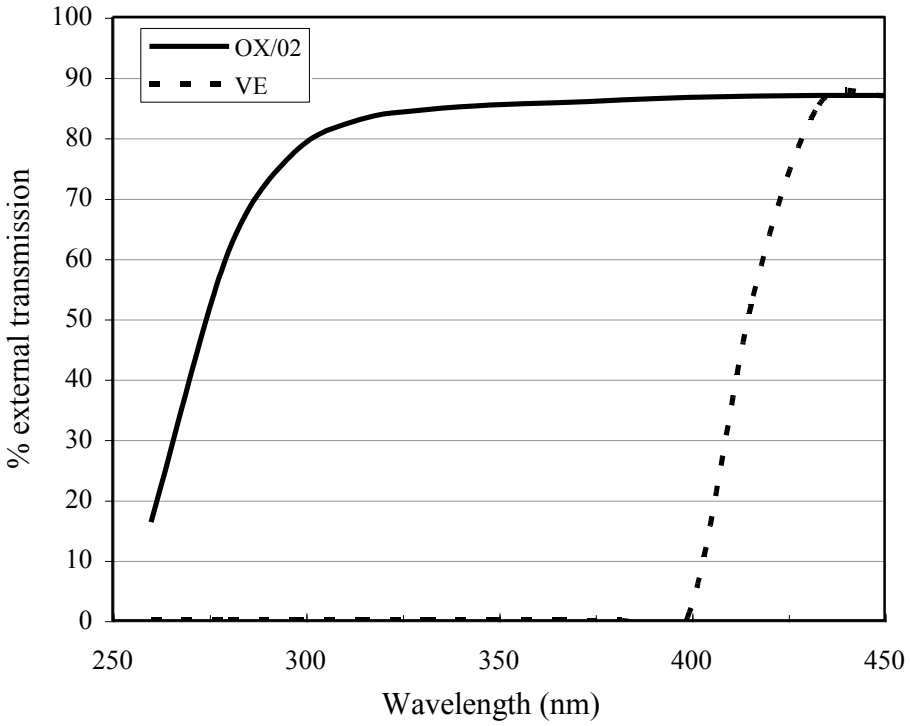


Figure 2.3 Transmission characteristics of the two main cloche materials used in the study; 'OX/02' and 'VE'. Samples were scanned using a Shimadzu UV-2100 UV-vis spectrophotometer.

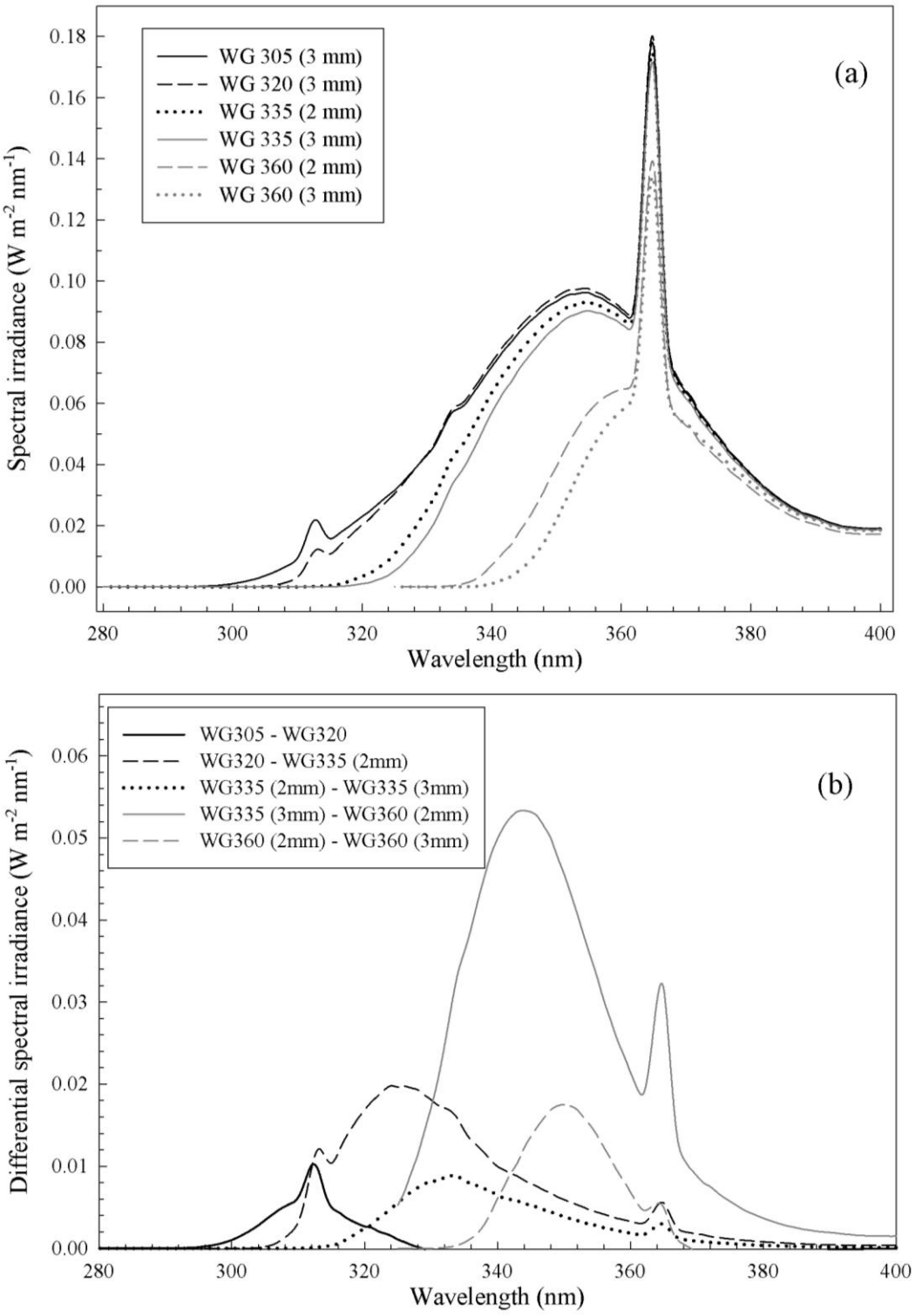


Figure 2.4 Effect of the long pass filters on the transmission of UV radiation (280-400 nm) in the Fi-totron™ growth cabinet. Irradiance measured in centre of cabinet directly under each filter using a MACAM SR9910 spectroradiometer.
(a) Spectral quality of the received irradiance.
(b) The differential irradiance of those filters ($\lambda_1 - \lambda_2$).

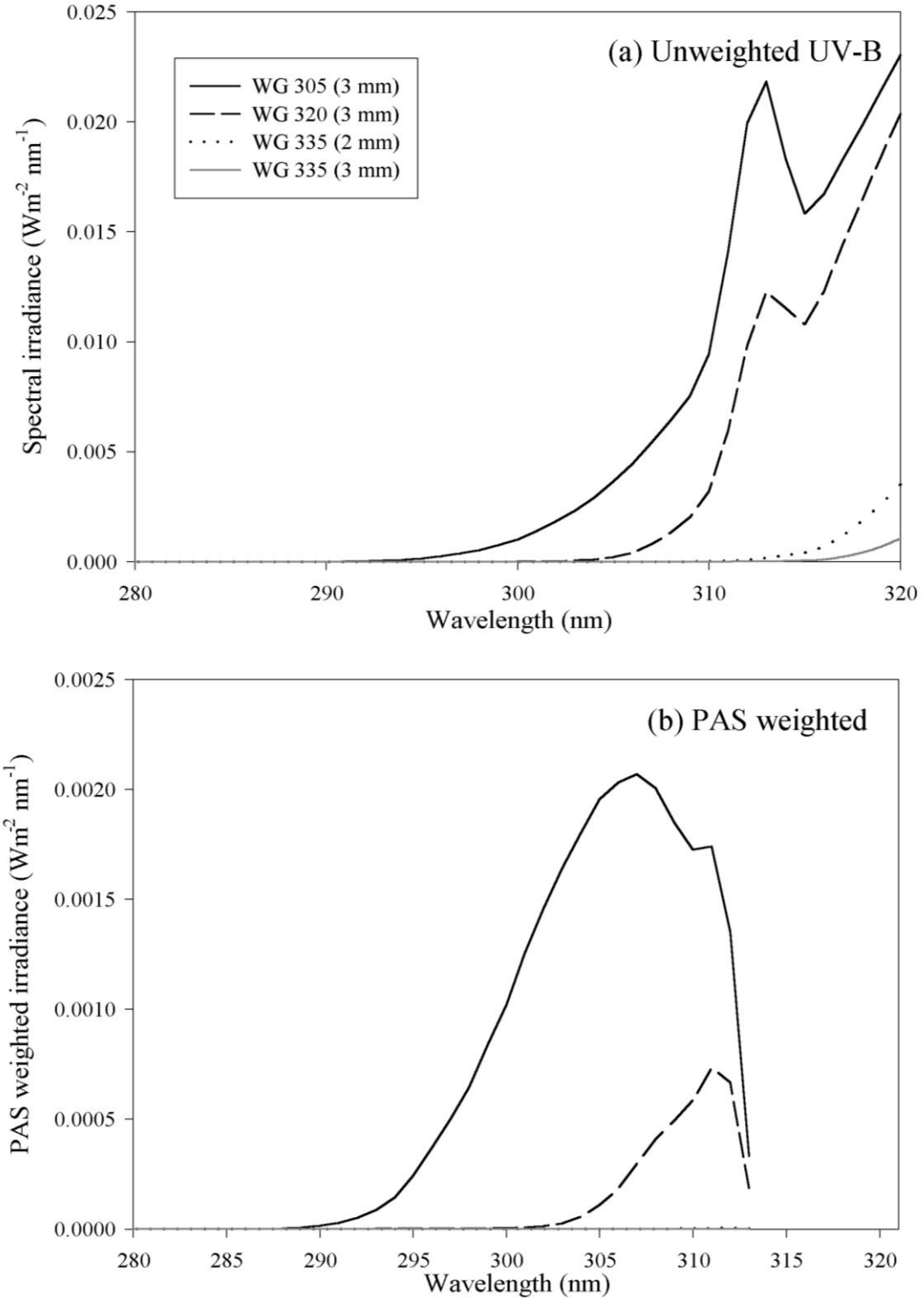


Figure 2.5 Effect of long-pass filters on the UV-B transmission characteristics in the Fi-totron™ growth cabinet.
(a) Unweighted UV-B radiation (280-320 nm).
(b) The same data weighted with the general plant action spectrum (PAS) as derived by Caldwell (see text for details).
See (a) for legend.

Table 2.1 Typical transmission properties of the Schott WG long-pass filters used in construction of the action spectra (see Figure 2.4 for spectral quality of the filters used in the study).

WG filter	Depth (mm)	50% internal transmittance (nm)
305	3	309
320	3	321
335	2	335
335	3	340
360	2	360
360	3	365

2.2.2.2 Interference filters

Interference filters (Oriel Co., USA) were used to study effectiveness/ action spectra work coupled with the xenon arc lamp. These filters allow for selective transmission of a narrow waveband (ca. 10 nm) of radiation. This is not possible with long-pass filters, as they completely exclude all lower wavelengths than their designation (Figure 2.4). All filters used were 25.4 mm diameter and the transmission properties are summarised in Table 2.2 below.

Table 2.2 Transmission properties of the interference filters used in construction of the action spectra.

Filter	Peak wavelength (nm)	50% transmission band-width (nm)	Minimum transmission (%)
290	289.4	11.2	12
300	300.0	11.4	15
310	310.0	12.0	15
320	321.2	9.2	25
360	360.7	9.1	25
380	381.7	10.5	25

2.2.3 Measurement of UV radiation and other microenvironment variables

The measurements and calculations of weighted UV-B doses were based on the function by Caldwell (1971), parameterised by Green *et al.* (1974), and termed 'plant action spectrum' (PAS); then normalised to 1 at 300 nm following Rupert (1982). The PAS-weighted dose is based on a summary of work done mainly using monochromatic action spectra. It has been widely used for plant studies involving UV-B manipulation (see Chapter 1 for full list of references). There have been criticisms of this weighting factor (e.g. Caldwell and Flint 1997), but because it is has been used so extensively it was thought the most appropriate weighting factor to use with emphasis on cross comparison between studies. Raw (un-weighted) values are also given wherever possible.

The (un-weighted) UV radiation from the xenon lamps was measured using a UV-enhanced silicon photodiode (SD112UV, Macam, UK) and connected to an LP102X power meter (Macam, UK), which was calibrated both for spectral response and linearity. The sensor was placed under the appropriate filter for recording of the fluence.

The spectral quality and output of the combined lamps used in the growth cabinet were measured using an SR9910 spectroradiometer (Macam, UK). This was calibrated against a National Physical Laboratory (NPL) standard light source. A representative spectrum during maximal Antarctic ozone depletion is shown for comparison (Figure 2.2). All measurements were taken at approximate growth height in the cabinet at 450 mm perpendicular to the lamp array. The UV-B exposures and those weighted to the PAS were calculated using the Macam proprietary software (Macam SPECTRO software version 6.17). PPFD, UV-A, UV-B, relative humidity and temperature were constantly monitored using a quantum sensor (Skye Instruments, UK), UV-A and UV-B broad-band sensors (both Delta-T Devices, UK), and a combined temperature and humidity sensor (Rotronic, UK). Whilst not accurately matching the transmission maxima of the lamps in the cabinet, the three light sensors were effective for continual, routine monitoring of the environmental conditions in the cabinet. The data from the sensors were collected using a Campbell 1000CRX data-logger, and automatically downloaded to a PC.

The output of the cabinet lamps was also measured using a Macam broad-band sensor under the individually lit UV lamps, with the sensor calibrated according to the maximal transmission of the lamp. This sensor was also used to measure the output of the xenon arc lamp.

Throughout the growth cabinet experiments, the sensors were periodically placed under the experimental cloches, to determine whether any of the UV parameters were changing with time. Periodic (rather than continual) placement was necessary because of space limitations. All data was collected and averaged over 300 s intervals.

The temperature in the cloches was maintained at 15^{o}C (day) and 7^{o}C (night). Whilst this does not reflect typical summer mean air temperatures of the maritime Antarctic nor Edmonson Point (both of which tend to be lower), the conditions provided were comparable with those that could be attained under cloches and on moss surfaces during the Austral summer (see Appendix 2). Earlier trials at lower, more realistic temperatures required the cabinet to undergo frequent defrosting cycles. This process regularly exposed samples to temperatures in excess of 20ºC, which are rare and irregular events in the Antarctic. Humidity was kept high within the cloches to prevent desiccation of the cryptogams, and also to prevent possible water stress in the vascular plants. Depression in photosynthetic productivity is known as a response to water stress in some plants, particularly mosses (Longton 1988).

The spectral data recorded in the cabinet show, at growth height, the broadly uniform fluence at the location of the plants in the central area of the cabinet. To alleviate any possible non-uniformity in the cabinet, the plants and the cloches were randomly rotated within this central area twice weekly. The UV-A:UV-B ratio remained constant through the working plane of the cabinet, and as such there was no particular bias in this ratio due to the location of the experimental material (Figure 2.6) This ratio was slightly lower than typical values recorded (using spectroradiometers) during the spring at three Antarctic sites (15-20 UV-A:UV-B; Rothera Point 67^{o}34’S, Palmer station 64^{o}46’S, and McMurdo station 77^{o}51’S). A summary table is given below (Table 2.3).

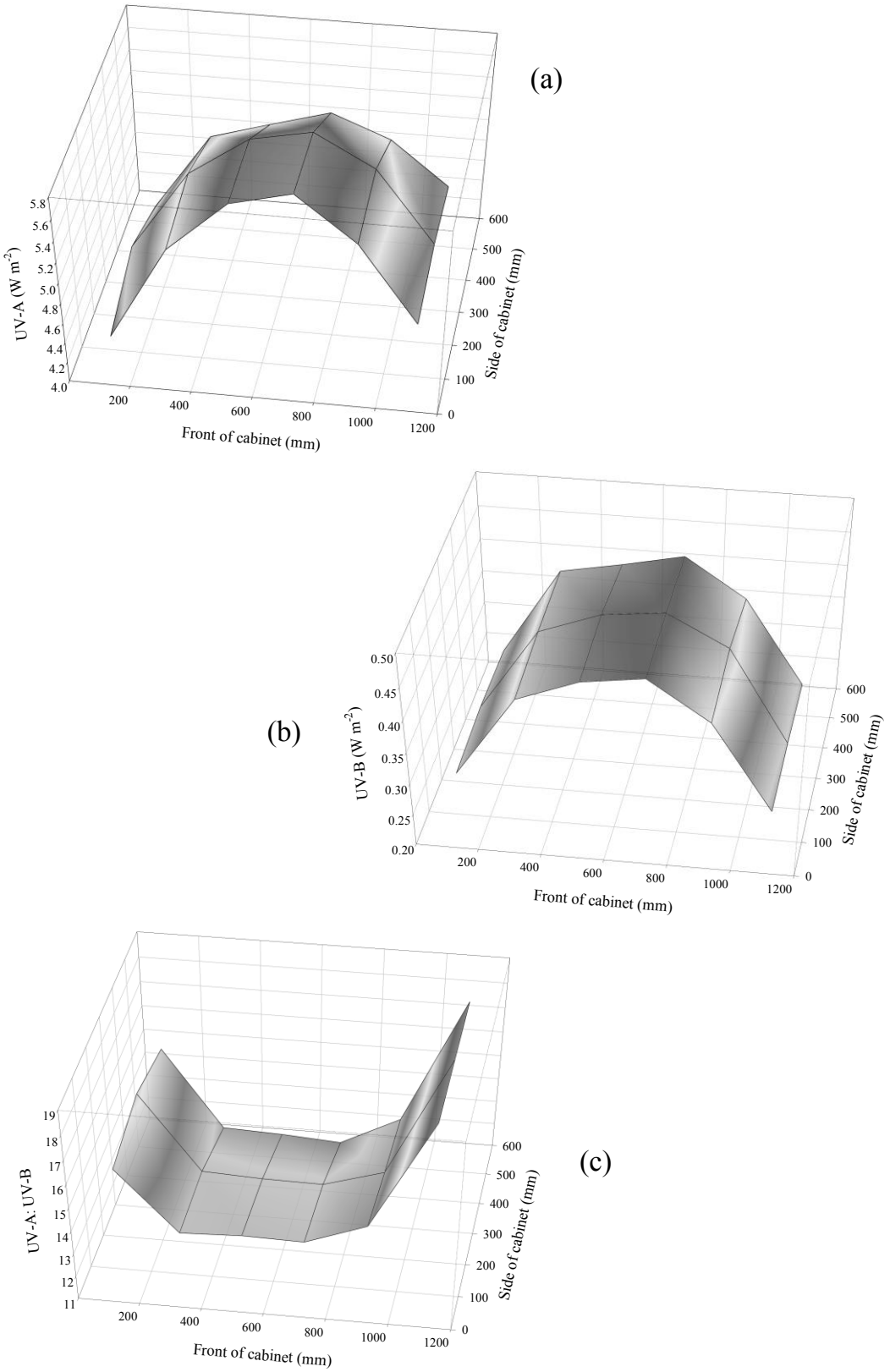


Figure 2.6 Relative spectral distribution in the Fi-totron™ growth cabinet.(a) UV-A, (b) UV-B, (c) UV-A:UV-B ratio. Irradiance recorded using MACAM SR9910 spectroradiometer.

Table 2.3 Summary data from spectral scans taken with the MACAM spectroradiometer at height of exposure. All values expressed as W m^{-2} except PPFD (μmoles m^{-2} s^{-1}).
n/a = value 10^{-5} or below (not within sensitivity range of instrument).

a. 12 points on working plane of 600 mm x 400 mm,

	Mean (± S.E)
UV-B (280-320 nm)	0.42 ± 0.03
PAS weighted	0.096 ± 0.007
UV-A (320-400 nm)	5.30 ± 0.07
UV-A:UV-B	12.6

b. Summary of data recorded at middle of working plane under different cloche types within the growth cabinet under different cloche materials. 'OX/02' (UV transparent), VA (UV-B opaque), 'VE' (UV opaque).

	Open	'OX/02'	VE	VA
PPFD	160.3	143.4	139.6	144.9
UV-B	0.442	0.384	n/a	n/a
UV-A	5.689	5.060	n/a	0.080
PAS weighted	0.101	0.085	n/a	n/a
UV-A:UV-B	12.87	13.17	n/a	n/a
PAR : UV-B (PPFD converted to W m^{-2})	362.7	373.5	n/a	n/a

The daily PAS-weighted dose under an 'OX/02' cloche calculated as 3.67 kJ m^{-2} and 4.89 kJ m^{-2} for a 12 h and 16 h day respectively. This is comparable to un-weighted UV-B values of 16.59 and 22.12 kJ m^{-2} for the same time periods. The UV-A irradiance under 'OX/02' was over 60 times greater than that for the VA material, despite the VA exhibiting 50% transmission at 400 nm.

The UV-B irradiance in the cabinet was compared to measurements taken between 1997-1999 from two Antarctic Peninsula sites, Rothera Point (67°34'S, 68°07'W) and Palmer Station (64°46'S, 64°03'W), and a continental site, McMurdo Station (77°51'S, 166°40'E) again using both raw and weighted values. The spectroradiometric data from the Antarctic sites all attained higher maximal instantaneous values than was recorded in the growth cabinet, and the daily weighted totals were also higher than the 12 h growth cabinet values (Table 2.4).

Table 2.4 Selected UV-B irradiance data from three spectroradiometer sites (maximum values for 1997/8 and 1998/9). PAS weighted data normalised to 300 nm (Palmer and McMurdo; data originally normalised to 280 nm, shown in brackets). Fi-totron™ cabinet shown for comparison (12 h total).

Site	Date	Instantaneous		Daily total (24 h)	
		UV-B ($W\ m^{-2}$)	Weighted ($W\ m^{-2}_{(PAS)}$)	UV-B ($kJ\ m^{-2}$)	Weighted ($kJ\ m^{-2}_{(PAS)}$)
Rothera	01 November 1997	3.46	0.45	n/a	n/a
	10 November 1997	3.56	0.46	n/a	n/a
	05 December 1998	3.12	0.36	n/a	n/a
Palmer	01 November 1997	3.82	0.55 (0.12)	100.6	12.1 (2.6)
	10 November 1997	3.60	0.51 (0.11)	108.6	13.4 (2.9)
	04 December 1998	4.38	0.55 (0.12)	113.5	12.6 (2.7)
McMurdo	01 November 1997	1.79	0.18 (0.040)	64.0	5.6 (1.2)
	22 November 1997	1.91	0.17 (0.036)	77.7	5.6 (1.2)
	28 November 1998	2.57	0.27 (0.059)	102.4	8.8 (1.9)
'Fi-totron'™ cabinet		0.44	0.10	19.0	4.32

As can be seen there is local variation in UV-B data especially between the Peninsula (Rothera and Palmer) where angiosperms occur, and the Continent (McMurdo) where the mosses and algae are dominant (see Figure 2.7 for locations). Moreover, looking at the calculated daily doses for McMurdo, the maximal values (normalised to 300 nm) are < 8.8 kJ m^{-2} (28 November 1998). This value is comparable with the 24 h continuous exposure achieved in the growth cabinets (7.3 kJ m^{-2}). The maximal values also occurred during the 1998 ozone depletion event, whilst in subsequent years, the maximal instantaneous values occur after the ozone 'hole' has reached its peak. The values at Rothera point for 1999-2000 yielded a mean daily dose irradiance of 4.1 kJ m^{-2} $_{(PAS)}$, with minimum and maximum values of 0.9 and 10.3 kJ m^{-2} (Newsham *et al.* 2002). The mean value is comparable with growth cabinet PAS-weighted exposures used (3.67 kJ m^{-2} and 4.89 kJ $m^{-2}{}_{(PAS)}$ for a 12 h and 16 h 'day' respectively), but are substantially lower than some published work (e.g. 32 kJ m^{-2}, Allen *et al.* 1997). The UV-B:PAR ratio was calculated using standard conversion factors (Table 2.3b, Holmes 1984). A similar ratio was found at the Antarctic Peninsula under lower ozone thickness (Newsham *et al.* 2002). Therefore it can be assumed that the light environment is not too dissimilar to what would be expected *in situ*.

The data from the broad-band sensors yielded slightly differing values compared to the absolute spectral data (Table 2.5). This was not unexpected given the different sensitivity of the broad-band sensors.

Table 2.5 Representative data measured using the broad band (Delta-T) and quantum sensors (Skye) in the 'Fi-totron'™ cabinet. All data expressed as mean ± S.E. Units, W m^{-2} except PPFD (μmoles m^{-2} s^{-1}).

	'VE'	'VA'	'OX/02'
PPFD	133 ± 0.18	136 ± 0.2	1.33 ± 0.10
UV-A	$4.2 \times 10^{-3} \pm 4 \times 10^{-5}$	$5.3 \times 10^{-3} \pm 5 \times 10^{-5}$	$2.69 \pm 3 \times 10^{-3}$
UV-B	$2.3 \times 10^{-3} \pm 6 \times 10^{-5}$	$2.9 \times 10^{-3} \pm 3.8 \times 10^{-5}$	$0.75 \pm 1 \times 10^{-3}$

2.3 Maintenance, cultivation and culture

The remit of the project was to try to determine sensitivity of photosynthesis to possible seasonal ozone depletion and thus UV-B enhancement amongst the Antarctic terrestrial vegetation. As such, a wide variety of plant material was used, coming from various locations throughout the Antarctic (sub-Antarctic, maritime and continental provinces). The three main locations for samples were Signy I. (60°43'S, 45°38'W) and Leonie I. (67°36'S, 68°20'W) both from the maritime Antarctic, and Edmonson Point, Victoria Land (74º20'S, 165º08'E) from the continental province (Figure 2.7 and 2.8).

2.3.1 Vascular plant (*Deschampsia antarctica*)

The plants used came from two sources. The first samples were clonal tufts propagated from material that had been collected from Signy I. during the 1994/95 season. These plantlets had been kept in cool storage on the transfer to the UK. Unfortunately there was only limited success (4%) with the regeneration of such plants, as most succumbed either to fungal infection, or did not survive transport. Surviving plants were maintained under 'VE' cloches for about 10 months before growing on for experimental work. The plants were grown in moss-peat based multi-purpose compost Fisons "Levington" (N : P : K ratio of 12 : 14 : 24), as this most resembled the soil conditions prevalent where the plants were collected (P. O. Montiel *pers. comm*. September 1995). Plants were watered twice weekly and additionally once weekly with a proprietary nutrient solution (P. O. Montiel *pers. comm.* September 1995, Miracle Gro, ICI, which contained urea, ammonium phosphate, potassium nitrate potassium phosphate and iron EDTA). Re-potting occurred when there were signs of the plants being pot-bound, with roots appearing from the base of the pot.

Numerous attempts were made to germinate seeds obtained from Signy I., which had also been returned in cool storage, but all had minimal (< 1%) success. Low germination rates were not unusual for plants from this location (R. I. Lewis-Smith *pers*. *comm*. June 1996).

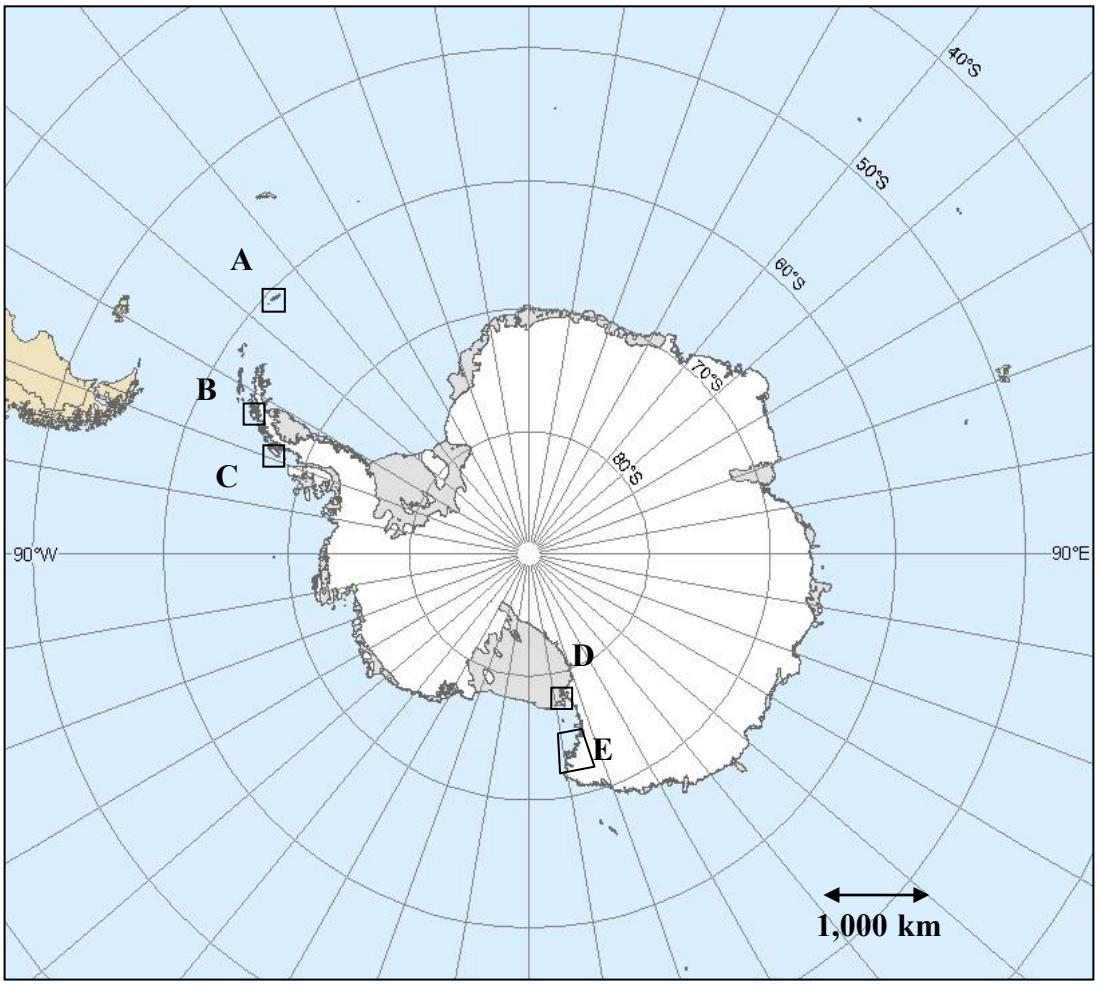


Figure 2.7 Showing locations where plant samples were obtained and the spectroradiometer sites from the Antarctic used in the thesis. A, Signy I. B, Palmer Station. C, Rothera and Leonie I. D, McMurdo Station. E, Edmonson Point Victoria Land. See Figure 2.8 for details of other Victoria Land locations within the Ross Sea sector.

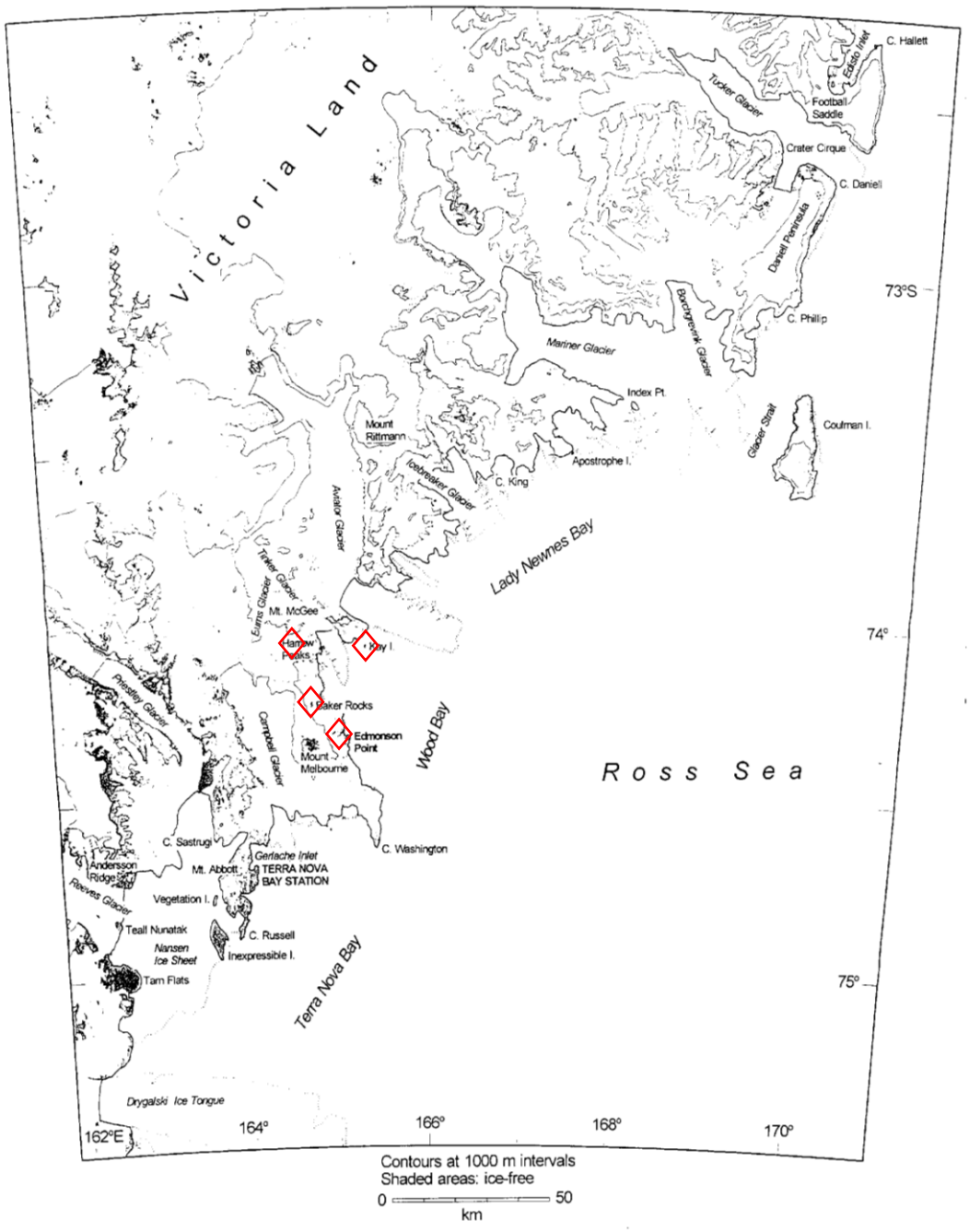


Figure 2.8 Ross Sea sector showing locations (◇) where plants were sampled in and around Wood Bay in relation to Victoria Land, East Antarctica. Modified from Wynn-Williams (1996 BAS internal report)

The second source of material comprised seed collected from Leonie I. during the 1996/7 season. Germination was attempted on seeds that were stored at -20ºC from fresh collection, again without success. Germination was also attempted with other seeds that had been collected at the end of the field season, air-dried then returned to the UK in bags containing a desiccant. The germination period, from imbibition to appearance of the coleoptile, took from one to three months. This latter group demonstrated a germination rate of ca. 20%. The regime that finally proved optimal was as described by Holtom and Greene (1967); seeds were removed from their caryopsis, using fine forceps, placed on moist filter paper and kept in darkness at about 15ºC. The authors had also found that varying the temperature during the 24 h cycle improved overall germination rate, which was not found to be the case for the limited amount of seeds in this study where all seeds received a chilling period prior to emplacement on the filter paper. The success rate was lower than Holtom and Greene (1967). After germination, maintenance of the seedlings was as described for the clonal tufts (above).

Physiological measurements using the infra-red gas analyser (IRGA) were obtained from intact, fully expanded leaves that displayed no evidence of senescence. One to three leaves were used for each recording (depending on cuvette type).

Germination trials of the pearlwort *Colobanthus quitensis* Kunth. Bartl. initially proved unsuccessful. However, seeds removed from the mature flowerheads collected Leonie I. during the 1996/7 season did germinate (50 to 75% germination). Unfortunately there was not sufficient time to expose these plants to altered UV-B fluence.

2.3.2 Mosses

Collection

All mosses used in this study were obtained from Edmonson Point. Samples were collected (where possible) in a frozen state in the field, and kept frozen until return to the UK. If this was not possible, the mosses were collected then air-dried prior to storage at -20ºC.

Cultivation

Attempts were made to cultivate a number of individual gametophyte shoots. Initially the shoots were grown on a number of types of inorganic nutrient agar, however the response was not different to that of isolated shoots grown on moistened filter paper. Neither technique provided sufficient biomass, during the time available for development, for experimentation. The mosses were also kept in their original turf, as both survival and, subsequently, new growth occurred in this state. Such new shoots, and/ or extended growth of the original shoots, were used as the experimental material wherever possible.

Prior to experimentation, plants were maintained for at least one month, initially at low PPFD (< 100 μmoles m^{-2} s^{-1}) for one week (bottom shelf of the growth cabinet under a UV-B opaque cloche) then transferred to the working shelf of the cabinet. The mosses were moistened regularly, with distilled water, and were also sprayed weekly with a nutrient solution (as used for *D. antarctica*) to minimise leaching of mineral nutrients.

Physiological measurements were taken by slicing approximately 5 mm from the top of the shoots, which were then spread on a moistened felt-pad (1000 mm^2) for use in the oxygen electrode. Cores (50 mm diameter) were taken from the moss turf for use in the IRGA.

2.3.3 Liverwort

The only liverwort species cultivated was *Marchantia berteroana*, collected from Fossil Bluffs, Signy I. Again this was kept continually moist, as it has been reported that even one instance of drying out caused loss of pigmentation and poor recovery of photosynthesis (Davey 1997). Accordingly, plant material was sprayed daily with distilled water and nutrient solution once weekly (as above). The liverwort was trialled on various growth media; agar, filter paper, vermiculite, and peat-based compost, which proved the most effective. Gemmae from the cups that were produced were removed using a cotton-wool bud and placed on the compost to grow. This allowed temporal separation of the material collected from Signy I., even though the material would be genetically identical.

Physiological measurements were taken using sections of undamaged thallus.

2.3.4 Algae

The only alga that was used in these studies, *Prasiola crispa*, was collected in a naturally frozen state from Victoria Land. Plants were split into individual thalli, placed on moist filter paper (Whatman N^{o} 1) in a 50 mm diameter Petri dish; this was found to give greatest longevity of the alga. As before, the plants were again sprayed with a nutrient solution once weekly, and kept moist with distilled water. The plant material was placed under a UV-B opaque ('VE') cloche until required. Physiological measurements undertaken in the oxygen electrode were obtained from a single piece of thallus (ca. 1000 mm^2), which was placed on moistened felt pad.

Time constraints prevented additional work on Antarctic lichens.

2.4 Set-up of combined oxygen electrode and fluorimeter

2.4.1 Equipment overview

Measurement of photosynthesis (net oxygen exchange) and photosystem II (PSII) fluorescence were taken using a customised semi-automated system. Control of this system was co-ordinated by a dedicated Acorn BBC microcomputer with enhanced memory and custom software. The cassette motor output controlled the saturating light-pulse shutter, whilst actinic (LED) light intensity was controlled using the 8-bit user port, allowing a total of 256 intensities (including zero).

The basic polarographic oxygen measuring system consisted of the following (Hansatech unless stated otherwise): LD2 gas-phase electrode chamber, CB1 control-box, LH36 High intensity 36 LED array light-source, and LC1 light-source control box.

PSII fluorescence was measured using an MFMS/2S single channel modulated fluorimeter. This had a light source of 8μE m^{-2} (maximum intensity) with a modulation frequency of 4.8 kHz and central wavelength of 583 nm, coupled with a 700 nm PIN diode detector. During the measurements, response time was set to 1.0 s, whilst modulation intensity and gain were adjusted depending upon the type of material used. Fluorescence maximum was determined by comparing each fluorescence reading with the previous maximum over the 1 s duration of the flash, sampling every 10 ms (i.e. 100 values sampled to obtain maximum; and compared visually with chart output).

Saturating light pulses were supplied from a FLS1 source, using a 75 W dichroic quartz-halogen light source (Osram) coupled with IR blue filter (Corning 4-96) to reduce heat load, and a single neutral density filter. A NiCd thermocouple (RS Components) inserted through gas port of the LD2 unit was used to record chamber temperature.

A Rinkadenki chart recorder provided a permanent fluorescence trace. The chart-recorder was typically set to 100 mV full deflection, with a speed of 5 mm min^{-1}. The chamber temperature was controlled using a circulating water bath (Haake EK12 cooling unit coupled with a Techne TE8J circlotherm unit), containing ultrapure water and 0.05% w/v sodium azide to inhibit algal/ bacterial growth. LED fluence was measured using a Skye SKP200 red-light sensor dedicated for use with the LED source. Oxygen evolution was measured for 180 s per light level with a linear regression fitted to approximately 1000 points collected over the final 30 s. The fluorescence characteristics were defined and calculated according to van Kooten and Snel (1990).

The electrolyte solution used for the oxygen electrode was based on that recommended by Walker (Walker 1988). Saturating (5%) CO_2 concentration was provided by a felt-pad moistened with 1.0 M $NaHCO_3$ adjusted to pH 9 with 2.0 M K_2CO_3. This concentration was used so as to overcome any diffusion limitations within the plant, and was standard practice when using the oxygen electrode (Walker 1988). Distilled water was used to moisten the pad that the living material was placed on. All chemicals used were BDH ANALAR grade. The computer control program was written in BBC BASIC.

2.4.2 Set-up and operation

The electrode was set-up following Walker (1988), and given (if necessary) up to 2 h to stabilise. Leaks were checked by injecting a set volume of air and also by withdrawal of a set amount (see below).

The electrode was considered suitable if the response time after injection of 0.5 ml of air was greater than 80% of full deflection in less than 5 s and that there was no appreciable loss in voltage for at least 15 s. Once set up, such an electrode would typically be stable for up to 36 h, after which time either excess deposition of silver chloride on the silver anode, or drying of the electrolyte led to failure of the electrode and/ or slowed response time.

Calibration of the electrode was achieved by injecting 0.5 ml of air with a known volume of oxygen. The screen display was adjusted to a set voltage response, which was automatically corrected for temperature, and changes in overall chamber volume that could vary depending upon sample.

The light response settings were set according to location of work. For field work in the Antarctic, 14 steps to ca. 1000 μmoles m^{-2} s^{-1} were used. The maximum light setting for samples in the growth cabinet studies was set to approximately 500 μmoles m^{-2} s^{-1}, rising in 15 steps. The differential between the two resulted from the maximum PPFD in the growth chamber being 160 μmoles m^{-2} s^{-1}. There should therefore be no induction of photoinhibition amongst the growth cabinet samples, which could interfere with, or mask any UV-B effect. Temperature was set at that used in the growth chamber (approximately 14^{o}C), unless otherwise stated. All samples were acclimated in the chamber for approximately 5 to 10 min, or until a stable reading was achieved. The initial light limiting stage should give an indication of the quantum

efficiency of photosynthesis, whilst the maximum rate should give an indication of the capacity of photosynthesis and of stress induced effects (Walker 1988).

The fluorimeter was set up according to the instructions in the Hansatech manual, and following the procedures established by Schreiber *et al.* (1986). A modulated fluorimeter has the advantage over an ordinary fluorimeter, in that it allows for direct measurement of F_o, which is not otherwise possible in a non-modulated system. Moreover, it allows the sample to be continuously illuminated whilst fluorescence measurements are taken. Accurate measurement of F_o is vital as many other important fluorescence parameters are derived from it (Bolhàr-Nordenkampf and Öquist 1993). The setting used for the plants are summarised in Table 2.6. The dark adapted PSII fluorescence ratio F_{vm} was calculated as $F_{vm} = (F_m - F_O) / F_m$. The measured parameter F_O is considered to be the fluorescence level where all reaction centres are open (not reduced) and F_m is when all reaction centres are closed (reduced) where photochemical quenching (qP) is maximal. Both qP and non-photochemical quenching (qN) were calculated using the equations of van Kooten and Snel (1990):

$$qP = (F'_m - F) / (F'_m - F'_O)$$

$$qN = 1 - \{(F'_m - F'_O) / (F_m - F_O)\}$$

The quenching parameter qP decreases to a minimum with increasing light intensity and is proportional to the number of open reaction centres. The ratio qN is thought to be related to changes in transthylakoid pH gradient, CO_2 fixation heat dissipation or xanthophyll cycle (Krause and Weiss 1991). It tends to increase to a maximum with increasing light intensity. Fluorescence yield, given by $(F'_m - F) / F'_m$, is related to rate of electron transport and proportional to quantum yield of CO_2 fixation (Genty *et al.* 1989).

2.5 OS-100 Fluorimeter

An OS-100 modulated fluorimeter (Opti-Sciences Inc.) was used for stand-alone fluorescence measurements. This used a 35 W quartz-halogen lamp as saturating source coupled with a 690 nm short-pass filter. Modulated light was supplied by a 660 nm LED source, in conjunction with a 690 nm short pass filter. This had an adjustable intensity of 0 to 1 μE; increasing from 1 to 225 points. Detection was by a PIN photodiode with a 700-750 nm band-pass filter. The frequency lock-in amplifier had auto switching from a sampling rate of 10 to 1000 points s^{-1} according to the test phase selected. The fluorimeter was used as a stand-alone system, or as part of the windows-based set-up using the OSLOG proprietary software. The plastic clips supplied with the fluorimeter were used to keep the probe at the same distance from the sample.

There were two ways of adjusting the system to provide a suitable fluorescence signal; the modulation intensity (MI), and the duration of the saturating pulse (SP). Generally a SP of 0.8 to 1.0 s was found to be sufficient. The lowest MI value was used, as it was less likely to stimulate variable fluorescence (although the maximal value of MI is still very low (1 μE at the tip of the probe). A signal from the plant of 60 to 100 fluorescence units provided stable results. This was induced by a MI of 100 (< 0.5 μE) using *Prasiola crispa* at a distance of 15 mm from the probe tip.

There were a number of precautions that had to be observed when using either the stand-alone or PC-driven system. Most of these were determined empirically during use of the equipment. The stand-alone system required a minimum fluorescence (40 units) before a pulse could be triggered. This signal was dependent upon MI value, which was again dependent on the amount of active reaction centres exposed to the beam. This was a problem, particularly when using lichens. It was only possible to save one trace during operation, so each sample had to be manually recorded if there were a need to study a number of traces. Furthermore, the OSLOG program was found to have a number of software problems, which resulted in the program terminating, with loss of all current data. Fluorescence parameters and derivatives were calculated as described above (i.e. F_{vm} and fluorescence yield. Settings used are shown below (Table 2.6).

Table 2.6 Summary of fluorimeter setting used in the thesis.

Species	OS-100	Hansatech fluorimeter
Prasiola crispa	MI 100 SP 0.8 20 min dark	LSI 6 Gain 0.1 20 min dark
Marchantia berteroana	MI 100 SP 0.8 20 min dark	Not done
Sarconeurum glaciale	Not done	LSI 8 Gain 0.1 30 min dark
Tortula princeps	MI 100 SP 0.8 20 min dark	LSI 6 Gain 0.2 30 min dark
Bryum argenteum	Not done	LSI 7 Gain 0.1 30 min dark
Ceratodon purpureus	Not done	LSI 5 Gain 0.2 30 min dark
Deschampsia antarctica	MI 100 SP 0.8 5 min dark	Not done

2.6 Infra-red gas analysis

2.6.1 Equipment overview

Measurements of CO_2 and H_2O exchange were taken using an open, differential infrared gas analysis (IRGA) system (CIRAS-1, PP-Systems). The analyser operates using two separate pairs of IRGAs, one optimised for CO_2 measurement and the other for H_2O measurement. Circulating air within the leaf cuvette can be conditioned to a known CO_2 concentration as the CIRAS has its own integral CO_2 supply. Similarly, humidity can be controlled using water vapour absorption and/ or moistened filter paper placed over the humidity conditioner. Accuracy is maintained by automatic self-balancing using an absolute zero protocol and also by differentially correcting each of the pair of IRGAs.

Three different cuvettes were used, allowing a variety of plant material to be analysed. A narrow-leaf type PLC(N), designed for use with grass blades and similar structures, which can accommodate a leaf up to 45 mm long. A broad-leaf type PLC(B), designed for dicotyledonous leaves with a maximal enclosed area of 250 mm^2 and had temperature and PAR control. The third cuvette, a moss type PLC(M) (mk I); a prototype set-up that was only tested and used at Edmonson Point (see Appendix 2 for a description of use and evaluation in the field).

The design and assessment of the moss cuvette was one of the objectives for the Antarctic field season in which the author took part (Appendix 2).

The IRGA and cuvettes were used for four types of recording:

1. Instantaneous field measurements using PLC(M) mk I (5 cm diameter moss cores, Edmonson Point).
2. Measurements taken at two levels of PPFD using the PLC(N) (single leaf, *D. antarctica*).
3. Recordings taken under non-limiting PPFD at varying CO_2 concentrations (A/c_i response) again utilising the PLC(N) (single leaf, *D. antarctica*).
4. Recordings taken under saturating CO_2 concentration (greater than 1000 μmol mol^{-1}) at varying PPFD; a light response curve utilising the automated PLC(B) (three leaves, *D. antarctica*).

Instantaneous measurements taken in the field were defined by making a record of gas exchange as soon as the response had stabilised upon enclosure within the cuvette (typically 20 to 30 s). Owing to the small chamber size of the standard cuvettes, coupled

with technical difficulties in maintaining tissue water content (and its known influence on photosynthesis in cryptogams), no UK-based experiments utilising the IRGA were taken on the cryptogams. The use of a water-saturated cryptogam in a small cuvette and use of flowing air with a high relative humidity presents considerable technical difficulties with possible deposition of the water vapour droplets in the analyser tubes, leading to loss of accuracy and possible damage to the IRGA (for details see CIRAS user manual). Whilst later work did show potential use for the liverwort, time limitations didn't allow for expansion of this work.

2.6.2 Set-up of the IRGA (and palm-top control)

CO_2 conditioning for the CIRAS system was applied by a 'Sparklet' soda gas cylinder, with each cylinder typically lasting up to 48 h. The raw measured data from the IRGA were; CO_2 reference, CO_2 differential, H_2O reference, H_2O differential, atmospheric pressure, flow rate, and temperature. Set values were also input; transmission co-efficient of the glass fronted cuvette (for leaf temperature determination), and boundary layer resistance (calculated using a replica leaf). Calculations made using the IRGA from the raw data were based on the equations derived by von Caemmerer and Farqhuar (1981). Flow rate settings varied, depending on the cuvette used, and were as recommended by the manufacturer.

The IRGA could be used as a manual stand-alone system, or under semi-automated control, by connection to a palm-top computer (Hewlett Packard HP1000cx), following manufacturer's instructions.

2.6.3 Use of the IRGA in laboratory work

Three methods were utilised; all using attached, fully expanded leaves from *D. antarctica*. For measurements at two levels of PPFD (varying PPFD), settings were chosen that matched the growth chamber as much as possible (ambient CO_2 concentration and humidity). Illumination was by a quartz-tungsten halogen (QTH) lamp.

For light response curves, the combined PPFD source (QTH lamp, PP Systems) broad-leaf cuvette was used. The light response settings were set to rise to a PPFD of 900 μmoles m^{-2} s^{-1} in twelve steps. Saturating CO_2 was maintained at 1000 μmol mol^{-1}. The linear regression at limiting light intensities gives quantum yield and the maximal rate at non-limiting light intensity provides the maximal rate.

The A/c_i response curves were constructed in 10 to 11 steps to an external CO_2 concentration of about 1200 μmol mol^{-1}. A PPFD of 500 μmoles m^{-2} s^{-1} was used as this was found to be sufficient vis-à-vis due the growth cabinet PPFD of ca. 160 μmoles m^{-2} s^{-1}. Furthermore, this level should not induce any photoinhibitory events. All measurements were taken at ca. 15°C (similar to the ambient temperature of the growth cabinet during the photoperiod). The linear regression for the initial part of the individual data curve gives an estimation of the A/c_i derived parameter carboxylation efficiency ($V_{c,\ max}$). This indicates efficiency of the primary carbon-fixing enzyme Ribulose 1,5 bisphosphate carboxylase/ oxygenase (Rubisco) (Long and Hällgren 1993).

2.7 Compound extraction

Chlorophyll extractions were used to provide a basis for rate calculations. Surface area and fresh mass may not always be the most appropriate measure of photosynthesis for many of the cryptogams (as a result of their poikilohydric nature and morphology). Photosynthesis rates were expressed per unit chlorophyll when possible on account of the heterogeneity of the plant material. Furthermore, alteration in the amount of these pigments has previously been reported as an effect of UV-B exposure (Teramura 1983). Extractions were carried out under minimal visible light.

Phenolic compounds in both higher plants and some (but not all) bryophytes have been postulated having a protective role against UV-B exposure (see Chapter 1 p. 14 *et seq*. for references). To ascertain whether this was also being undertaken in the Antarctic vegetation, crude extractions of these compounds were carried out in the bryophytes and the hair grass. Mycosporine like amino-acids (MAAs) have been postulated for use as a UV-B screen in algae (Karentz *et al.* 1991, Garcia-Pichel and Castenholz 1993), thus crude extracts were taken from samples of the alga *P. crispa* to determine if the quantity of these compounds was altered by UV-B exposure.

Ultimately, changes in productivity as a result of UV-B exposure should impact on carbohydrate acquisition and thus composition of water and ethanol-soluble sugars. Possible changes in primary productivity and the effect on non-structural carbohydrates with regard to UV-B exposure have not been studied in any detail in the Antarctic flora, though there has been some work on general carbohydrate metabolism of *D. antarctica* (Zúñiga *et al.* 1996).

2.7.1 Extraction of chlorophylls and carotenoids

Whilst it was hoped to minimise the number of methods used, this was not always possible as a result of the variety of experimental material used. Where possible, solvents contained calcium carbonate to minimise phaeophytinisation. Extract volumes were made to a total of 5 or 10 ml depending on mass of material. Three methods of extraction were employed.

i. *Deschampsia antarctica*. Fresh mass was determined, then samples were (if required) cut into approximately 1 cm long pieces prior to quenching in liquid nitrogen. The samples were then extracted immediately or stored at -80ºC. For extraction, the plant fragments were plunged into 4 ml of calcium carbonate saturated methanol (HPLC grade, Sigma-Aldrich, UK). The samples were then steeped at 40^{o}C (until the leaves appeared bleached), before centrifugation at 8,000 r.p.m. for two min. to remove particulates (P. O. Montiel *pers*. *comm*. June 1996). Total chlorophyll and carotenoid content was determined by UV-vis spectrophotometer (Shimadzu UV-2100). Pigment concentrations were calculated using the standard equations of Lichtenhaler (1987). The rest of the sample was stored at -80^{o}C for HPLC analysis. This method showed no substantial phaeophytinisation in the subsequent HPLC analysis.

ii. Bryophytes and *D. antarctica* samples taken after photosynthesis measurements. Plant material was quenched in liquid nitrogen, freeze-dried, and stored in darkness under vacuum until needed. Each sample was then weighed and ground using a pestle and mortar. A known mass of the sample was extracted overnight in calcium carbonate saturated methanol, centrifuged at 8,000 r.p.m. and the pellet re-extracted until white. Samples were made up to a known volume with the solvent. The sample was then used immediately or stored at -80^{o}C. This method was found to be most effective and allowed for easy transfer to HPLC analysis.

iii. Chlorophylls from *P. crispa* were extracted by steeping small pieces of thallus in calcium carbonate saturated dimethyl sulphoxide (DMSO) following the method of Ronen and Galun (1984), but kept at room temperature. This was found to be the most effective solvent for chlorophyll extraction of this alga, as other solvents still left green tissue despite repeated grinding and re-extraction.

2.7.2 Extraction of phenolic compounds.

Two slightly different methods were used, depending on source of material or original extract.

i. *D. antarctica*. Glacial acetic acid (GAA) was added to the ethanolic extracts (already used for soluble sugar analysis, see below) to a final concentration of 0.1% v/v (P.O. Montiel *pers*. *comm*. June 1996).

ii. *D. antarctica* and bryophytes. Extraction followed the basic procedure of Markham (1982). Samples (either fresh or dried) were extracted first in a methanol and water mixture (ratio 9:1) then ground to a slurry. After centrifugation and storage of the supernatant, the pellet was re-extracted in a 1:1 v/v mixture of methanol and water. The supernatants were combined and then freeze-dried *in vacuo* and kept at -80ºC until used. The residue was re-suspended in 1 ml ultra-pure water for HPLC.

A comparison between method (i) and (ii), showed that no differences in either peak number or relative height of the peaks could be discerned in chromatograms from *D*. *antarctica* regardless of sample preparation and extraction method.

2.7.3 Extraction of mycosporine-like amino-acids

All extractions were performed using dried tissue. The extraction followed that of S. Ertz (*pers*. *comm*. September 1995). Small pieces of tissue (ca. 100 mg) were extracted in 20% v/v methanol. The extract was kept at 12ºC, and agitated for 24 h in darkness. Samples were then centrifuged and passed through 0.5 µm filters. Immediately afterwards samples were scanned from 280 to 400 nm using a UV-vis spectrophotometer (Shimadzu UV-2100).

2.7.4 Extraction of water and ethanol soluble sugars.

This method was based on that used for extraction of water and ethanol-soluble sugars from barley seedlings (Smith unpublished m/s, Farrar 1992) and that of P. O. Montiel (*pers*. *comm*. June 1996). The tissue was weighed, and then ground in 84% v/v aqueous ethanol under liquid nitrogen. A known amount of an internal standard (treitol) was added, and the extract was incubated for 2 h at 60^{o}C. The sample was centrifuged after extraction for clarification; the resulting supernatant was decanted and stored at 4^{o}C. The pellet was re-suspended in ultra-pure water and further incubated for 15 h at 40^{o}C. Following incubation the extract was sequentially rinsed then centrifuged, and

the resulting supernatants combined. The extract was dried *in vacuo*, and stored at -80^{o}C. The dried sample was re-suspended in a known volume of ultra-pure water for HPLC/ electrochemical detection.

2.7.5 High Performance Liquid Chromatography (HPLC)

2.7.5.1 HPLC and diode array detection (DAD).

HPLC was performed using a Kontron automated gradient system consisting of a ternary pump system (model 325), auto-sampler (model 360) and a diode array detector (DAD). All mobile phases were filtered (to 0.5 μm), kept under helium sparging and in-line de-gassed. The DAD unit is capable of measuring at seven separate wavelengths and also a continuous spectrum with 4 nm resolution throughout the chromatogram run.

2.7.5.1a Chlorophylls and carotenoids.

This method was based on that modified by P. O. Montiel (*pers*. *comm.* June 1996). A silica bonded 5 μm size column (Prodigy ODS3 40 x 150 mm) coupled with a 40 x 50 mm guard column. The injection loop for the experiment was 60 μl, flow rate was 1.25 ml min^{-1} over the 30 min. run time, with column temperature kept constant at 30^{o}C. The following mobile phases used: A- ammonium acetate (40 g l^{-1} in water) 1: 4 absolute methanol. B- absolute methanol 3:2 absolute acetone (all solvents used were HPLC grade). A typical solvent phase protocol is shown in Figure 2.9.

The following standards (all from Sigma) were also used: chlorophyll-a, and chlorophyll-b, alpha- and beta-carotene and xanthophyll.

2.7.5.1b Phenolic and related compounds.

This method was based on that by Lunte (1987) and was further developed in conjunction with P.O. Montiel (BAS). The column described above was used, temperature was kept at 30^{o}C, with a flow rate of 1.5 ml min^{-1} for the run time of 45 min. The injection loop was 60 µl. The mobile phases used were: A- 50 mM ammonium dihydrogen phosphate, pH 2.5. B- absolute acetonitrile. Again all solvents were HPLC grade. A typical solvent phase protocol is shown in Figure 2.10.

External standards, orientin and tricin (obtained from Plantech, Reading), were used for work on the hair grass. These flavonoids were the only ones previously identified in *D. antarctica* (Webby and Markham 1994), also known to occur in other plant species, and thus readily available. They were used to try to identify the resultant peaks from the chromatograms. The standards (catechin and flavone) were used for the analysis of the moss samples, which were obtained after the experimental period.

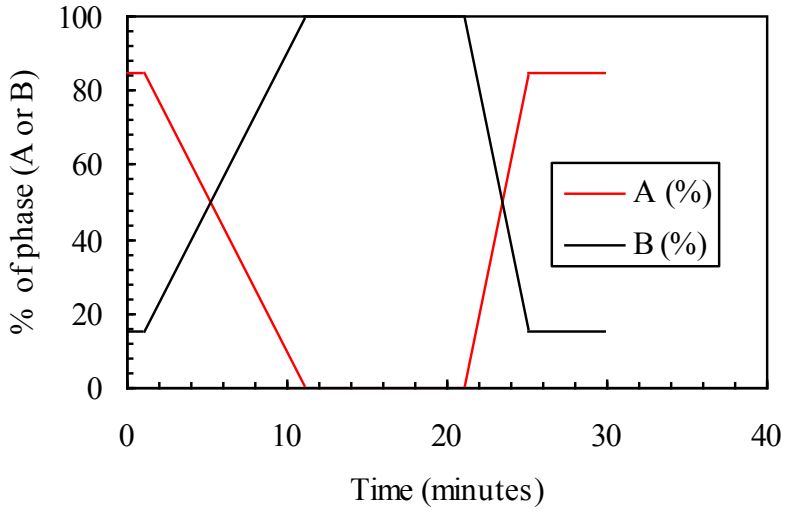


Figure 2.9 Solvent phase used for chromatographic analysis of chlorophylls and carotenoids.

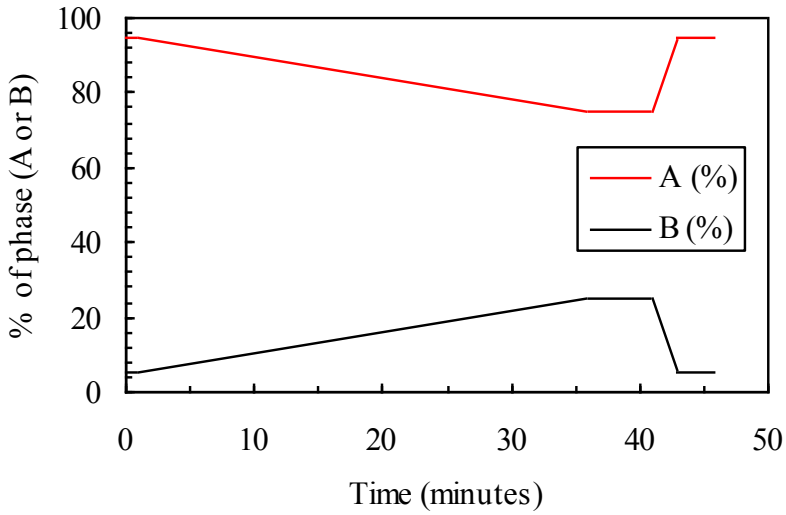


Figure 2.10 Solvent phase distribution for chromatographic analysis of phenolic-like compounds.

2.7.5.2 HPLC and electrochemical detection of carbohydrates.

The equipment was as detailed above but detection was by an ANTEC DECADE (Digital Electrochemical Amperometric Detector) detector. This method was based on that used on Antarctic specimens by P. O. Montiel (*pers*. *comm*. June 1996). It used a CARBOPAC MA1 column at constant temperature of 30^{o}C and 0.4 ml min^{-1} flow rate (run-time 46 min.). The volume of the injection loop was 50 µl. The mobile phase used was 600 mM sodium hydroxide. Standards used were *m*-inositol, treitol, sucrose, fructose, and glucose (all HPLC grade from Sigma).

2.7.6 Spectrophotometry

All UK-based spectral analysis of samples was carried out using a Shimadzu UV-2100 scanning spectrophotometer, with optional integrating sphere attachment. UV transparent quartz cuvettes (10 mm path length) were used throughout. Zeroing was achieved using a solvent extract blank over the desired wavelength range. Estimation of phenolic and MAA content was achieved by taking selected absorption maxima and dividing by the sample mass. Similar mass and volume values were used for each experimental extraction.

2.8 Morphological and developmental changes

2.8.1 Gross morphology

A photographic record was taken of the hair grass after taken where appropriate. For the seed grown material measurements of width and length of mature fully expanded tillers were recorded.

2.8.2 Scanning electron microscopy (SEM)

Leaf waxes and stomatal distribution

Samples of the UV-B-exposed and UV-B-excluded hair grass were also taken for observation under the SEM. The method used was broadly following that of Pugh (1996) and K. Robinson (*pers*. *comm*. May 1997). The pieces of leaf were frozen by plunging into liquid nitrogen. Specimens were then mounted on aluminium stubs using double-sided adhesive carbon discs (Agar Scientific Ltd.), gold coated in a Bio-Rad SC502 sputter coating unit, examined and photographed in a model S-360 Leica SEM. Low magnification images were obtained using an acceleration voltage of 10 kV, probe current of 200 pA, working distance of 10-15 mm and the in-built frame store.

2.9 Experimental design and statistical analysis

The basis of this thesis was to assess potential effects of altered UV-B exposure with concurrent exposure to or screening from UV-B. A wide variety of plants were to be used for preliminary screening, then some were to be studied in more detail and if any were damaged by UV-B exposure then to find out if ozone depletion could also cause these changes. Thus the first experiment was a simple exclusion/ inclusion experiment. It was hypothesised that UV-B alters photosynthesis, in particular PSII, thus F_{vm} measurement was to be the predominant screening tool. As the fluorescence analysis in itself is not conclusive (Walker 1988, Maxwell and Johnson 2000), measurements were also corroborated with concurrent gas-exchange analysis. Furthermore, putative UV-B absorbing pigments were extracted as a result of the prevalent data indicating a common effect (Searles *et al.* 2001). Once an effect could be identified then it would be necessary to deconvolute it to the wavelengths most altered by ozone depletion and thus ecologically relevant.

Plant stress responses are difficult to be attributed to a particular effect thus water stress may also result in responses that may be caused by other factors. To negate any other ameliorating debilitating factors in this thesis it was considered important that the plants studied had as little as possible growth under UV-B exposure. To ensure this, where possible plants were grown from seed (*D. antarctica*) or by vegetative propagation (liverwort). When this was not achievable the plants were sampled from new growth that occurred under UV-B exclusion in the growth cabinet (mosses). When this was not feasible then the plants were kept for up to one month under UV-B exclusion (*P. crispa*). Coupled with this known UV-B-excluded developmental history the plants were kept in as far as possible non-limiting conditions regarding nutrient and water status, and PAR was kept to an intensity that was itself at least not photoinhibitory. In this respect then any response of the plant should be primarily to the UV-B. Whilst it is understood that these conditions could be considered unrealistic, if the plant was not responsive now when resources would be available then it would be unlikely to respond when UV-B is one of many limiting factors and thus harder to elucidate a specific UV-B effect.

This work was carried out using a single growth cabinet which necessarily limited space available. Thus replication itself was limited, sampling however though small should still be meaningful. Limited availability of material and generational time

of the material to attain the required lack of UV-B exposure prior to manipulation also precluded the possibility of a block/ split plot type analysis. As the samples were kept in the same growth cabinet, this could be considered to be pseudo-replication (Hurlbent 1984). Strict replication of sampling is, in the plant physiological field, based on the use of agricultural plots. Thus one growth cabinet is only one plot, therefore no replication. Following this strategy, appropriate replication (i.e. $n \geq 3$) would require at least six growth cabinets alone.

Statistical software used for analysis was 'Statgraphics for Windows 2.1' (Statistical Graphics Corp.). Where there were only two sets of data, i.e. before and after exposure then a non-paired t-test was performed after checking for equal variance. Otherwise a one-way ANOVA was applied to multiple samples after checking for normal distribution using to Cochran's C-test for homogeneity of variance, thence Fisher's least significant difference test for multiple samples to identify those significantly different (considered to be $P < 0.05$). If there was unequal variance then the Kruskal Wallis test followed by the Mann Whitney (Wilcoxon) test were applied.

Curve-fitting, where appropriate, was applied to the data using the computer program 'Sigmaplot' (version 4.01, SPSS Inc.). Unless otherwise specified the functions noted below were used. A/c_i data were fitted to a mono-exponential function ($y = y_0 + ae^{-bx}$), where $a = A_{max}$ and compensation point is calculated from $y = 0$; based on that used by Smith (unpublished m/s) and Delgado *et al.* (1993). This function usually gave a very good fit to the data ($r^2 > 0.90$). This was also used for the light response as it also gave a good fit to the data. For the fluorescence quenching analysis (when appropriate); qN data were fitted to the exponential function $y = y_0 + a(1-e^{-bx})$, whilst qP and fluorescence yield were fitted to the exponential function ($y = y_0 + ae^{-bx}$) as it also yielded a good fit. Initial rates for both A/c_i and light response were fitted using a least squares linear regression. Sample size used throughout was always $n \geq 3$, when greater than three it is stated in the work.

CHAPTER 3
EFFECTS OF SHORT-TERM EXPOSURE TO UV-B RADIATION ON CRYPTOGAMS

3.1 Introduction

This chapter considers the possible effects of a relatively short-term enhancement (maximum 34 days) of UV-B radiation on the photosynthetic activity of cryptogams collected from the Antarctic. The possible production of UV-B screening compounds was also investigated. The study covering a variety of relevant plant types and methods, is subdivided into species groupings as this format was considered to be most appropriate. The main aim of this work was to screen a variety of organisms to assess whether UV-B exposure to plants that had been previously excluded from such radiation would induce an effect on photosynthetic competence parameter F_{vm}. This would allow for rapid measurement. Furthermore, whilst there has been much work on the effects of altered UV fluence on non-Antarctic species (see Chapter 1), only limited work has been published on the Antarctic flora (e.g. Post and Larkum 1993, Lud *et al.* 2001, Newsham *et al.* 2002, Robinson *et al.* 2005), there is still a paucity of data on effect of UV-B on the photosynthesis. Thus more detailed experiments were carried out using the oxygen electrode to record response of photosynthetic gas exchange to increasing PPFD coupled with chlorophyll-quenching analysis. There has also been conflicting reports about the presence and importance of UV-B absorbing pigments within cryptogams (Bjorn 2007). Thus extracts of putative UV-B absorbing compounds were also conducted, namely MAAs for the alga and flavonoids for the bryophytes. This study addresses the first Chapter 1 objective, namely screening a variety of plants for effects of short-term UV-B exposure. Any modifications to the methods described in Chapter 2 are stated in the relevant sections.

Prasiola crispa is a cosmopolitan nitrophilous green alga occurring in many locations throughout the Antarctic; particularly areas associated with penguin rookeries. Previous work has included physiological effects of exposure to UV-B radiation. A field study using differential screening of UV-B radiation (Jackson and Seppelt 1997), highlighted changes in UV-absorbing compounds and a small, yet significant, drop in F_{vm} of the UV-B-exposed samples after one month compared to those excluded from

UV-B radiation. Using artificial UV sources Post and Larkum (1993) found a drop in quantum efficiency and lowered maximal photosynthetic rates following four weeks exposure to elevated UV-B; however, despite observing a change in UV-absorbing compounds depending on UV-B environment, they found no increase, expressed per mg chlorophyll content, as a consequence of UV-B exposure. Conversely work on the Antarctic Peninsula by Lud *et al.* (2001) found no evidence of an alteration in F_{vm} fluorescence yield, net photosynthesis nor increase in UV absorbing pigments as a result of either UV-B supplementation or screening from UV-B radiation. They found an increase in CPD frequency in UV-B-exposed samples; it must however, be noted that the value for F_{vm} was low (typically < 0.6).

Marchantia berteroana is a thallose liverwort with a limited distribution on the maritime Antarctic islands, the limited physiological studies on this species e.g. Davey (1997) showed that estimated yearly productivity of the plant was higher than other Antarctic cryptogams, but that it was also sensitive to desiccation, which resulted in poor recovery of photosynthetic capacity. Work has been carried out on the effect of UV-B exposure on flavonoid composition in the non-Antarctic species *M. polymorpha* (Markham and Porter 1974, Markham *et al.* 1998), which noted no change in bulk flavonoid content, but an alteration in ratios certain flavonoids and increased UV-B exposure.

The moss *Sarconeurum glaciale* occurs over a more restricted range than the more cosmopolitan mosses, *Bryum* spp. and *Ceratodon purpureus*, but is locally abundant in Victoria Land (Lewis-Smith 1999). It was thought to be endemic, but has been located in Patagonia (Greene 1975). *S. glaciale* is often found desiccated and heavily pigmented in exposed sites compared to samples from more sheltered areas (R. I. Lewis-Smith *pers. comm.* January 1996). There has to date been no published work on this species in relation to UV-B exposure.

Tortula princeps is another moss with a limited distribution compared to the more cosmopolitan mosses, *Bryum* spp. and *Ceratodon purpureus*, but locally abundant in Victoria Land (Lewis-Smith 1999). A blackened form of the moss is found in exposed locations in the field; a 'sun ecotype', compared to a verdant 'shaded ecotype', (R.I. Lewis-Smith *pers. comm*. January 1996). There is limited published Antarctic context work on the productivity of this moss (e.g. Convey 1994), and none assessing the effect of UV-B exposure. However, a study on the effect of UV-B radiation on the

temperate species *Tortula ruralis* (Takács *et al.* 1999), found the moss to be unaffected by UV-B exposure.

The cosmopolitan moss *Bryum argenteum* has a wide distribution, including the UK, some work has been carried out on this species in the Antarctic e.g. Rastorfer (1970), found optimum temperature for photosynthesis in this moss to be 25 to 30^{o}C. Work by Markham *et al.* (1990) and Ryan *et al.* (2009) are of particular interest as they show a possible correlation of flavonoid content in *Bryum* spp., and declining historical ozone levels in the Antarctic. If it could be demonstrated that *B*. *argenteum* produces flavonoids as a direct result of UV-B exposure then it would also be possible to quantify this correlation.

Ceratodon purpureus, another cosmopolitan moss that occurs at Edmonson Point, has been noted to show a change in colouration of the moss between gametophyte populations found at solar exposed and sheltered areas (R. I. Lewis-Smith *pers. comm.* November 1995). Exposed samples exhibit more of a brown colour compared to the greener, shaded moss. Investigations into possible photoprotective strategies on the moss from Antarctic populations (Post 1990) on a sun 'ginger' ecotype and a shade 'green' ecotype noted that the 'ginger' exposed ecotype has increased levels of carotenoids and anthocyanins, which were thought to be responsible for the colour. Other studies on this moss in an Antarctic context include photosynthesis (Convey 1994), and pigments (Lovelock and Robinson 2002, Dunn and Robinson 2006). Dunn and Robinson (2006) investigated the effect of seasonal UV-B variation and found that *C*. *purpureus* contained a high amount of putative UV-B screening compounds with anthocyanin concentration appearing to be most responsive to alteration in UV-B exposure.

This study should be able to identify potentially UV-B sensitive plants to then elucidate whether any observed effect would be expected under those UV-B wavebands most altered by ozone depletion. Furthermore as the species cover a wide range of maritime to continental Antarctic locations, from cosmopolitan to monotypic of very restricted distribution, it should also be possible to discuss/ speculate on possible strategies to cope with UV-B exposure and the origin of those strategies.

Some of the species described here were also studied for preliminary data in the locality of their collection (Edmonson Point and surrounding areas, Appendix 2). These results have also been included for discussion.

3.2 *Prasiola crispa*

3.2.2 Materials and methods

Work on the terrestrial alga was carried out on samples obtained from Edmonson Point while modifications to experimental details described in Chapter 2 are outlined below. For the fluorescence relaxation experiment, samples were removed from UV-B exposure and the fluorescence immediately recorded without any dark adaptation.

For the first part of the experiment, the plant material was divided as per experimental design and placed under either UV-B opaque ('VE') or UV-B transparent ('OX/02') cloches. After the initial 206 h cumulative UV-B exposure, the UV-B-exposed plants were also placed under a UV-B opaque cloche to recover (initial exposure, Figure 3.2). At the same time, the control (un-exposed) samples were placed under the 'OX/02' cloche for 200 h (control to UV Figure 3.2). Then the samples were placed under the UV-opaque cloche for a recovery period. No combined oxygen electrode/ fluorimeter measurements were made during the recovery period.

Statistical analysis of F_{vm} was carried out using the Kruskal-Wallis test and the Mann Whitney (Wilcoxon) test for comparison on particular sampling times (as variance throughout the sampling period proved to be unequal).

3.2.3 Results

3.2.3.1 Appearance and morphology

There was no apparent change in thallus colouration between the control and the exposed samples for the duration of the exposure.

3.2.3.2 Dark adapted-fluorescence and fluorescence yield

UV-B exposure caused a dramatic decline in selected fluorescence parameters. This was apparent after only 2 h exposure to altered UV fluence, and continued for the duration of exposure (206 h). Examination of the $F'_m - F_s / F'_m$ (fluorescence yield) characteristic, which is indicative of electron transport capacity, clearly demonstrates this change (Figure 3.1).

The changes in F_{vm} during UV-B exposure were also rapid and sustained. The samples exposed to UV-B showed a significant ($P < 0.05$) loss of F_{vm} after 2 h. This dropped (significantly) after 6 h, and again at 71 h, compared to the previous exposure (Figure 3.2a). However, increased duration of exposure did not further significantly alter F_{vm}, although the significant difference ($P < 0.05$) was maintained compared to the control. Loss in F_{vm} was primarily attributed to an increase in the F_o parameter, which typically rose by a third of its initial value after prolonged exposure to the altered UV fluence. F_m tended to be more variable, with mean values both falling and rising during the exposure. The F_o value of the control samples also varied, though to a lesser degree.

The UV-B-exposed samples partially regained the initial F_{vm} values during the recovery period under the UV-B opaque cloche (Figure 3.2b). This recovery was accompanied by a concurrent fall in F_o.

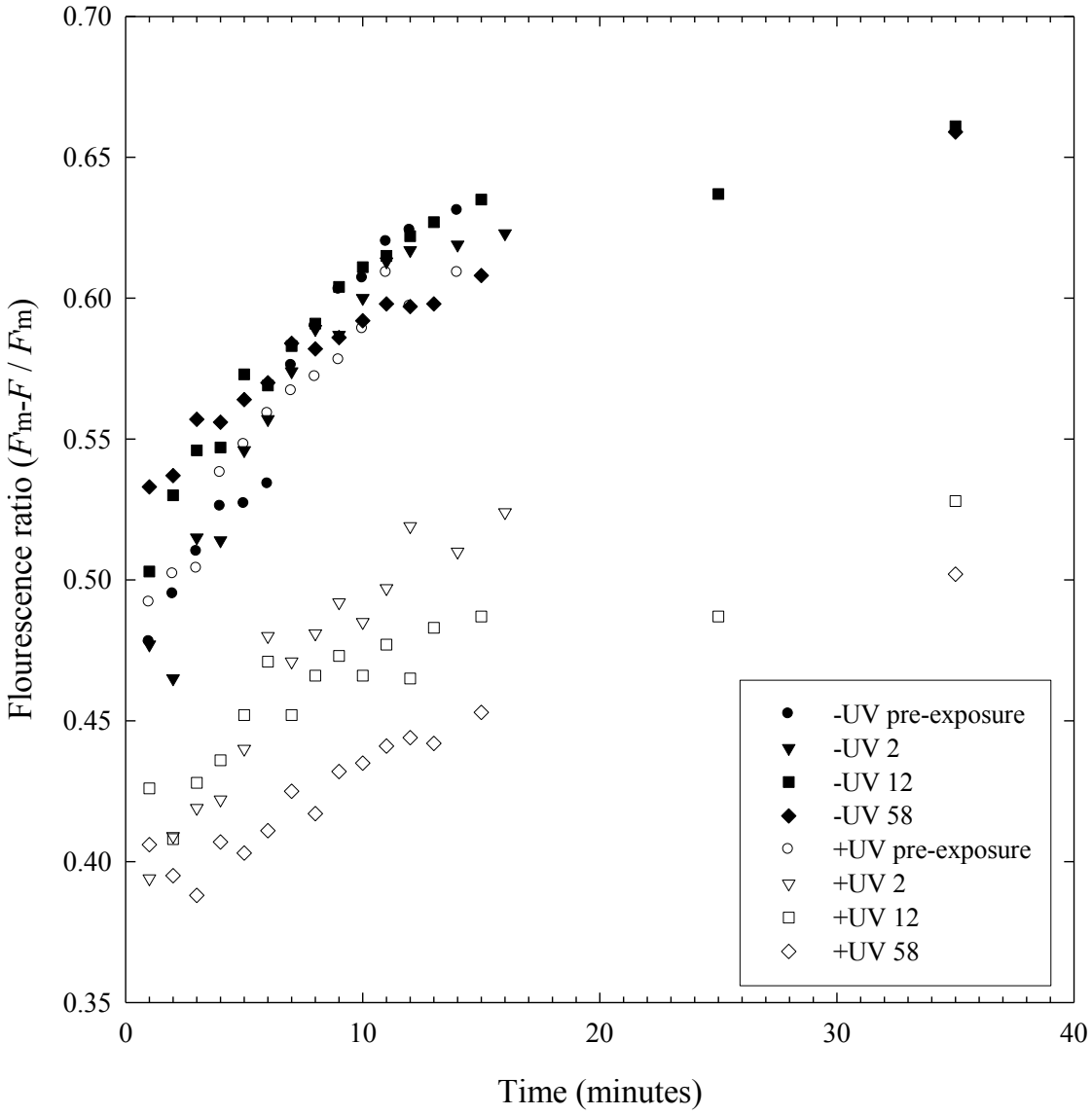


Figure 3.1 Effect of UV-B exposure on the recovery of fluorescence yield (*F*'m-*F*/*F*'m) in the alga *Prasiola crispa*. Measurements taken (in darkness) immediately after removal from exposure. See legend for cumulative period of UV-B exposure (h) (-UV, UV-B excluded; +UV, UV-B exposed).

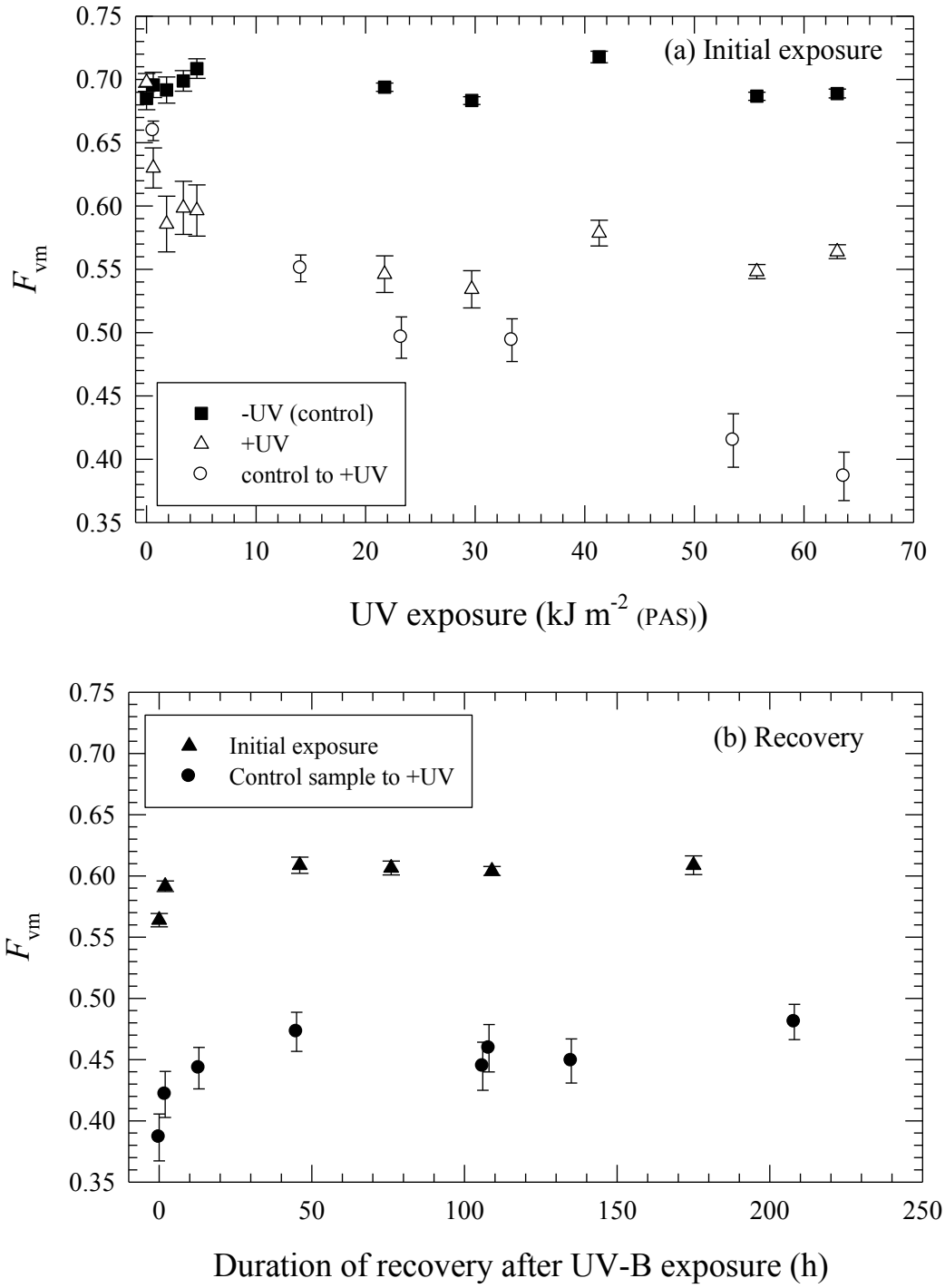


Figure 3.2 Plot of F_{vm} values attained during and after exposure to, or exclusion from, UV-B radiation in the alga *Prasiola crispa*.
(a) Initial sample and control sample subsequently exposed to UV-B radiation.
(b) Exclusion from UV-B radiation (recovery period) for both initial and control samples following 63 h UV-B exposure.
Mean ± S.E. (n = 9).

Samples from the UV-B opaque cloches that were subsequently placed under the UV-B transparent cloche also suffered a substantial and significant ($P < 0.05$) loss of F_{vm}. In this instance values fell by nearly half to 0.38 (Figure 3.2a). Again, this was attributed to a rise in F_o values, rather than an obvious drop in F_m. As before there was a rapid, but this time limited, recovery of F_{vm} (Figure 3.2b). This may have been the result of the control samples being maintained for a longer overall duration than the samples initially exposed to altered UV fluence.

3.2.3.3. Light response; gas exchange and fluorescence quenching analysis

Rates of net oxygen evolution measured under saturating CO_2 were comparable throughout the exposure period. There was some variation in the photosynthetic data within samples. Maximal rates (A_{max}) were comparable at each sampling time. Figure 3.3 shows the measurements taken after 29.4 kJ m^{-2} (PAS) exposure (96 h). Light compensation points (LCP) were typically 20 to 30 μmoles m^{-2} s^{-1}. Apparent quantum yields were typically between 0.08 to 0.09 g (dry mass)$^{-1}$ min^{-1}/m^{-2} s^{-1}. However, after 96 h the UV-B-excluded sample had a much shallower gradient of 0.038 compared to the UV-B-exposed value of 0.081 g (dry mass)$^{-1}$ min^{-1}/m^{-2} s^{-1} . Dark respiration rates were in the range of 1.1 to 1.5 μmoles g (dry mass)$^{-1}$ min^{-1}, except after 96 h when the 'OX/02' sample had a mean value of 2.8 μmoles g (dry mass)$^{-1}$ min^{-1} 96 h (though not significant, $P > 0.05$, Figure 3.3).

Changes in F_{vm} described earlier were also concurrent with those obtained from the Hansatech fluorimeter. The F_{vm} of the UV-B-exposed sample fell by more than 21% of the starting value after 206 h of exposure to UV-B radiation. Again the rise in F_o was the main reason for the decline in F_{vm}.

There were no marked differences between the UV-B-exposed and UV-B-excluded samples as regards the quenching parameters, qN and qP. There was more variability between the replicates than the treatments.

There was a noticeable and consistent fall in fluorescence yield at low PPFD (Figure 3.4). When the data for apparent quantum yield and fluorescence yield were plotted together there was general positive trend between the two parameters, but, a strong linear relationship between fluorescence yield and quantum yield (demonstrated by Genty *et al.* 1989) was not apparent in the results.

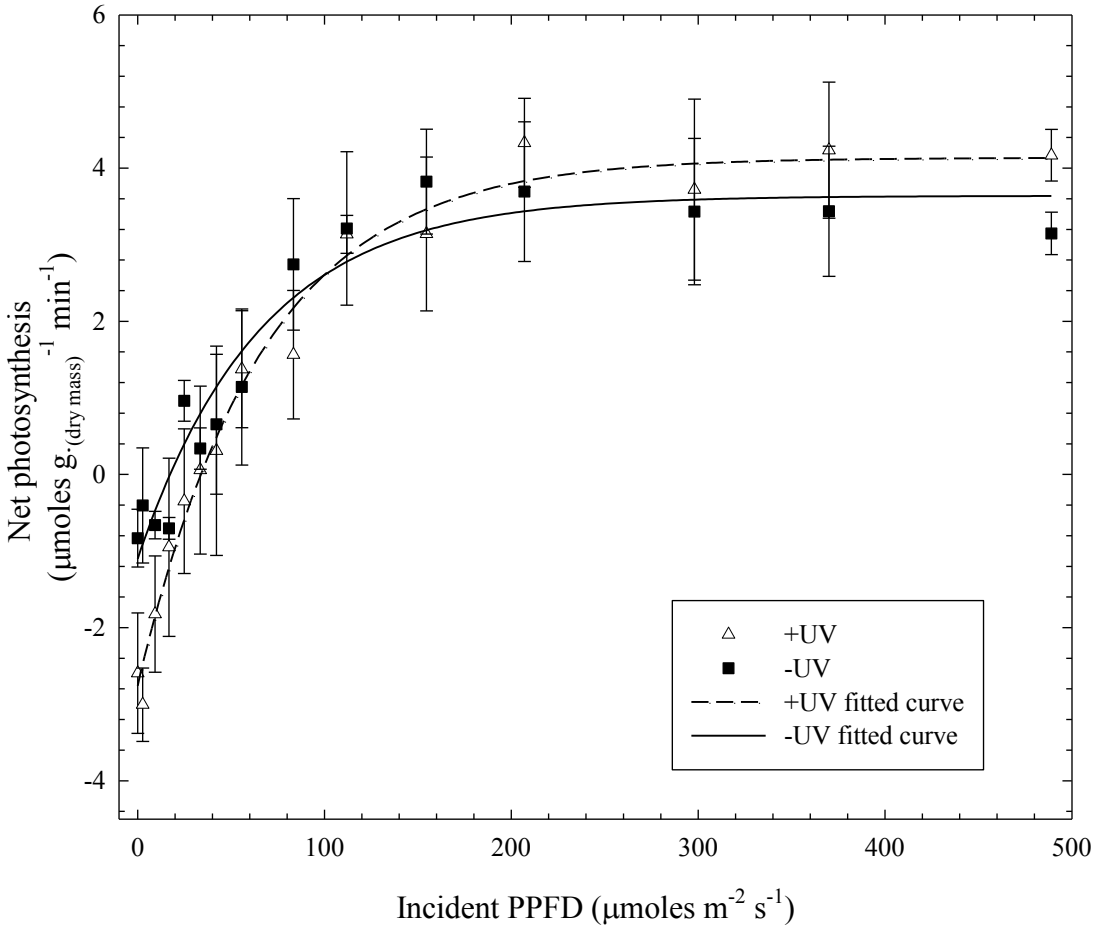


Figure 3.3 Photosynthesis (net oxygen evolution) vs light plot obtained from the alga *Prasiola crispa* after 96 h (29 kJ m^{-2} (PAS)) exposure to, or exclusion from, UV-B radiation (+UV, UV-B exposed; -UV, UV-B excluded). Mean ± S.E. (n = 3).
(r^2, +UV 0.78; -UV 0.70).

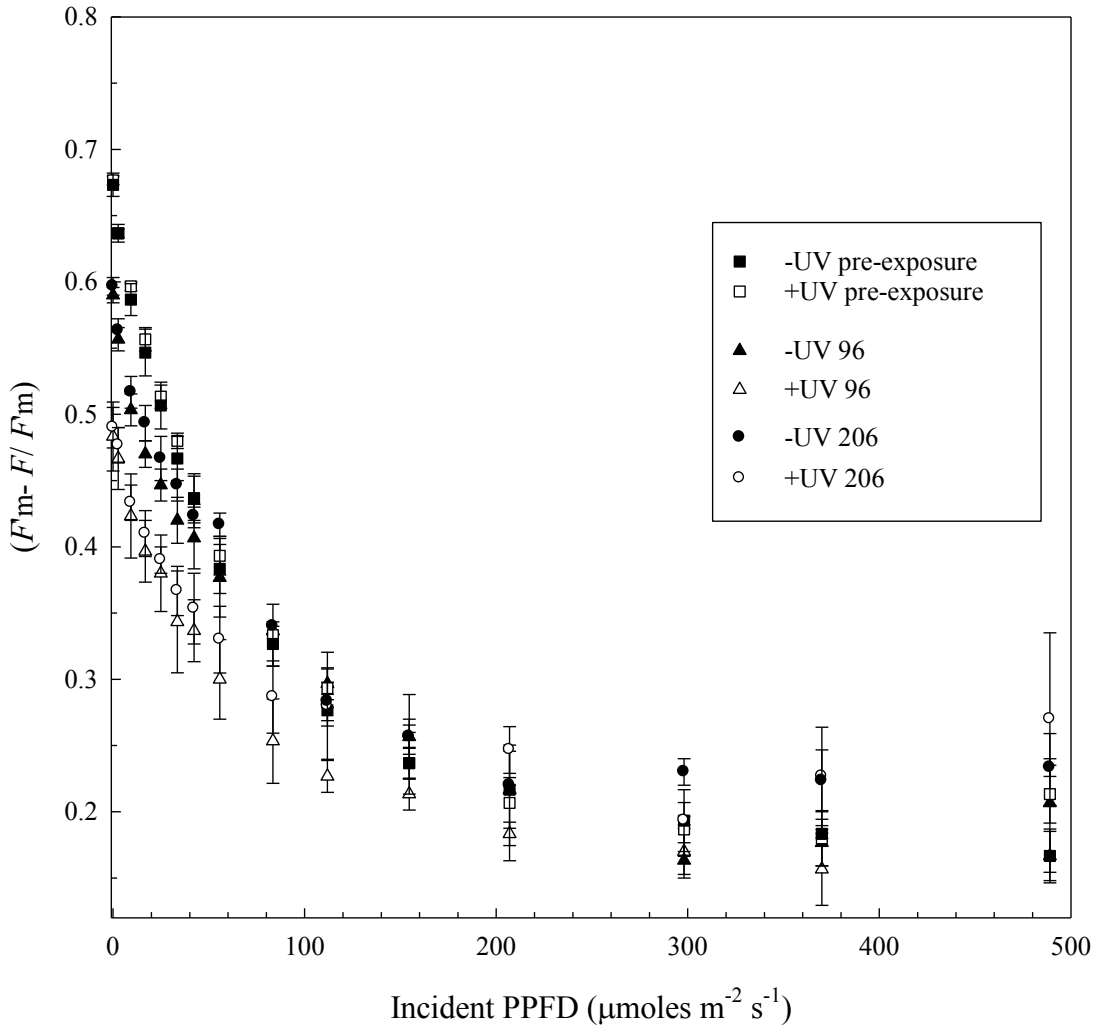


Figure 3.4 Fluorescence yield (*F*'m -*F*/ *F*'m) vs light plot obtained from the alga *Prasiola crispa* after exposure to, or exclusion from, UV-B radiation applied at 0.085 W m^{-2} (PAS) (-UV, UV-B excluded; +UV, UV-B exposed).
See legend for duration of exposure (hours)
Mean ± S.E. (n = 3).

3.2.3.4 Biochemical analysis

Chlorophyll content (recorded at the end of the experiment) showed no significant difference ($P > 0.05$) expressed on a dry mass basis as a result of UV-B exposure with mean (± S.E.) contents of 8.02 (± 0.23) and 7.51 (± 0.75) μg mg^{-1} for UV-B-excluded and exposed respectively. Extraction of putative MAA like compounds yielded a spectrum with a distinct absorption peak at 325 nm. The mean amount (expressed as a function of absorbance and dry mass) for the UV-B-excluded samples was 0.098 (± 0.004) $a.u.mg^{-1}$ dry mass, whilst for the UV-B-exposed samples, mean amount was 0.110 (± 0.006) $a.u.mg^{-1}$ dry mass. This was not found to be significantly different ($P > 0.05$).

3.3 *Marchantia berteroana*

3.3.1 Materials and methods

Plants were exposed for 85 h resulting in a total UV-B$_{(PAS)}$ weighted dose of 26 kJ m$^{-2}{}_{(PAS)}$. All other details regarding exposures and other methods are as described (Chapter 2).

3.3.2 Results

3.3.2.1 Appearance and morphology

There was a change in the colouration of the exposed material, which occurred over a period of three to five days. The appearance of this purple/ brown pigmentation was most distinct around the older base of the thallus and at the tips, whilst the region of the plant around the gemmae maintained a distinct green colour. Attempts to extract this purple pigment were unsuccessful.

3.3.2.2 Dark-adapted fluorescence

There was a marked fall in F_{vm} for the UV-B-exposed material (Figure 3.5), which occurred within 2 h of exposure to UV-B. The difference between the UV-B-excluded and exposed was significantly different at each sampling time. The fall in F_{vm} of the UV-B-exposed sample could be attributed to both a rise in F_o and a fall in F_m. There was also a transient decline in F_{vm} of the control sample (< 10% loss), but it did recover to within 6% of the initial value.

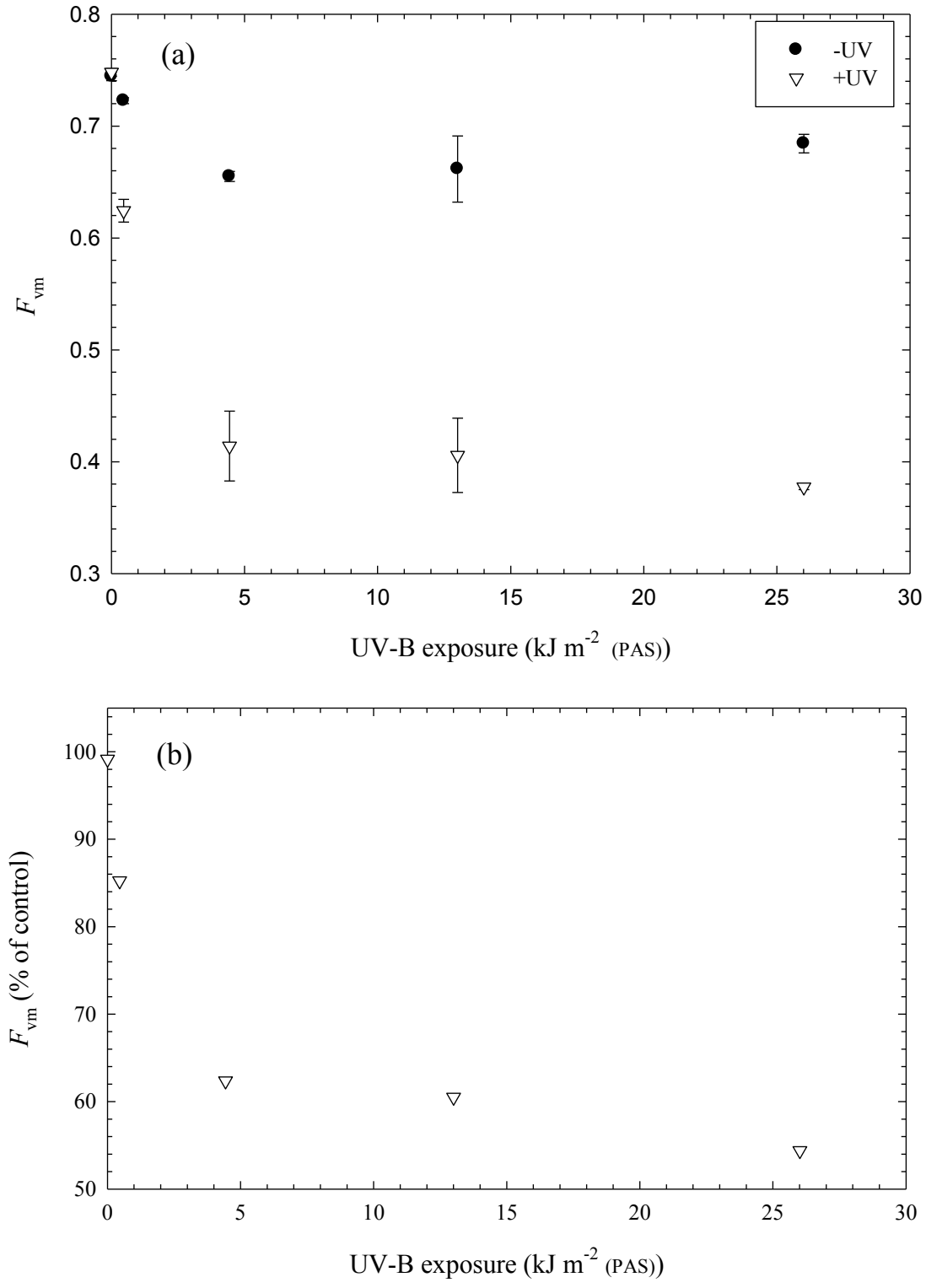


Figure 3.5 Plot of F_{vm} values obtained from the liverwort *Marchantia berteroana* during exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed). (a) F_{vm}. (b) The same data expressed as % of non-exposed sample. Mean ± S.E. (n = 3).

3.4 *Sarconeurum glaciale*

3.4.1 Materials and methods

Plants were exposed for 320 h resulting in a total UV-B (PAS) weighted dose of 98kJ m^{-2} (PAS). All details have been described previously.

3.4.2 Results

3.4.2.1 Appearance and morphology

No development of a dark pigmentation was observed throughout the culture and experimentation period.

3.4.2.2 Dark-adapted fluorescence

There was no significant difference in F_{vm} values, which remained at ca. 0.78 throughout the exposure period.

3.4.2.3 Light response; gas exchange and fluorescence quenching analysis

The initial values of the fluorescence yield were marginally lower for the UV-B-exposed sample after 2 h, and only when below 150 μmoles m^{-2} s^{-1} PPFD. However, after prolonged exposure there was no discernible difference between the exposed and non-exposed samples. Measurements of qP showed no alteration as result of UV-B exposure. Furthermore, qN only exhibited a small transitory effect. Thus after the initial 2 h exposure, there was a slight increase in qN at low irradiance (< 150 μmoles m^{-2} s^{-1} PPFD) for the UV-B-exposed moss compared to the control. Subsequent measurements of qN were almost identical (Figure 3.6).

Results for gas exchange showed no obvious differences, indeed there was more divergence between sampling dates than the exposed or control samples on those dates. The light response data measured after 320 h exposure (98 kJ m^{-2} (PAS)) are shown in Figure 3.7. Results from light response measurements taken at Edmonson Point were slightly lower than those presented above, but were recorded at 10 ^{o}C. Extrapolation to 15 ^{o}C would realise an A_{max} of ca. 4 μmoles g (dry mass)$^{-1}$ min^{-1}, which would imply that transportation and the conditions in the growth chamber had no deleterious effect on the moss.

3.4.2.4 Biochemical analysis

The HPLC results showed no evidence of any flavonoid-like compounds being present within the moss following the standard extraction procedure.

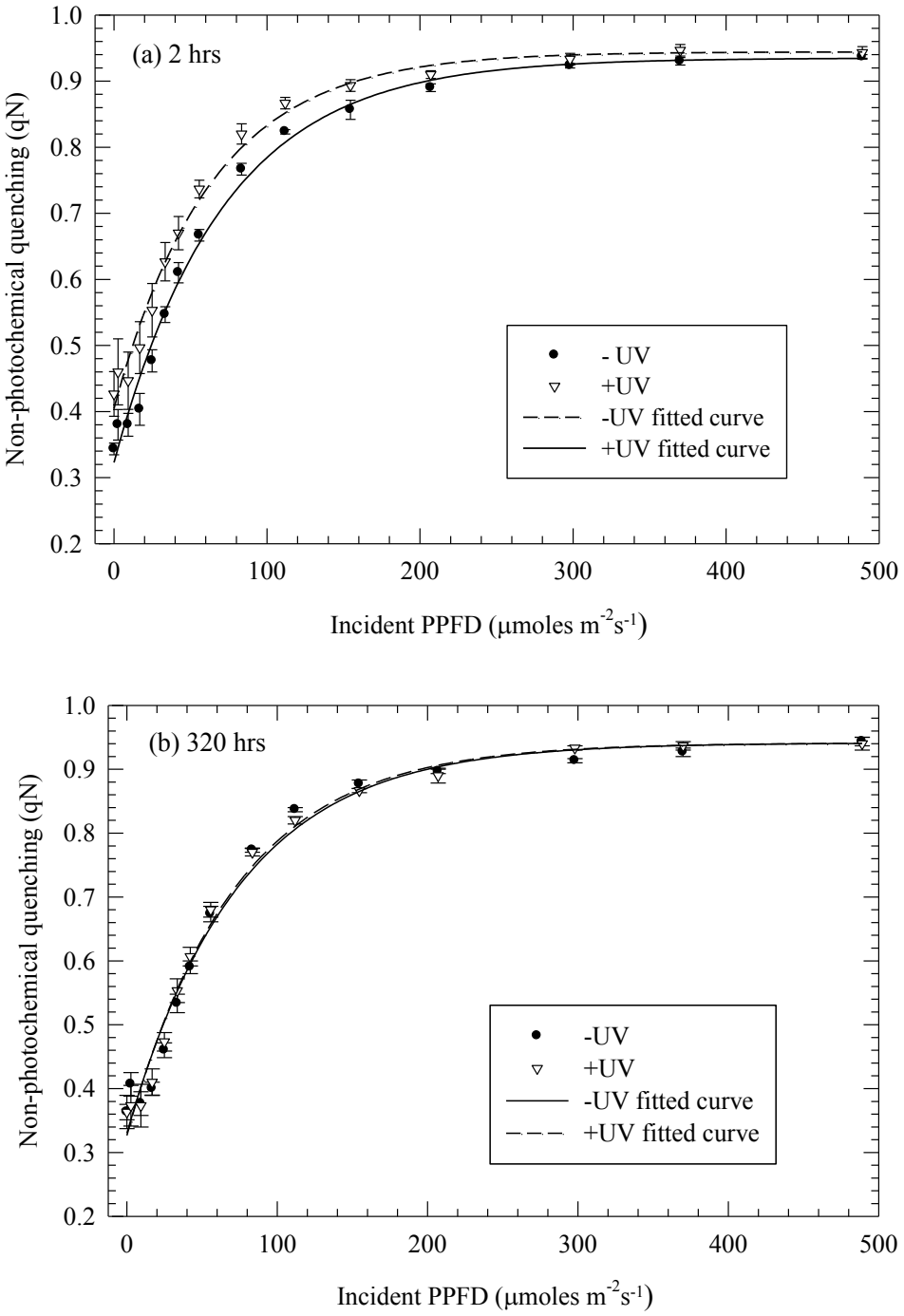


Figure 3.6 Non-photochemical quenching (qN) vs light plot obtained from the moss *Sarconeurum glaciale* after exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).
(a) 2 h (0.6 kJ m^{-2} (PAS))
(b) 320 h (98 kJ m^{-2} (PAS))
Mono-exponential curve fitted to each data-set (all $r^2 > 0.9$).
Mean ± S.E. (n = 3).

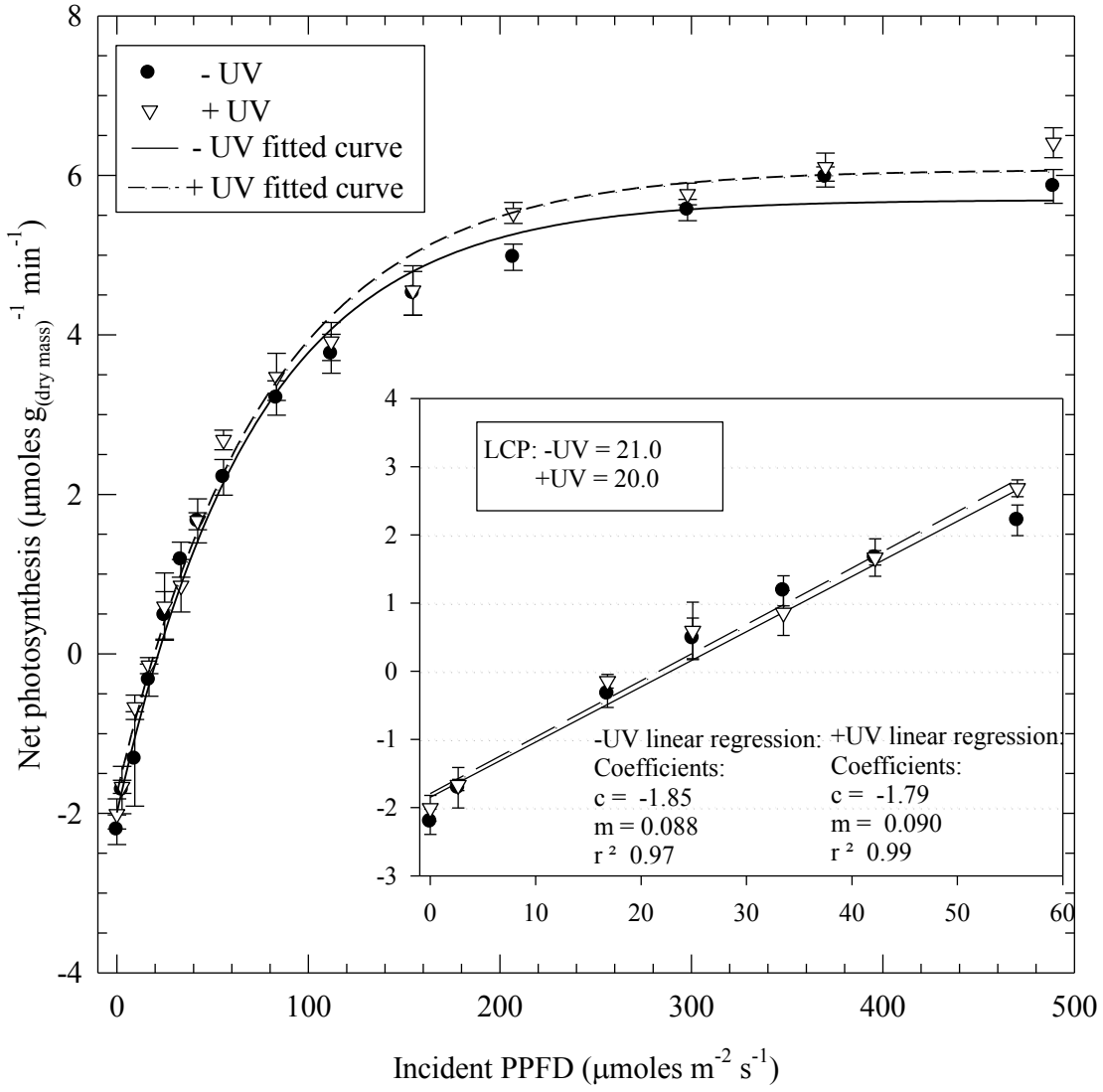


Figure 3.7 Photosynthesis (net oxygen evolution) vs light plot obtained from the moss *Sarconeurum glaciale* after 320 h exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed). Mono-exponential curve fitted to each data-set ($r^2 = 0.99$ for both fits). Inset linear regression ($y = mx + c$). LCP (light compensation point). Mean ± S.E. (n = 3).

3.5 *Tortula* (= *Syntrichia*) *princeps*

3.5.1 Materials and methods

All details are as described in Chapter 2. The plant material was exposed to UV-B radiation for up to 546 h (equivalent to 167 kJ m^{-2} (PAS)).

3.5.2 Results

3.5.2.1 Appearance and morphology

There was no apparent visible change between the samples after 546 h exposure to UV-B radiation.

3.5.2.2 Dark-adapted fluorescence

The F_{vm} data only exhibited significant alteration at 320 h exposure. There was a very small, but significant ($P < 0.05$), difference between the UV-B-exposed sample (0.75) and the UV-B-excluded sample (0.77).

3.5.2.3 Light response; gas exchange and fluorescence quenching analysis

Measurements of fluorescence yield showed no discernible difference between the UV-B-exposed and the UV-B-excluded samples during the experiment. Moreover, there was no apparent difference in either qP or qN. Alteration in photosynthetic rates varied according to which method (dry weight or chlorophyll) was used in the rate calculation. Only when data was expressed on a chlorophyll basis was there any difference between UV-B-exposed and non-exposed samples (Figure 3.8). Thus slightly lower apparent quantum yields were found in the UV-B-exposed samples after 2 h and 546 h cumulative exposure. Moreover, A_{max} for the UV-B-exposed samples were almost a third lower than the non-exposed sample (Figure 3.8). However, the decrease in apparent quantum yield was not reflected in any substantial loss of F_{vm}. Comparison with the field study shows that growth chamber samples exposed to UV-B had a similar response to that recorded at Edmonson Point. However, the UV-B-excluded samples exhibited a much higher rate of net photosynthesis. This would imply that the moss had not been adversely affected by the transport to the UK. However, the similar response

between the field study and the UV-B-exposed growth chamber sample would suggest that the UV-B exposure in the growth chamber was depressing net photosynthesis (i.e. oxygen evolution) under saturating PPFD and CO_2 concentration.

3.5.2.4 Biochemical analysis

HPLC analysis of sample extracts did not show substantial quantities of any flavonoid-like compounds that could be considered to serve a UV-B protective/ screening role irrespective of treatments. The lack of flavonoids is not unexpected as *T. princeps* is a member of the Pottiaceae, which are not considered to contain flavonoids (Markham 1990). Likewise the extraction of chlorophyll for the light response data did not reveal any significant difference in amount between UV-B-exposed and non-exposed samples.

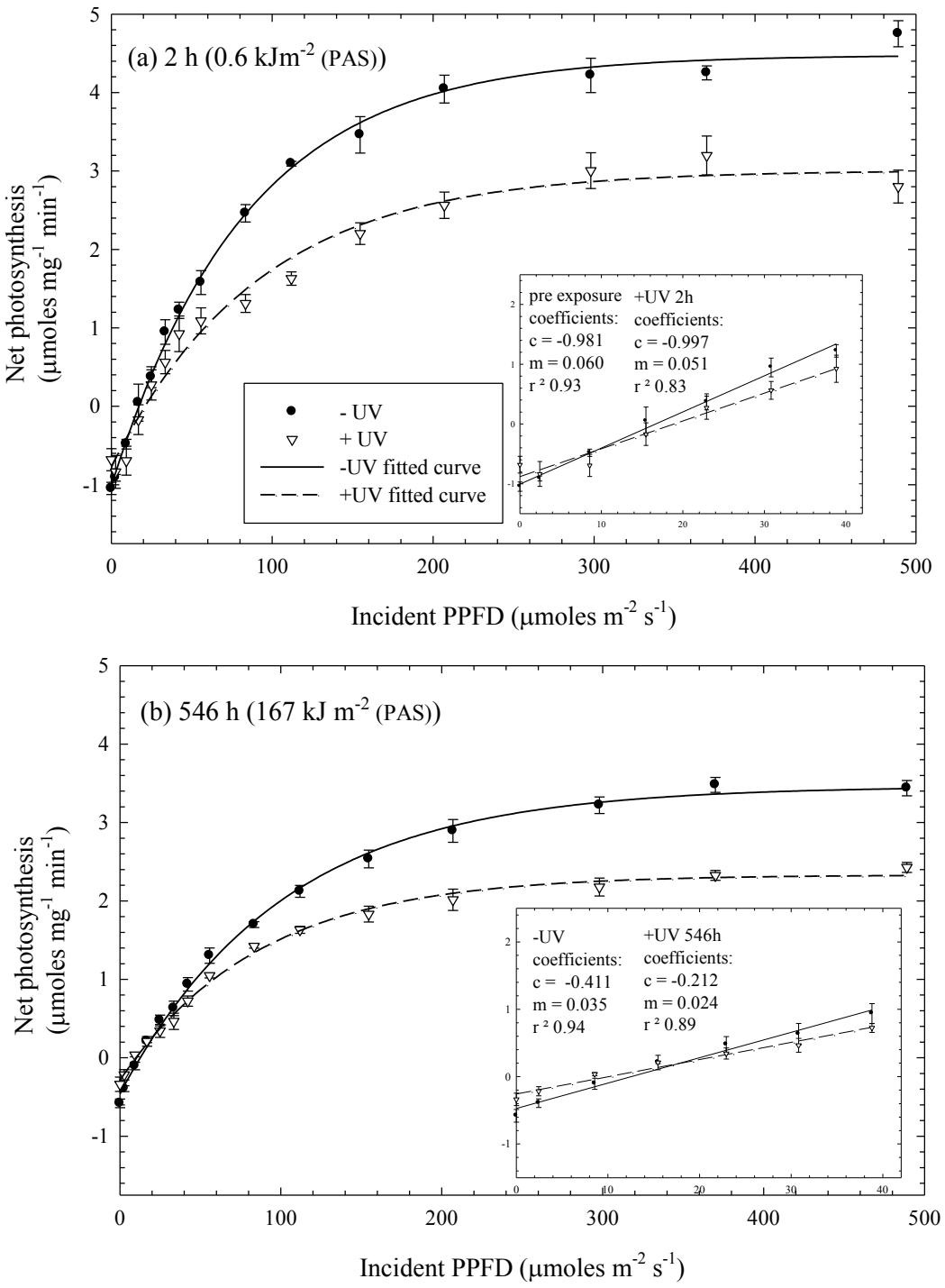


Figure 3.8 Photosynthesis (net oxygen evolution) vs light plot obtained from the moss *Tortula princeps* after exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).
(a) 2 h. (b) 546 h.
Mono-exponential curve fitted to each data-set (all $r^2 > 0.95$).
Inset linear regression ($y = mx + c$).
Data plotted on a chlorophyll (a + b) mass basis.

3.6 *Bryum argenteum*

3.6.1 Materials and methods

The methods followed were as stated in Chapter 2. Plants were exposed to an increased UV-B fluence for up to 70 h (21.4 kJ m^{-2} (PAS)).

3.6.2 Results

3.6.2.1 Appearance and morphology

There was no obvious visual difference between the exposed and non-exposed samples.

3.6.2.2 Dark-adapted fluorescence

F_{vm} values taken after 70 h UV-B exposure (22 kJ m^{-2} (PAS)) showed a very small but highly significant ($P < 0.001$) decrease in the UV-B-exposed sample, with mean values of 0.81 and 0.79 for the UV-B-excluded and UV-B-exposed samples respectively.

3.6.2.3 Light response; gas exchange and fluorescence quenching analysis

UV-B exposure did not alter qP, but qN was slightly depressed at low PPFD. This was not apparent however, after 70 h of exposure (Figure 3.9). Conversely, fluorescence yield values were slightly lower at low PPFD for the control samples, whilst further exposure led to the UV-B-exposed samples having a slightly lower yield value over the light response range.

There was a slight alteration in photosynthetic characteristics with the control exhibiting a lower dark respiratory rate, both initially and after 10 h exposure. However, A_{max} were similar (Figure 3.10a). After 70 h exposure, dark respiration was similar in both samples, but A_{max} was markedly lower in the UV-B-exposed sample (Figure 3.10b). Light compensation points (LCPs) were variable, but the control samples were always lower than the UV-B-exposed samples. The apparent quantum yields were also variable, with no clear trend between the UV-B-exposed and the UV-B-excluded samples. The gas exchange data was broadly similar to that recorded at Edmonson

Point, where A_{max} (at 20°C) was approximately 14 μmoles g (dry mass)$^{-1}$ min^{-1}, which would imply no significant deterioration in the material during subsequent transport, storage and regeneration.

3.6.2.4 Biochemical analysis

HPLC analysis of methanolic extracts from the moss confirmed the presence of flavonoid compounds, as would be expected from the literature (Markham and Given 1988). There was, however, no consistent difference between the chromatogram profiles of either UV-B-exposed or control extracts.

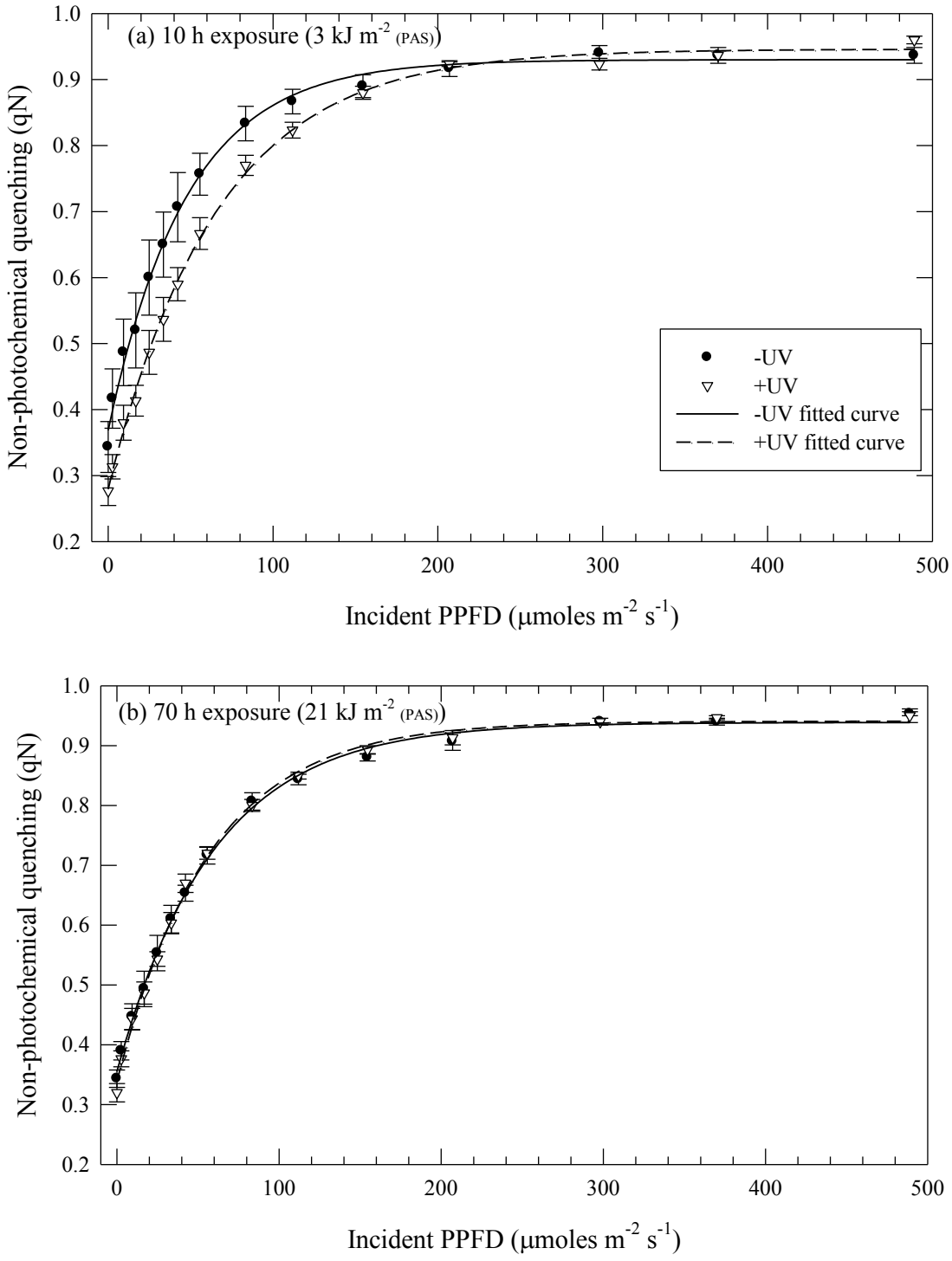


Figure 3.9 Non-photochemical quenching vs light plot obtained from the moss *Bryum argenteum* after exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).
(a) 10 h. (b) 70 h.
Mono-exponential curve fitted to each data-set (all $r^2 > 0.98$).
For legend see (a)
Mean ± S.E. (n = 3).

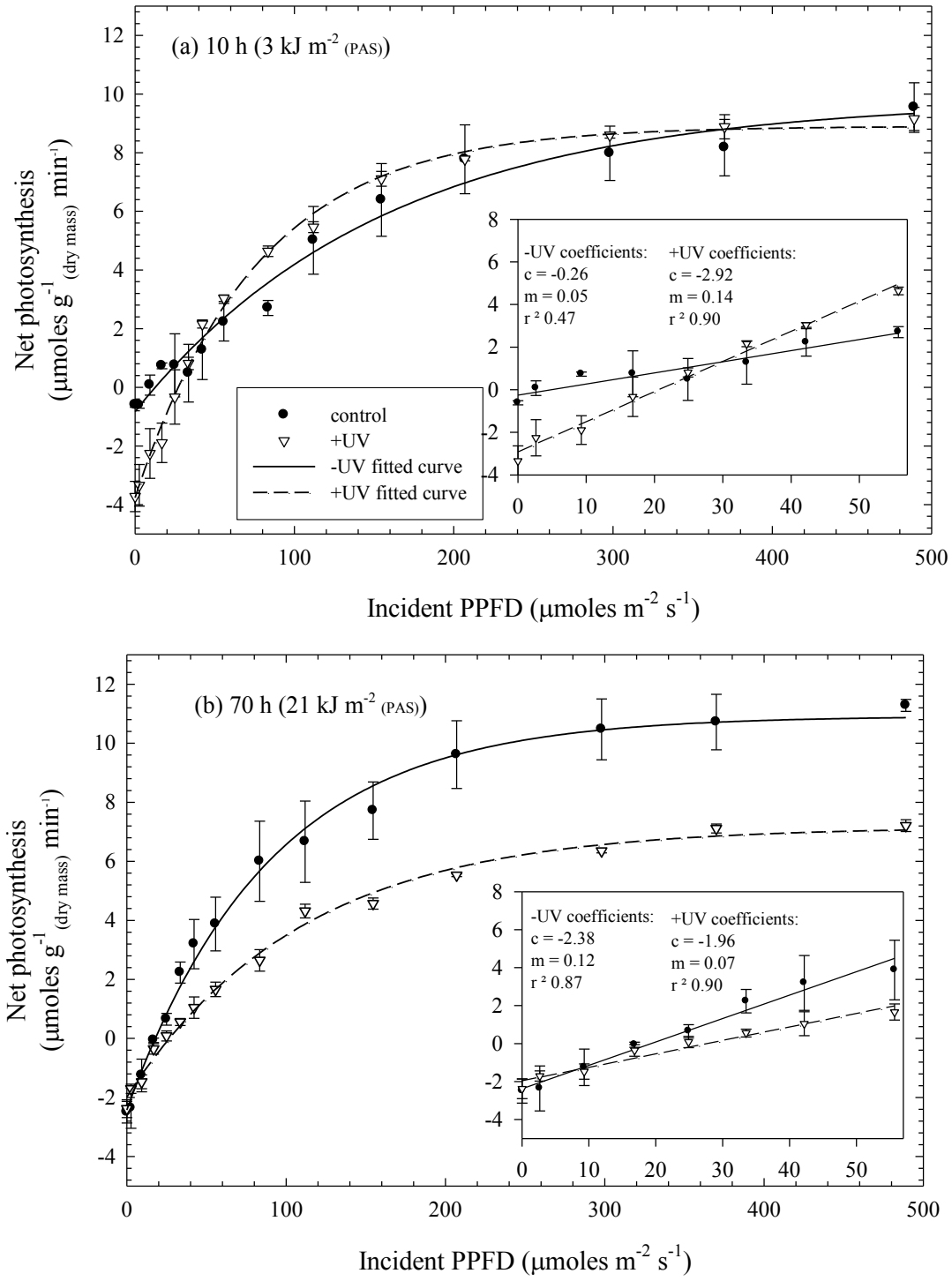


Figure 3.10 Photosynthesis (net oxygen evolution) vs light plot obtained from the moss *Bryum argenteum* after exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).

(a) 2 h. (b) 546 h.

Mono-exponential curve fitted to each data-set (all $r^2 > 0.87$).

Inset linear regression ($y = mx + c$).

Data plotted on a chlorophyll (a + b) mass basis.

3.7 *Ceratodon purpureus*

3.7.1 Materials and methods

This was as described earlier in Chapter 2. The moss was exposed to UV-B for up to 70 h (21.4 kJ m^{-2} (PAS)). The F_{vm} data was compared using the Kruskal-Wallis test and the Mann Whitney (Wilcoxon) test for comparison on particular sampling times (as variance throughout the sampling period proved to be unequal).

3.7.2 Results

3.7.2.1 Appearance and morphology

No obvious alteration in appearance of the moss was noted for the duration of the exposure.

3.7.2.2 Dark-adapted fluorescence

The F_{vm} of the UV-B-exposed samples dropped slightly, but not significantly ($P > 0.05$) after exposure for 70 h, with a mean of 0.78 compared to 0.80 for the UV-B-excluded sample. The drop in F_{vm} and F_v was attributed to a reduced F_m value.

3.7.2.3 Light response; gas exchange and fluorescence quenching analysis

There was no substantial alteration in qP, but qN did decrease after 70 h exposure compared to control. This was lower for the initial part of the light response curve for the UV-B-exposed sample. The fitted curves for 70 h exposure (Figure 3.11) illustrate this fall. Fluorescence yield values were similar throughout the light range for both UV-B-excluded and UV-B-exposed samples.

The apparent quantum yields were broadly similar between treatment and control after the first exposure of 10 h. Following 70 h exposure (22 kJ m^{-2} (PAS)), the UV-B-exposed sample exhibited a slightly higher ($P < 0.05$) apparent quantum yield than the control (Figure 3.12). 10 h of UV-B exposure did not significantly alter dark respiration; mean rates of around 3 μmoles g^{-1}(dry mass) min^{-1} were recorded in both samples. However, dark respiration for the UV-B-exposed samples after 70 h exposure was

significantly higher ($P < 0.05$) compared to the UV-B-excluded samples (Figure 3.12). Moreover, light compensation point (LCP) did increase from ca. 20 to ca. 30 μmoles m^{-2} s^{-1} for the UV-B-exposed sample after the same exposure (Figure 3.12). This was probably a result of the increase in dark respiration as apparent quantum yield remained slightly higher for the UV-B-exposed samples. A_{max} values were found to be similar at all sampling times at ca. 5.8 μmoles g^{-1} (dry mass) min^{-1}. The photosynthesis measurements recorded at Edmonson Point were lower (A_{max} at 20°C being ca. 2 μmoles g^{-1} (dry mass) min^{-1}) relative to the growth chamber study. This could mean that any UV-B effect was ameliorated by what would appear to have been favourable conditions in the growth chamber but, comparison was by way of dry mass and there may have been more non-photosynthetic tissue in the field samples compared to that used in the growth chamber.

3.7.2.4 Biochemical analysis

HPLC confirmed the presence of flavonoid like compounds (Figure 3.13). The relative area of each of the seven peaks was greater in the UV-B-exposed samples (three significantly, $P < 0.05$, Table 3.1). This would suggest a differential effect of UV-B exposure on flavonoid composition. However, an absence of new peaks in the UV-B-exposed samples would indicate no *de novo* synthesis of flavonoid compounds.

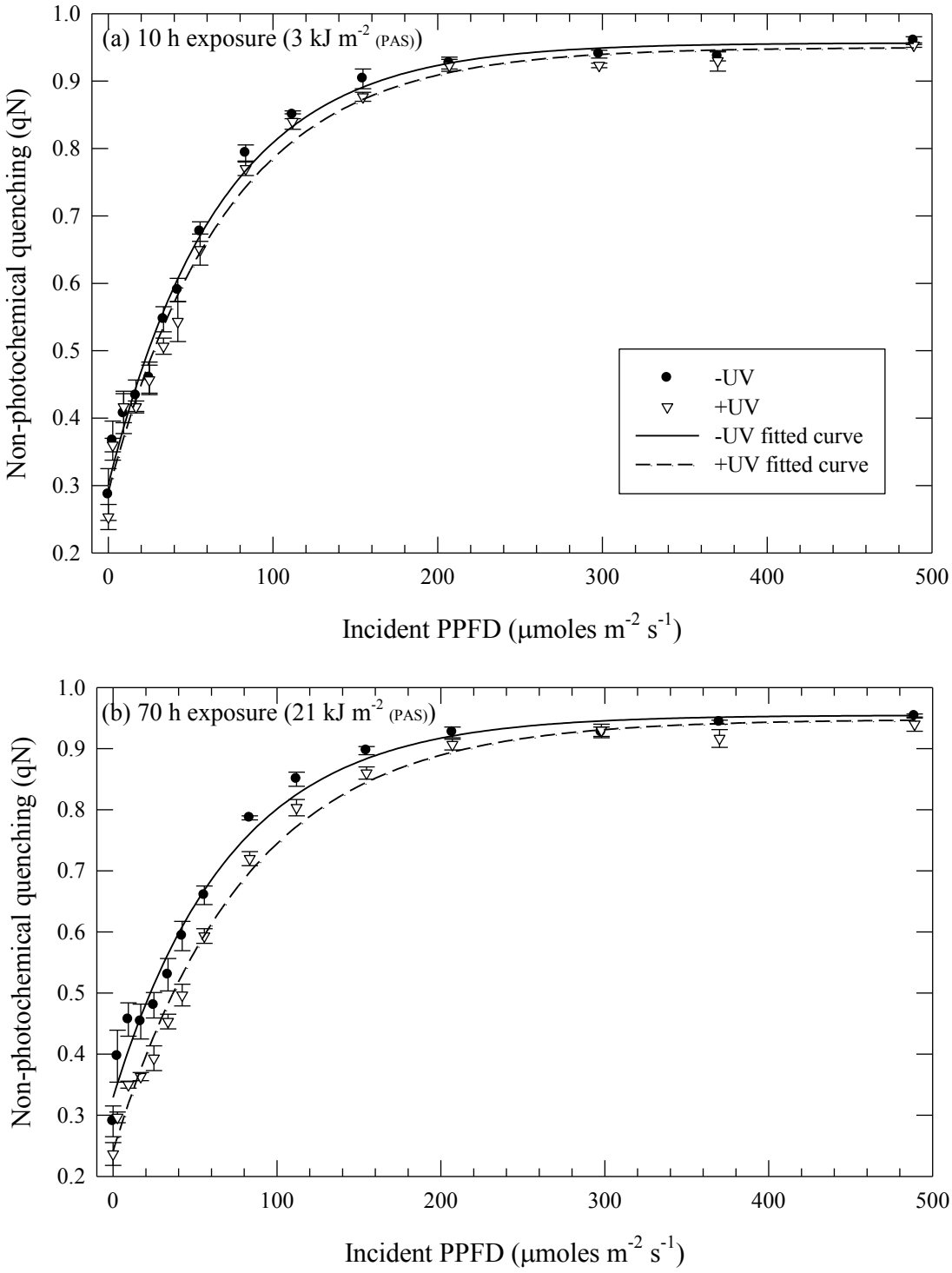


Figure 3.11 Non-photochemical quenching (qN) vs light plot obtained from the moss *Ceratodon purpureus* after exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).
(a) 10 h. (b) 70 h.
Mono-exponential curve fitted to each data-set (all $r^2 = 0.99$).
For legend see (a). Mean ± S.E. (n = 3).

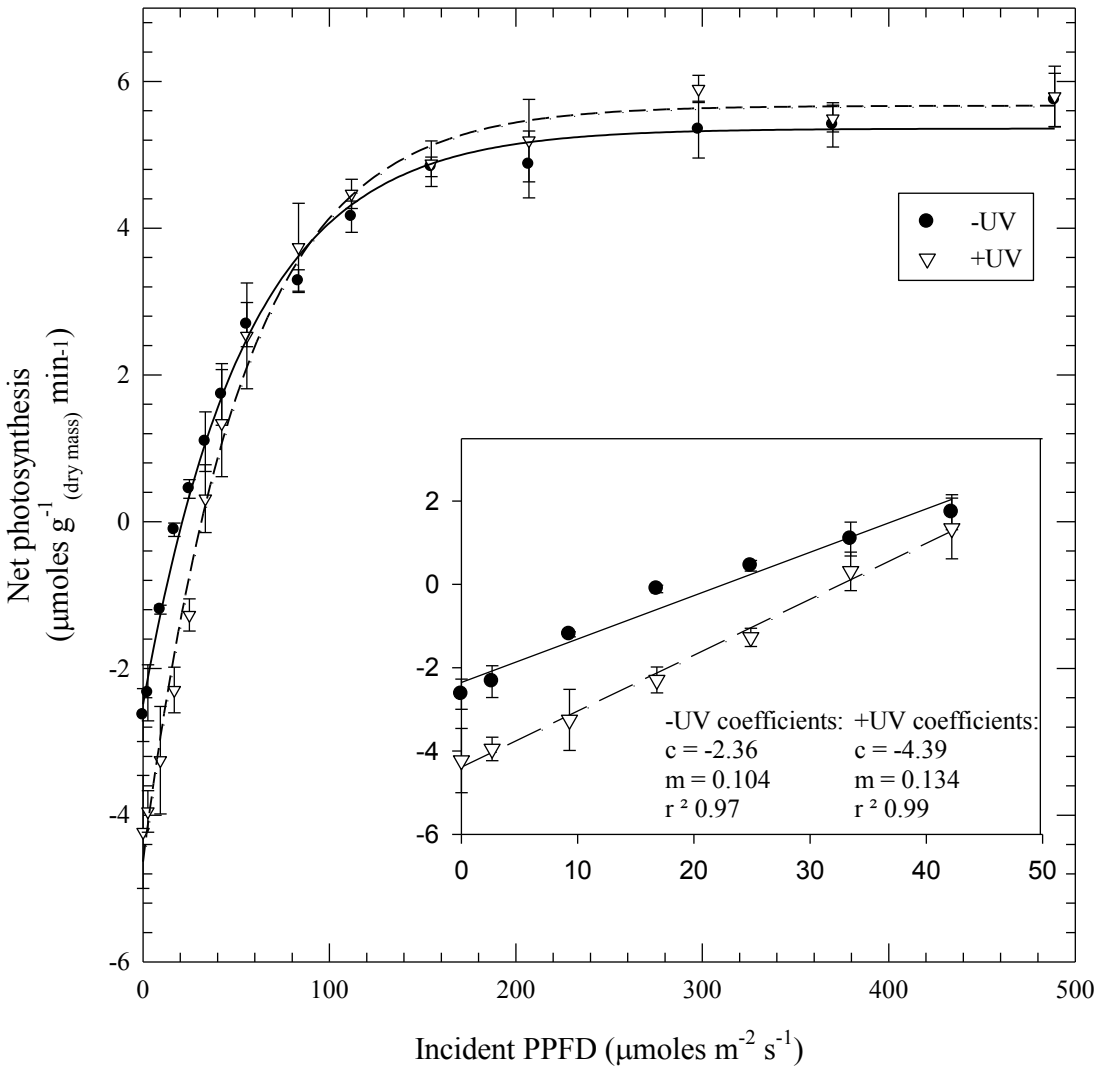


Figure 3.12 Photosynthesis (net oxygen evolution) vs light plot obtained from the moss *Ceratodon purpureus* after 70 h (21 kJ m^{-2} (PAS)) exposure to, or exclusion from, UV-B radiation (-UV, UV-B excluded; +UV, UV-B exposed).
Mono-exponential curve fitted to each data-set (all $r^2 > 0.97$).
Inset linear regression ($y = mx + c$).
Mean ± S.E. (n = 3).

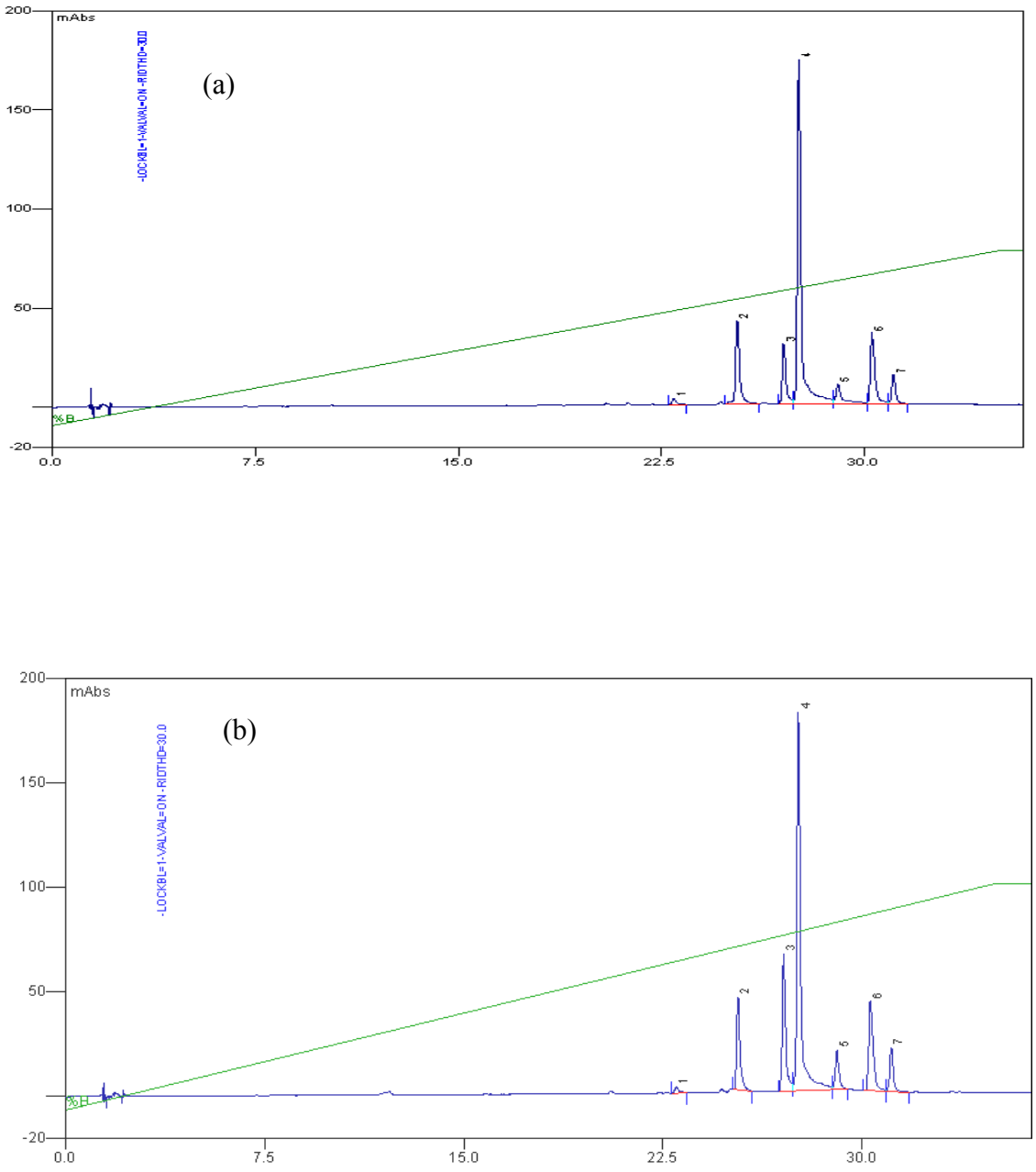


Figure 3.13 Representative chromatogram profiles of flavonoid extracts obtained from *Ceratodon purpureus* shoots taken after 70 h exposure to, or exclusion from, UV-B radiation. Similar mass used for tissue extraction.
(a) UV-B-excluded.
(b) UV-B-exposed.
See Table 3.1 for mean peak areas.

Table 3.1 Mean area of eluted peaks expressed per unit mass of sample, taken from chromatogram profiles of flavonoid extracts obtained from the moss *Ceratodon purpureus* taken after 70 hours of exposure to, or exclusion from, UV-B radiation
(t-test, * $P < 0.05$, $n = 3$).

Peak number	Mean peak area mg^{-1} (dry mass)		Ratio UV-B-exposed/ UV-B-excluded
	UV-B-excluded	UV-B-exposed	
1	0.04	0.05	1.25
2	0.51	0.72	1.41
3	0.28	0.95*	3.39
4	2.02	2.83	1.40
5	0.16	0.28	1.75
6	0.46	0.86*	1.87
7	0.19	0.34*	1.78

3.8 Chapter Discussion

This study has found a clear effect of UV-B exposure on the electron transport potential of *Prasiola crispa* (i.e. depression of F_{vm}). This effect was also partially reversible if UV-B radiation was excluded, with recovery of F_{vm} over a similar time-scale to that of the initial exposure. The alga showed a drop in F_{vm} in a field study (Jackson and Seppelt 1997), but there was no difference between UV-B, and both UV-A and UV-B exclusion; implying that the effect on F_{vm} can be attributed to UV-B exposure. However, Lud *et al.* (2001) could find no effect of UV-B on F_{vm} or fluorescence yield in a field study; although dark adaptation may not have been sufficient in their study as F_{vm} values for both control and exposed were lower than would be anticipated (Bolhár-Nordenkampf *et al.* 1989). Recovery of F_{vm} after UV-B exposure has been found in other chlorophyte algae (Herrmann *et al.* 1997). Increases in F_o have also been reported as a consequence of UV-B exposure (Tevini *et al.* 1988); however, such increases have also been correlated with loss of D1 protein as a result of photoinhibitory treatments in the moss *Ceratodon purpureus* (Rintamäki *et al.* 1994). Conversely, photoinhibition can also lead to a decrease in F_o (Lovelock *et al.* 1995a); in this case, the recovery of F_o was associated with recovery from photoinhibition. The work presented here does show some similarities with the effects of photoinhibition; furthermore, a study on the marine alga *Ulva rotundata* showed similar characteristics of loss of F_{vm}, primarily a result of increases in F_o, then recovery after photoinhibitory exposure (Franklin *et al.* 1992). However, photoinhibitory type damage was indicated from the gas-exchange data; there was no loss in apparent quantum yield or change in qN, both of which would be expected as a consequence of photoinhibition. Post and Larkum (1993) demonstrated a reduction in quantum efficiency, but this fall was not noted here. There were however, differences between exposures; Post and Larkum (1993) used an aqueous as opposed to gas phase electrode. Nevertheless, their work correlates with the drop in fluorescence parameters noted here. This observation highlights the difficulty in interpreting the possible responses to UV-B exposure on the basis of a single measurement technique (i.e. fluorescence). Lud *et al.* (2001) using an IRGA at ambient PAR could not detect any effect of enhanced UV-B exposure. The decrease in fluorescence yield with increasing PPFD of the UV-B-exposed samples would infer that the loss of PSII competence as observed from F_{vm} measurements continues, and would be most important at lower light levels. There was no strong

correlation between fluorescence yield and apparent quantum yield as has been shown with photoinhibitory stress (Demmig and Björkman 1987). This would imply that had there been an effect of UV-B exposure on the kinetics of the photosynthetic rate under light limiting conditions (i.e. a lowering of quantum efficiency), then it was not reflected in net oxygen evolution. There may be a number of reasons for this; UV-B damage, by way of PSII inactivation, may not follow the same pathway as photoinhibitory damage (Hideg *et al.* 2000). It would be possible that, as the fluorimeter beam only focuses on a (relatively) small population of chlorophylls, different populations of the photosystems may have been effected, resulting in the decline in fluorescence, whereas the measurement of net oxygen evolution is for the whole algal sample. Moreover, sensitivity of the photosynthetic apparatus to changes in fluorescence may be greater than gross measurements of gas exchange. Furthermore, other potential sites of damage such as the light independent stage would not necessarily be highlighted using polarographic measurement of oxygen.

The presence of MAAs in *P. crispa* was not unexpected (Post and Larkum 1993), but could not be confirmed as a having a protective role against UV-B radiation. This MAA absorbance peak has been found in *P. crispa* regardless of exposure to UV radiation (Post and Larkum 1993), where MAA concentration found in this study was similar to that reported by Jackson and Seppelt (1997). The continued presence of these compounds after exclusion from UV-B exposure would suggest slow turnover rates (or long half-lives) and/ or that they perform other functions within the alga other than a UV screening role. Proline is known to accumulate in high amounts in *P. crispa*, and to have a possible cryo-protective role (Jackson and Seppelt 1997); and it is possible that MAAs have a similar function (i.e. as an osmo-compatible solute) other than purely a UV-B screening function. Alternatively, the presence of these nitrogen-containing compounds may be a consequence of location of the alga (close to water run-off from penguin colonies).

The purple pigment observed in the work on *Marchantia berteroana* could be a terpenoid as it appeared to be cell wall-bound. Work on *M. polymorpha* (Markham *et al.* 1998), which is chemically, very similar to *M. berteroana* (Asakawa and Campbell 1982), has demonstrated an alteration in the ratio of B-ring *ortho*-dihydroxyflavones to B-ring monohydroxyflavones as a result of exposure to supra-ambient levels of UV-B radiation. These authors concluded that flavonoids were not being used as an active screen against UV-B radiation, but as free radical scavengers or antioxidants. However,

recent work on an Antarctic liverwort and *M. polymorpha* (Snell *et al*. 2009) demonstrated the presence of UV-B-inducible anthocyanidin. Whilst it was not possible to confirm that this was the pigment observed in this study, it would be expected to be at least of a similar composition. Importantly, the anthocyanidin reported has a maximum absorption at 490 nm, so would not obviously act as a UV-B screen. The decline in F_m, and concurrent rise in F_o, has been shown in higher plants by other workers (e.g. Tevini *et al.* 1988). The results presented here show that the electron transport potential in this species was highly sensitive to exposure to UV-B radiation. Decreased F_{vm} is related to reduced photosynthetic competence, in particular PSII activity, hence the effect of a relatively low dose (0.085 W m^{-2} s^{-1} (PAS); 4.89 kJ m^{-2} (PAS) day^{-1}) similar to (or lower than) during the Austral summer (see Chapter 1) has the potential to have a negative impact on the photosynthesis of this liverwort. The transient loss of F_{vm} in the UV-B-excluded sample was unusual as the samples had been kept under identical conditions both before, and throughout the experiment. There was no clear reason for this decline in F_{vm} other than an insufficient dark adaptation period, or an asymmetric distribution of reduced reaction centres used for the fluorescence measurement.

The work on *Sarconeurum glaciale* is the first known to the author, and has demonstrated (at most) only minor and reversible changes in photosynthesis after an exposure of 98 kJ m^{-2} (PAS). The only discernible differences occurred after the initial 2 h exposure (620 J m^{-2} (PAS)). Some investigators have previously concluded the need for experiments to be undertaken under a high level of PAR, resulting from the possible disproportionate effects occurring under low irradiance (e.g. Allen et *al.* 1998). The PAR received by the plants in this experiment could be considered to be unnaturally low, and therefore to enhance any UV-B effect or damage; but this was not the case. The apparent lack of flavonoid-like compounds was not unexpected, as they are not (necessarily) ubiquitous throughout the Musci, with perhaps 50% not known to produce flavonoids (Stafford 1991). The current classification of *S. glaciale* places it in the Pottiaceae, which are not all considered to contain flavonoids (Markham 1990). However, the methods employed for extraction and analysis may not have been exhaustive, or effective. Flavonoids have probably been used as UV-B screens in the evolutionary past however; but also associated with other protective functions (Dixon and Paiva 1995). Presumably *S. glaciale* does have protective mechanisms against both/ or the high PAR and low temperatures and water availability. Barsig *et al.* (1998) reported no increase in UV-screening pigments in an Arctic moss. There are other

compounds present in mosses that could respond to increases in UV-B e.g. carotenoids (Newsham *et al.* 2002, Robinson *et al.* 2005) and/ or be able to perform an equivalent function to that of the flavonoids e.g. biflavonoids (Geiger 1990).

The pigmentation responsible for blackened form of *Tortula princeps* in the field would not appear to be formed as a direct consequence to UV-B exposure for the duration of this study. The fluorescence data provides little convincing evidence of a UV-B effect as regards F_{vm} and the quenching parameters, qN and qP. This also corroborates work on a montane species of *Tortula* (Takács *et al.* 1999), which demonstrated no change to F_{vm} in the moss when exposed to 136.1 kJ m^{-2} day^{-1} (un-weighted UV-B) for up to 8 days (1.1 mJ m^{-2}). The reduced A_{max} was only marked when expressed on a chlorophyll mass basis. This reduced value was apparent after 2 h exposure and continued for the duration of the experiment (relative to the moss excluded from UV-B). The lack of change in chlorophyll content would infer that the chlorophyll itself was inactive in the UV-B-exposed samples. Loss of chlorophyll content in a moss has been noted as a consequence of UV-B exposure (Robinson *et al.* 2005). However, the lack of F_{vm} depression and only a small loss in quantum efficiency would indicate that the efficiency of PSII was unaffected at low PPFD in samples exposed to UV-B radiation. Whilst this was not a long-term experiment, the preliminary conclusion is that if there is a response of this moss to UV-B radiation, it may not alter overall productivity. This is because the effect on A_{max} was only on a chlorophyll basis (not mass based) and under non-limiting PPFD and CO_2 concentration.

There was no obvious blackening or change in pigmentation in *B. argenteum*; this is in line with observations in the Antarctic where, even at the most solar exposed sites, the moss does not exhibit a blackened state. The lack of substantial F_{vm} loss (< 3%) implies PSII was not a primary target. However, the lower initial qN values indicate possible damage to thylakoid membranes, leading to a slower build-up of the trans-thylakoid pH gradient (Krause and Weis 1991). The quantum yield values for the UV-B-exposed samples indicate a loss of photochemical efficiency; yet, this decline was not reflected in F_{vm} depression. The alteration in A_{max} was more marked (falling by 30%). This loss would imply damage/ restriction to the light independent stage. As there is conflict in the data it is not possible to conclude PSII damage has occurred in *B. argenteum*. Furthermore, there was a lot of variability with the oxygen electrode data particularly regarding the non-exposed samples (large S.E. bars shown in Figure 3.10).

HPLC confirmed the presence of flavonoid-like compounds as found in other Bryaceae (Webby *et al.* 1996). However, any protective role against UV-B could not be substantiated as a result of inter-sample variability. It is possible that the flavonoids were already present in adequate concentration within the moss samples and the UV-B exposure in the growth chamber was not sufficient enough to elicit a change in production. Accordingly, this work could not support the observation that herbarium specimens of *Bryum* spp. had increased flavonoid levels as a result of ozone depletion (Ryan *et al.* 2009). Nevertheless, further work is needed to fully substantiate the role of these compounds in UV-B protection in this moss.

The work presented on the moss *C. purpureus* has shown that it may exhibit sensitivity to UV-B exposure, but the effects on photosynthesis are slight, and need further study to be fully understood. Photoinhibition in *C. purpureus* has been shown to lead to a rise in F_O (Rintamäki *et al.* 1994), however, a large rise in F_O was not found in this study, which would imply that any damage/ changes noted were not related to photoinhibitory effects. Loss of qN would infer that there was a problem in the thylakoid membrane, but the lack of F_{vm} response does not support this, as photochemical efficiency of PSII was not altered. The quenching parameter qN can have a number of possible sources (Krause and Weis 1991, Müller *et al.* 2001), so it is possible that some external factor had caused this decrease. Moreover, the light response data would indicate the UV-B-excluded samples were less efficient in light limited photosynthetic activity due to the lower apparent quantum yield. The increased level of flavonoids again supports the (previously noted) general response to UV-B exposure. Newsham (2003) found an increase in UV absorbing compounds in a moss, however, other workers did not find sufficient UV absorbing compounds to link flavonoids in *C. purpureus* to UV-B protection (Lovelock and Robinson 2002). It would not be possible to state whether the change in flavonoids noted in the work presented here would afford the moss protection from increased UV-B exposure. This alteration in relative amount of flavonoids merits further investigation.

This work has demonstrated differential responses to UV-B by different plant groups and species within those groups with respect to F_{vm}. Other parameters that exhibited sensitivity to UV-B exposure were qN, apparent quantum yield, and A_{max}. Changes in F_{vm} were the most frequent and significant effect seen in this study. These effects are summarised in Table 3.2. The alga and the liverwort would appear to be most sensitive species to UV-B as judged by this measurement. These both experienced

significant drops in F_{vm} within a few hours of exposure. F_{vm} depression is associated with PSII damage, so would imply UV-B exposure had a negative effect on photosynthesis within this sensitive group. However, there was no supporting alteration in gas-exchange response using the oxygen electrode.

Table 3.2 Sensitivity to UV-B exposure ranked by maximum significant depression of the F_{vm} parameter (values per kJ m^{-2} (PAS) shown in parentheses).

Species	Maximum significant depression of pre-exposure value (%) [X]	Exposure (hours) [Y]	Maximum exposure (for F_{vm}, hours)	Ratio of depression: exposure [X/Y]
Marchantia berteroana	49.6	85 (26)	85 (26)	0.58 (1.9)
Prasiola crispa	23.0	97 (30)	206 (63)	0.24 (0.8)
Bryum argenteum	0.4	70 (21)	70 (21)	0.006 (0.019)
Tortula princeps	1.6	320 (97)	546 (167)	0.005 (0.017)
Ceratodon purpureus	Not significant	N/a	70 (21)	N/a
Sarconeurum glaciale	Not significant	N/a	320 (98)	N/a

The alteration in qN was variable and transient. Other work has suggested that qN would be expected to rise as a result of UV-B exposure (Bornman and Teramura 1993). However a rise in qN only occurred in the least sensitive moss, *S. glaciale*. The origin and magnitude of qN has been attributed to a number of sources including (1) a change in the trans-thylakoid pH gradient (2) state1-state2 transitions, and (3) photoinhibitory quenching (Krause and Weis 1991). The actual effect of UV-B exposure on qN could vary between the sensitive species as contributions of each of these could not be fully resolved (in this study). Membrane damage has also been associated with exposure to UV-B radiation (Iwanzik *et al.* 1983), and may be related to falls in qN, if membrane damage prevents establishment of pH gradient (particularly during the light limiting period). Interestingly, there was no effect on qN in the alga, which exhibited a marked fall in F_{vm}. Recent fieldwork on an Antarctic liverwort (Snell *et al*. 2009) noted a rise in qN as a result of UV-B exposure, though qN was calculated using a different formula (Maxwell and Johnson 2000). The value of qN depends on the accurate measurement of F_o, F_m, F'_o, and F'_m. F'_o is ideally measured after a pulse of far-red light at > 700 nm (van Kooten and Snel 1990). The measurement of F'_o in this study was not carried out using a far-red light source. Thus, there may have been a slight error in the measurement of F'_o which would also affect the calculation of qP, but not F_{vm}.

Alterations in light response A_{max} and apparent quantum yield (when they did occur) were dramatic. The loss of A_{max} would indicate a problem with the light independent stage (Walker 1988), which would not necessarily be highlighted in the fluorescence response. Apparent quantum yields were also lower on these occasions. However, these alterations were not always fully reflected in the fluorescence data, in particular fluorescence yield and F_{vm}.

When alteration in flavonoid production did occur, there was no evidence of *de novo* synthesis as a result of UV-B exposure in the species studied. The species-specific differences in apparent flavonoid composition are of interest as it would appear to reflect differing strategies to counter the effects of UV-B radiation. Confirmation of the presence of these putative screening compounds was not always possible, although it can be stated with confidence that neither *T. princeps* nor *S. glaciale* contain measurable quantities of similar compounds that were extracted from *B. argenteum* and *C. purpureus*. Interestingly, both *T. princeps* and *S. glaciale* develop a deeply pigmented state in exposed areas (R. I. Lewis-Smith *pers. comm*. January 1996); this is not the

case for the cosmopolitan mosses, where in *C. purpureus* only browning occurs as a result of an increase in carotenoids (Post 1990).

The recovery from UV-B exposure shown by *P. crispa* needs further investigation. It would appear that there could have been photoinhibitory-type damage, similar to that shown by Rintamäki *et al.* (1994). However, there was no other supporting evidence for this as the cause of F_{vm} loss.

Similar UV-B-induced effects that have been found in this study have previously been described in other plants (See Chapter 1 for general references). Importantly, there appear to be different mechanisms of protection and damage between the alga and liverwort compared with the mosses. Damage to the light dependent stage was the primary effect on the alga and the liverwort (loss of F_{vm}, no alteration in A_{max}). If the mosses were damaged by UV-B exposure, then the evidence was conflicting; there was loss of A_{max} and decrease in apparent quantum yield, but no concurrent effect on F_{vm} or qP.

The results obtained in this study broadly concur with the preliminary measurements taken at Edmonson Point (Appendix 2). Maximal photosynthetic rates of the mosses followed a similar ranking with *B. argenteum* having the highest A_{max}. Furthermore, comparison of those mosses studied at Edmonson Point showed that, at the very least, all mosses sampled achieved comparable, or higher, maximal net photosynthetic rates. Thus, the environmental conditions in the growth cabinet were not deleterious to photosynthetic activity. This is important as it demonstrates that shipping to the UK and subsequent regeneration did not adversely affect the physiology of the cryptogams. Any deterioration of the plant material could lead to enhanced UV-B exposure having a greater impact on photosynthesis (Jordan 1996).

To place this work in an Antarctic context; the alga is widespread throughout the maritime and coastal areas of the continent, and the sensitivity exhibited to UV-B exposure by way of F_{vm} reduction would be expected to impact on productivity, algal survival and possible future spread. It also would seem that UV-B would be a limiting factor and thus may have historically limited the distribution of the nitrophilous alga. The effect on the liverwort was again rapid and sustained. Unlike the mosses, both the alga and liverwort species exhibit a sheet-like form and thus would receive, both within the growth cabinet and in the field, a higher UV-B dose than the mosses. This may have played a part in the similar response of the alga and liverwort compared to the mosses.

As regards the mosses studied, a number of factors could be responsible for the responses recorded. The two mosses that showed no evidence of containing putative flavonoids and no change in photosynthesis (on a dry mass basis in *T. princeps*) are both considered to have a very restricted distribution compared to the other two mosses *B. argenteum* and *C. purpureus*; both of which are cosmopolitan species and exhibited the presence of flavonoids. The coping strategies of the cosmopolitan mosses must be important in their distribution and global success, but there must be a metabolic cost such as flavonoid production. Another moss, the endemic *Grimmia antarctici*, was not found to contain appreciable amounts of putative UV-B absorbing compounds compared to two cosmopolitan species that were also investigated (Dunn and Robinson 2006). The pigmented form of the restricted mosses could provide a lower albedo and increase warming at the cost of less penetration of PAR and the consequent loss of photosynthetic productivity. Kershaw and Watson (1983) noted that heavily pigmented lichens maintained a 6^{o}C differential temperature to bare rock surface on which they were growing. On the other hand, those mosses with inducible flavonoids would be able to screen from UV-B radiation, but not alter the potential efficiency of photosynthesis. Hence the cosmopolitan mosses, particularly *Bryum* spp., have a higher photosynthetic rate as was shown in this work. If these mosses had evolved under differing pressures, i.e. low temperature and low UV-B, compared to the cosmopolitan mosses which would have had to cope with a higher UV-B; it could be possible that together with water stress, freezing, and nutrient paucity that production of UV-B absorbing compounds is the limiting and restricting component especially now with regards to the recent ozone thinning. The more restricted species have probably been present in Antarctica for a considerable time at least since the last glacial maximum, 5 to 6 ka B.P. (Convey *et al.* 2008), and are as such probably well adapted to the Antarctic environment. Whether this early environment would have included higher levels of biologically effective UV-B is unclear, but the exposures in this study did nothing to effect these mosses. Moreover, there is considerable debate to the origin of Antarctic terrestrial life (e.g. Convey *et al.* 2008). There is very little endemism in the moss flora (possibly up to 5%, Peat *et al.* 2007), and the results from this study would indicate that the restricted species *S. glaciale* may well have been a pre-glacial relic which has survived in refugia; it was thought to be endemic until discovery in Patagonia. However, it is a trans-Antarctic species; being found in both the west and east of Antarctica. For this moss to have spread from Patagonia to East Antarctica since the last glacial maximum would be

some considerable achievement, and with such an advance not realising production of flavonoid like compounds to shield from a higher UV-B exposure as with the cosmopolitan species is also remarkable. Furthermore, the cosmopolitan species are found in areas of greater water availability than the restricted species (Lewis-Smith 1999). It would be expected that the cosmopolitan species would be of a generalist type and adapted to wide range of conditions, whilst the restricted species would be limited by a rapid change in the light environment, namely increased UV-B exposure. This was not found to be the case.

To conclude, there does appear to be differential sensitivity to UV-B exposure at relatively low and environmentally relevant UV-B fluence amongst the species examined. There are differing sites of action; PSII being the major target in some cryptogams and the light independent stage altered in some of the mosses. Depression of A_{max}, if it occurs, may also be substantial, with up to a 30% loss compared to un-exposed samples. Where the damage appears to be great, then there has also been rapid recovery (of F_{vm}) when UV-B exposure has ceased. Production of flavonoids is dependent on species, and not necessarily as a response to enhanced UV-B exposure. Furthermore, when there is an alteration, it is as a result of a change in relative amount rather than production of new compounds.

CHAPTER 4

STUDIES ON THE EFFECT OF EXPOSURE TO UV-B RADIATION ON PHOTOSYNTHESIS AND RELATED PROCESSES IN *DESCHAMPSIA ANTARCTICA*

4.1 Introduction

This work was carried out to try to identify possible effects of UV-B exposure on the efficiency of CO_2 assimilation, and to determine whether photosynthesis and other associated processes are also altered by exposure to UV-B radiation.

Prior to the current interest in Antarctic ozone depletion, *Deschampsia antarctica* was an understudied species and the subject of relatively few publications (e.g. Holtom and Greene 1967). Some of these were of a biochemical nature (Zúñiga *et al.* 1994), and some physiological (Edwards and Smith 1988). Edwards and Smith (1988) found the optimal temperature for photosynthesis in *D. antarctica* was ca. 13^{o}C, reducing to 30% at 0^{o}C. None had been concerned with the potential effects of UV-B radiation. Since then (and after the work described in this thesis was carried out) work on the response of *D. antarctica* to field and growth chamber manipulations of UV-B radiation have been reported (Rozema *et al.* 2001, Xiong and Day 2001). Some of this work has looked at the effect of UV-B on photosynthesis. Xiong and Day (2001) used an aqueous-phase oxygen electrode to record light response curves on excised leaves and only found a decrease in photosynthesis at high PPFD (800 μmoles m^{-2} s^{-1}) expressed as a function of chlorophyll content or dry mass, but not on an area basis. They concluded that any UV-B effect on photosynthesis was associated with the light-independent stage. Rozema *et al.* (2001) constructed light response curves using IRGA at ambient CO_2 concentration, but at 6^{o}C above growth temperature. They found no effect of enhanced UV-B exposure on these light response curves. Furthermore, fieldwork utilising the fluorescence ratio F_{vm} did not show any differences attributed to altered UV-B exposure (Lud *et al.* 2001).

Any effect on photosynthesis should also impact on the products of photosynthesis, namely soluble carbohydrates. Alterations in soluble carbohydrate content of grasses under conditions of water stress and low temperature are well documented, e.g. water stress (Kameli and Lösel 1993) or low temperature (Tognetti *et*

al. 1990). Work on the effect of UV-B exposure on the products of carbon fixation in other vascular plants has produced variable results. Mackerness *et al.* (1997) noted a fall in glucose concentration in pea, over five days of exposure to 164 $mWm^{-2}\ s^{-1}$ (PAS) UV-B radiation (concurrent with 150 μmoles $m^{-2}\ s^{-1}$ PPFD), a similar fall in glucose was also noted in maize (Barsig and Malz 2000). Whether changes to soluble carbohydrates indicate some co-ordinated response (or osmotic alteration) to stress, or whether the response is more specific is unknown. However, a study on conifer seedlings indicated that plants grown under high UV-B (6 kJ m^{-2} (PAS) day^{-1}) showed greater tolerance to high PPFD (2,000 μmoles $m^{-2}s^{-1}$) and drought (Poulson *et al.* 2002). *D. antarctica* is known to accumulate very high amounts of sucrose and fructans (Zúñiga *et al.* 1996), hence any alteration in photosynthetic activity could change the concentration of sucrose or other soluble carbohydrates. Moreover, sucrose accumulation in *D. antarctica* is linked with freezing tolerance (Zúñiga-Feest *et al.* 2005), so any alteration in sucrose concentration could impair the ability of the hair grass to withstand the low Antarctic temperatures. No work has to date been published on the effect of enhanced UV-B exposure on non-structural carbohydrate accumulation in *D. antarctica*.

Changes in leaf length as a consequence of UV-B exposure have been reported on a number of occasions (see Chapter 1). Reduction in leaf length of *D. antarctica* has been noted in field manipulations of the UV-B environment (Day *et al.* 1999). Furthermore Rozema *et al.* (2001) found reduced shoot length in plants exposed to enhanced UV-B radiation using a lamp-based system in the field, but did not find a significant decrease in specific leaf area. Any changes to leaf morphology should impact on photosynthesis by alteration in surface area available for absorption of PAR and thus productivity.

Alteration in flavonoid production in response to UV-B exposure is well documented (see Chapter 1). The flavonoid composition of the grass has been analysed (Webby and Markham 1994); with a total of five flavone compounds identified as unique to *D. antarctica*. Three other compounds were also found (orientin, isowertisin and tricin) that were also present in other grass species. Work has also been carried on the simpler phenolics, ferulic acid and coumaric acid (Ruhland and Day 2000), which were found in relatively large quantities within the hair grass. Subsequent studies did not note any significant change in composition or amount of soluble flavonoids as a result of increased UV-B exposure (van de Staaij *et al.* 2002, Ruhland *et al.* 2005). However, all of these studies have been on mature plants in the field or plants that have

been re-grown from field-collected tufts. Therefore all plants used would have a developmental history of UV-B exposure prior to experimentation.

Photosynthetic and related accessory pigments have also been investigated in relation to UV-B exposure. The xanthophyll cycle has been shown to have a role in excess light energy dissipation in many plants (Demmig-Adams and Adams 1996). Moreover, inhibition of the cycle by as a result of UV-B exposure has previously been demonstrated (Pfündel *et al.* 1992). Accordingly, xanthophyll cycle intermediates were extracted and analysed for this study. Work by Xiong and Day (2001) on the hair grass noted higher concentrations of chlorophylls and carotenoids in UV-B-exposed plants on a leaf area basis, but not dry mass. Lud *et al.* (2001) however, found no alteration in chlorophyll or carotenoids content after exposure to enhanced UV-B radiation for 15 days.

The purpose of this study was to determine whether altered UV-B fluence, at an environmentally realistic level, could induce and maintain a physiological or biochemical response in the hair grass previously un-exposed to UV-B radiation. Thus the exposure fluence was relatively low but maintained within a comparable ratio of UV-B:UV-A:PAR used previously (see Chapter 2). The work described here is a combination of two series of experiments, one series carried out on re-grown tufts (samples taken from Signy I.), and the other on younger plants grown in the UK (seed collected on Leonie I.) without prior developmental exposure to UV-B radiation.

4.2 Materials and methods

4.2.1 Environmental chambers and monitoring

As described in Chapter 2 with the following additions/ amendments. The regenerated tufts were grown under a 12 h light/ dark cycle. PPFD and UV radiation were measured continuously (where practicable, see Chapter 2). The biologically weighted (UV-B $_{(PAS)}$) values were as reported previously, thus the daily 12 h and 16 h weighted UV-B (UV-B $_{(PAS)}$) were 3.67 and 4.89 kJ m^{-2} respectively. The regenerated material was kept under a 12 h cycle as the growth cabinet did not originally function effectively at the planned setting of 16 h.

4.2.2 Plant stock

Regenerated material (48 tufts) was collected from Signy I. during the Austral summer 1994/95. Plants were initially excluded from UV-B radiation for eight weeks prior to exposure so that all new growth had not been previously exposed to UV-B radiation. This should ensure that if there were sensitivity to UV-B exposure then it would be exhibited by the hair grass. Plants were sampled before UV-B exposure and on subsequent times (Table 4.1). The limited amount of plant material prevented a more thorough sampling programme. Seeds collected from Leonie I. were germinated then placed directly under UV-B opaque ('VE') or UV-B transparent ('OX/02') cloches before the first cotyledon leaves had appeared.

Plants were randomly selected from each treatment for analysis. The mid-sections of young, fully expanded leaves (with no evidence of senescence) were used for all measurements, except for the non-structural carbohydrates. A photographic record was made of the seed-grown plants to show any possible morphological differences. Leaves were also taken for analysis under the scanning electron microscope (SEM) to observe any surface modifications that could be responsible for any alteration in photosynthetic activity.

4.2.3 Fluorescence measurements

Measurements of F_{vm} were taken on both the regenerated plants and on the seedlings using the OS-100 modulated fluorimeter. Five recordings from different leaves were made for each treatment.

4.2.4 Gas exchange using IRGA

For recordings at two levels of PPFD (varying PPFD), the regenerated plants were taken from the chamber and placed in a constant temperature room (12 to 14^{o}C). Two levels of PPFD used were 100 and 480 μmoles m^{-2} s^{-1}; the former approximating to the cabinet PPFD, the latter to the saturating intensity for the cabinet grown plants. Light response curves were recorded for the seed-grown plants (as described in Chapter 2). A/c_i measurements were taken on both sets of plants (see Chapter 2 for details). Typically four recordings were taken for each sampling date; the leaves were then removed and the total surface area calculated.

4.2.5 Biochemical analysis

Samples from the regenerated plants were taken periodically for biochemical analysis (Table 4.1). Three replicate samples were taken at ca. 1 h (am) and 10 h (pm) into the photoperiod. The shoots and the leaf blades were separated for sugar analysis, whilst for pigment analysis leaf blades alone were sampled. Samples were analysed for non-structural carbohydrates (simple sugars, HPLC-ECD) chlorophyll content (spectrophotometric/ HPLC-DAD), carotenoid composition (spectrophotometric/ HPLC-DAD), and flavonoids. Flavonoid samples (for HPLC-DAD) came from the soluble sugar extracts, or from oven dried (100^{o}C) samples taken after gas exchange measurements. Full details of extraction and analysis protocols are given in Chapter 2.

Flavonoids and chlorophylls were also extracted from the seed-grown plants. Leaves were oven-dried at 100^{o}C prior to extraction for HPLC-DAD analysis of flavonoids. Chlorophyll extraction was as described for the regenerated plants (described in Chapter 2).

4.2.6 Schedule of sampling and statistical analysis

The plants grown from seed were sampled after approximately 100 days from germination. The regenerated plants were sampled over a period of 79 days (summarised in Table 5.1). Statistical analyses and curve-fitting (where appropriate and unless otherwise stated) were carried out as described in Chapter 2.

Table 4.1 Summary of analysis and schedule of sampling of the regenerated *Deschampsia antarctica* plants.

Day	Cumulative UV-B exposure (mid-point of photoperiod) kJ m^{-2} (PAS)	Physiological Sampling	Biochemical Sampling
-4	0	A/c_i	+
-3	0	2xPPFD F_{vm}	
3	9.2	2xPPFD F_{vm}	+
6	20.2	F_{vm}	
10	34.9	2xPPFD	+
14	49.5	A/c_i	
17	60.6	F_{vm}	+
21	75.2	F_{vm}	
25	89.9	A/c_i	
41	148.6	A/c_i	
45	163.3		+
59	214.7	A/c_i	+
75	273.4		+
79	288.1	2xPAR	

Key: A/c_i, effect of sequential alteration in CO_2 concentration on net photosynthesis 2xPPFD, *in situ* photosynthesis recorded at low (100 µmoles m^{-2} s^{-1}) and high (480 µmoles m^{-2} s^{-1}) PPFD.

4.3 Results

4.3.1 Overall appearance and changes in morphology

Plants that were subjected to UV-B exposure produced leaves that appeared shorter, less expanded, and more tightly rolled than the control plants. Also there appeared to be bronzing of leaves, and those that had senesced appeared darker compared to the UV-B-excluded samples. There was no noticeable difference in tillering, but tiller length was shorter and width narrower on the UV-B-exposed plants. These observations were most distinct in the seed-grown plants (Figure 4.1). Here the mean fully expanded tiller length was 36 mm for the UV-B-exposed compared to 59 mm for the UV-B-excluded plant (Significantly different $P < 0.01$, $n = 13$). Furthermore, mean width was 1.5 mm for UV-B-exposed compared to 2.1 mm for the UV-B-excluded tillers (again significantly different $P < 0.01$, $n = 13$). Spread of plant was also less, at 110 mm compared to 180 nm as a result of shorter tiller length.

The SEM images showed that the leaves from clonal tufts exposed to UV-B radiation appeared to have surface waxes of a rougher texture compared to the non-exposed samples (Figure 4.2), whilst those of the seedling appeared smoother than the re-grown tufts regardless of exposure protocol (Figure 4.3). Both sets of leaves contained appreciable amounts of waxes. There were however, insufficient samples to fully quantify stomatal distribution. The investigation did show *D. antarctica* grown in this study as having a similar morphology to both that of conspecifics described from field and greenhouse studies (Gielwanowska *et al.* 2005), and to that of other xeromorphic grasses (Humphreys *et al.* 1986, McNeilly *et al.* 1987).

4.3.2 Dark-adapted fluorescence

The regenerated plant material only exhibited a significant difference ($P < 0.05$) in F_{vm} after 75.2 kJ $m^{-2}{}_{(PAS)}$ exposure, when the mean F_{vm} of the UV-B-exposed sample was higher (0.79) compared to the UV-B-excluded sample (0.78). There were no other significant differences between the exposed and non-exposed plants ($P > 0.05$).

F_{vm} measurements on the plants grown from seed exhibited no significant difference between plants (t-test, $P > 0.05$) despite the considerable morphological differences already noted.

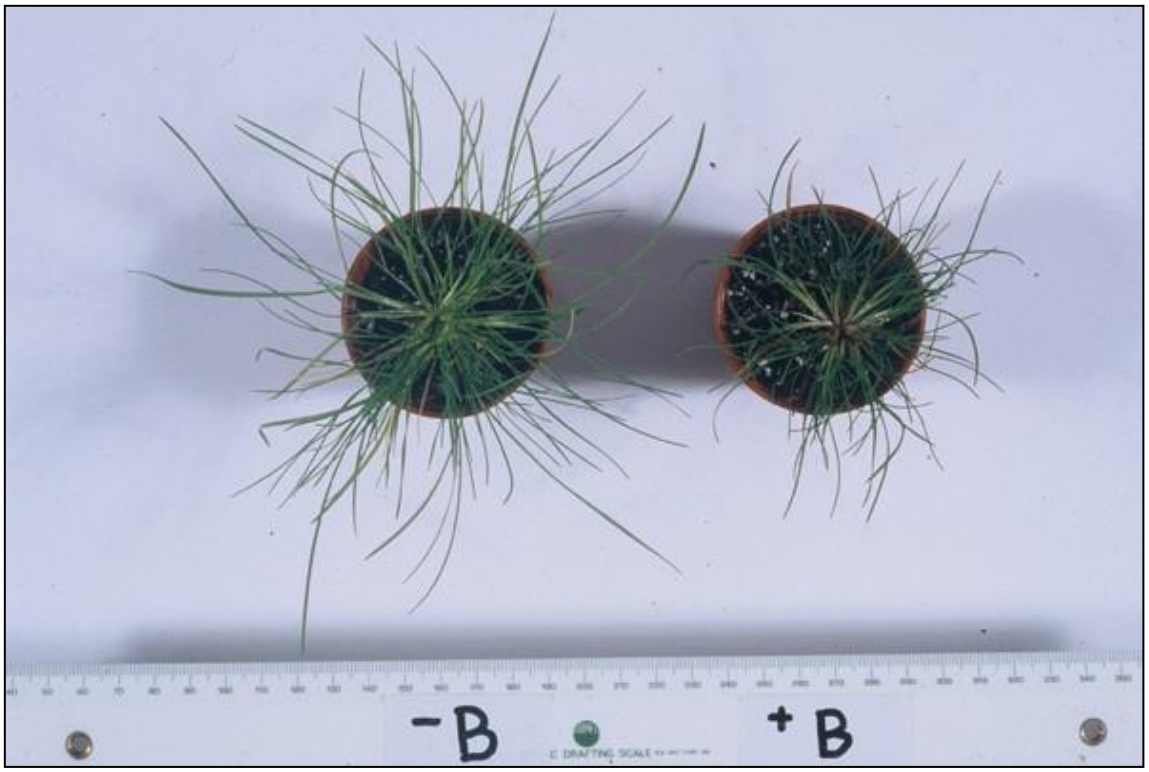


Figure 4.1 Effect of exposure to UV-B radiation on the morphology of *Deschampsia antarctica*. Plants grown from seed collected from Leonie I. Plants had been continually exposed/ excluded since germination. (-B excluded, +B exposed to UV-B radiation).

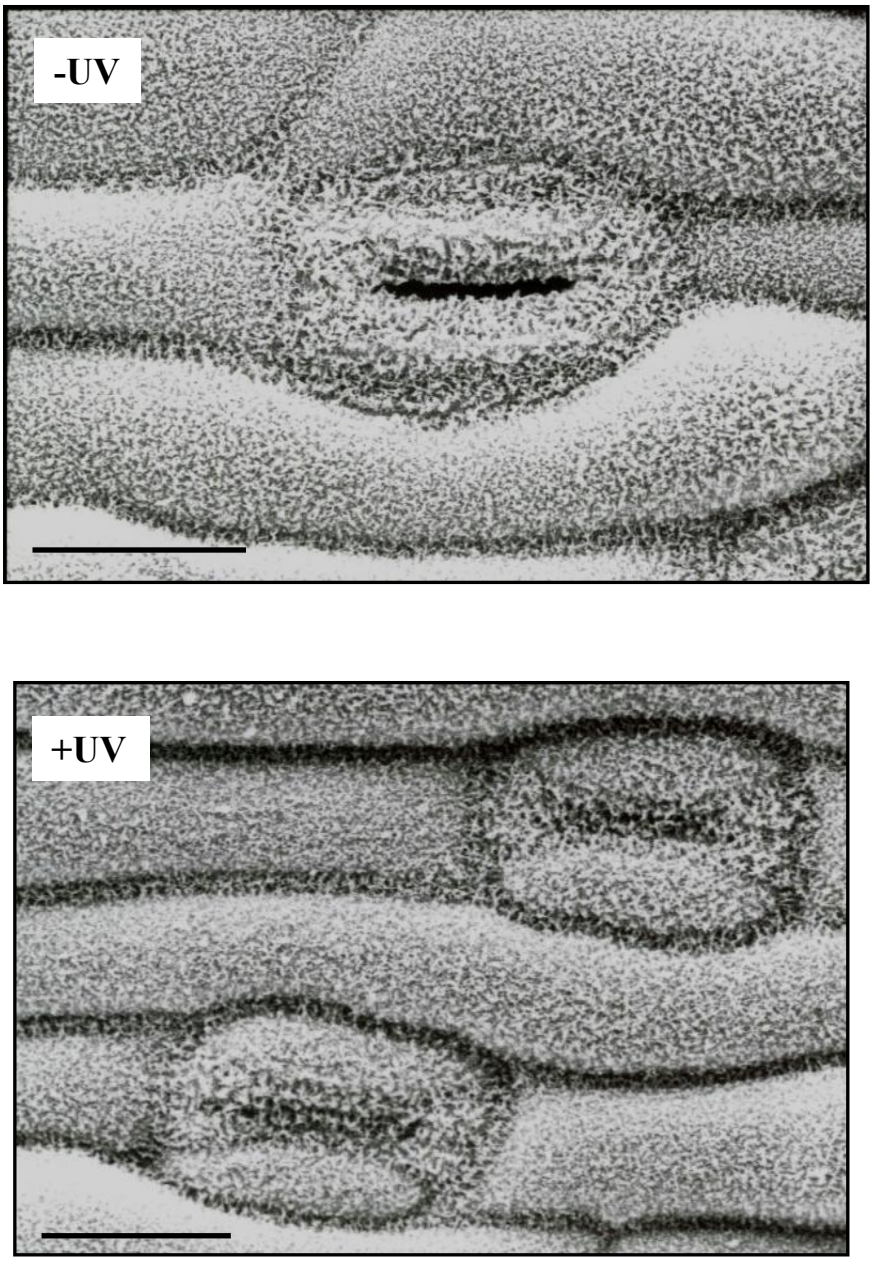
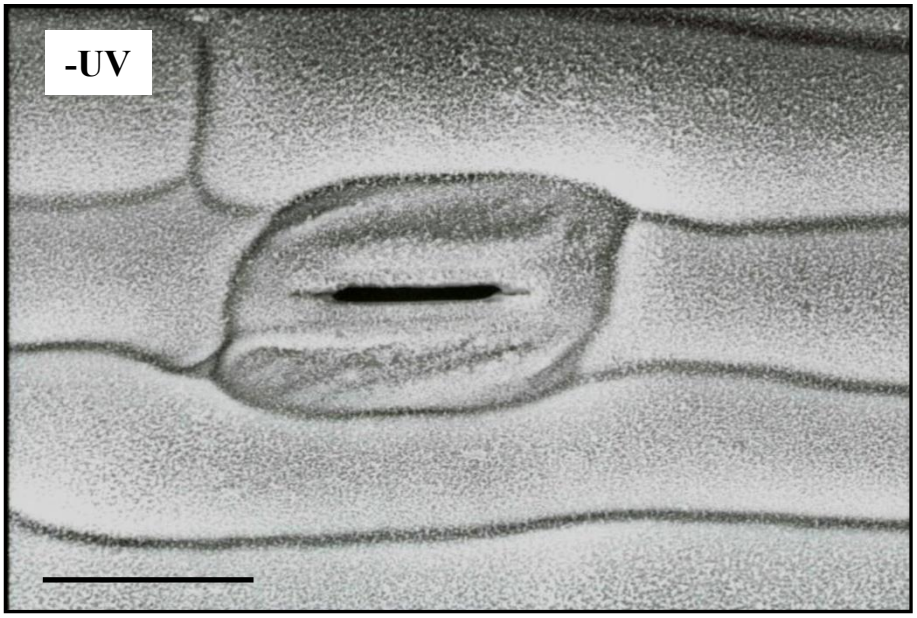


Figure 4.2 Representative SEM images of the adaxial surfaces of *Deschampsia antarctica* leaves from re-grown clonal tufts either excluded from (-UV) or exposed to (+UV) UV-B radiation since re-growth. Scale bar = 20 μm.



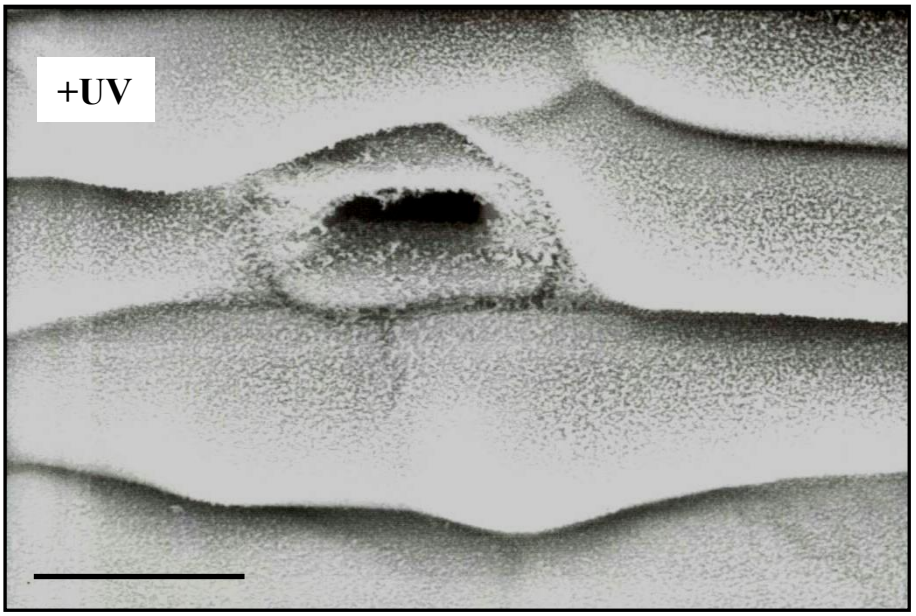


Figure 4.3 Representative SEM images of the adaxial surfaces of *Deschampsia antarctica* leaves from seedlings either excluded from (-UV) or exposed to (+UV) UV-B radiation since germination.
Scale bar = 20μm.

4.3.3 Varying PPFD and light response curves

The rate of photosynthesis (obtained at approximately growth height and ambient CO_2 concentration) of the regenerated plants were similar throughout the experiment with rates in the range of 3-5 μmoles m^{-2} s^{-1}, and 8-10 μmoles m^{-2} s^{-1} for 100 and 480 μmoles m^{-2} s^{-1} PPFD regimes respectively. Photosynthetic rates did not differ significantly ($P > 0.05$) between control and the UV-B-exposed plants throughout the duration of the experiment. These values were comparable to those recorded by Xiong *et al.* (2000), but slightly higher than those of Rozema *et al.* (2001).

Transpiration rates were between 0.5-1.0 mmoles m^{-2} s^{-1} at both 100 and 480 μmoles m^{-2} s^{-1} PPFD. The calculated water use efficiency (WUE, expressed as g (H_2O)/ g (CO_2) was high with values around 40 g (H_2O)/ g (CO_2), but falling as low as 17 g (H_2O)/ g (CO_2). This was probably an anomalous value as no other similar extreme values were subsequently attained. Light response curves (obtained from the seed-grown plants) showed no significant difference (t-test, $P > 0.05$) between samples in either apparent quantum yield or A_{max}.

4.3.4 A/c_i response curves

The overall values obtained for the A/c_i curves are shown in Table 4.2. The internal CO_2 concentration that was equivalent to ambient external CO_2 concentration was approximately 200 μmol mol^{-1} for both control and exposed leaves.

Calculated $V_{c,max}$ were slightly higher in the UV-B-excluded plants, and significantly ($P < 0.05$) after 14 days, (Figure 4.4, Table 4.2). Moreover, this pattern was most noticeable in the UK germinated plants, with UV-B-excluded samples exhibiting a significantly higher (t-test, $P < 0.01$) $V_{c,max}$ (0.039) compared to UV-B-exposed plants (0.027, Figure 4.5a). Similar findings were found when rates were expressed as function of chlorophyll content (Figure 4.5b).

Maximal assimilation rates (A_{max}) were again quite variable between sampling dates, but A_{max} was usually lower in UV-B-exposed compared to control leaves on most sampling dates (Figure 4.2, Table 4.2). The mean calculated A_{max} of the seed grown UV-B-exposed plants was slightly lower (12.6 μmoles m^{-2} s^{-1}) though not significant (t-test, $P > 0.05$) than the UV-B-excluded samples (14.3 μmoles m^{-2} s^{-1}).

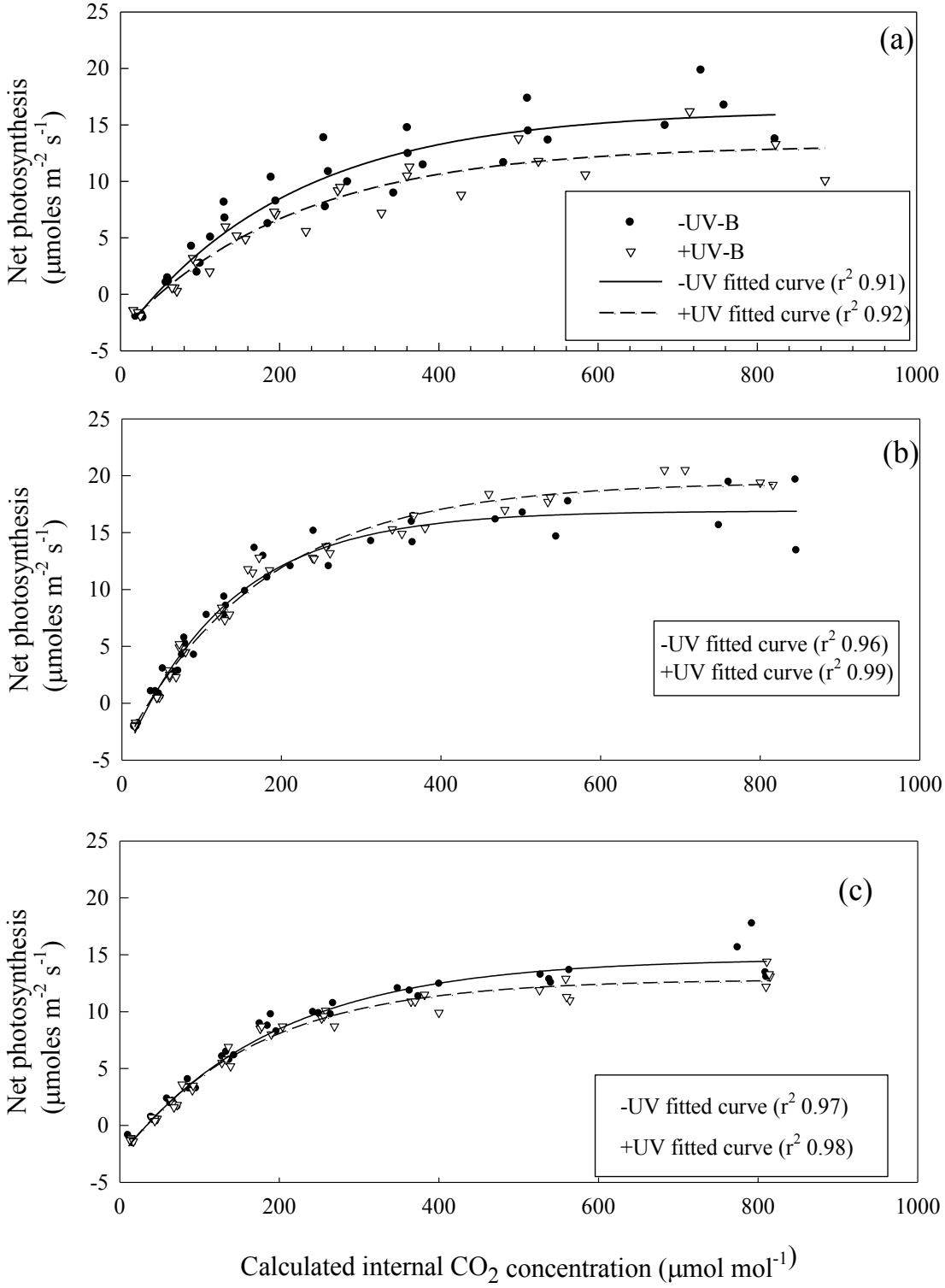


Figure 4.4 Effect of varying internal CO_2 concentration on net CO_2 exchange (photosynthesis) obtained from *Deschampsia antarctica* leaves re-grown from tufts either, exposed to UV-B (+UV) or, excluded from UV-B (-UV) radiation, for (a) 14, (b) 41, and (c) 59 days. Mono-exponential function fitted to each data-set. (n = 4 except, a +UV, n = 3).

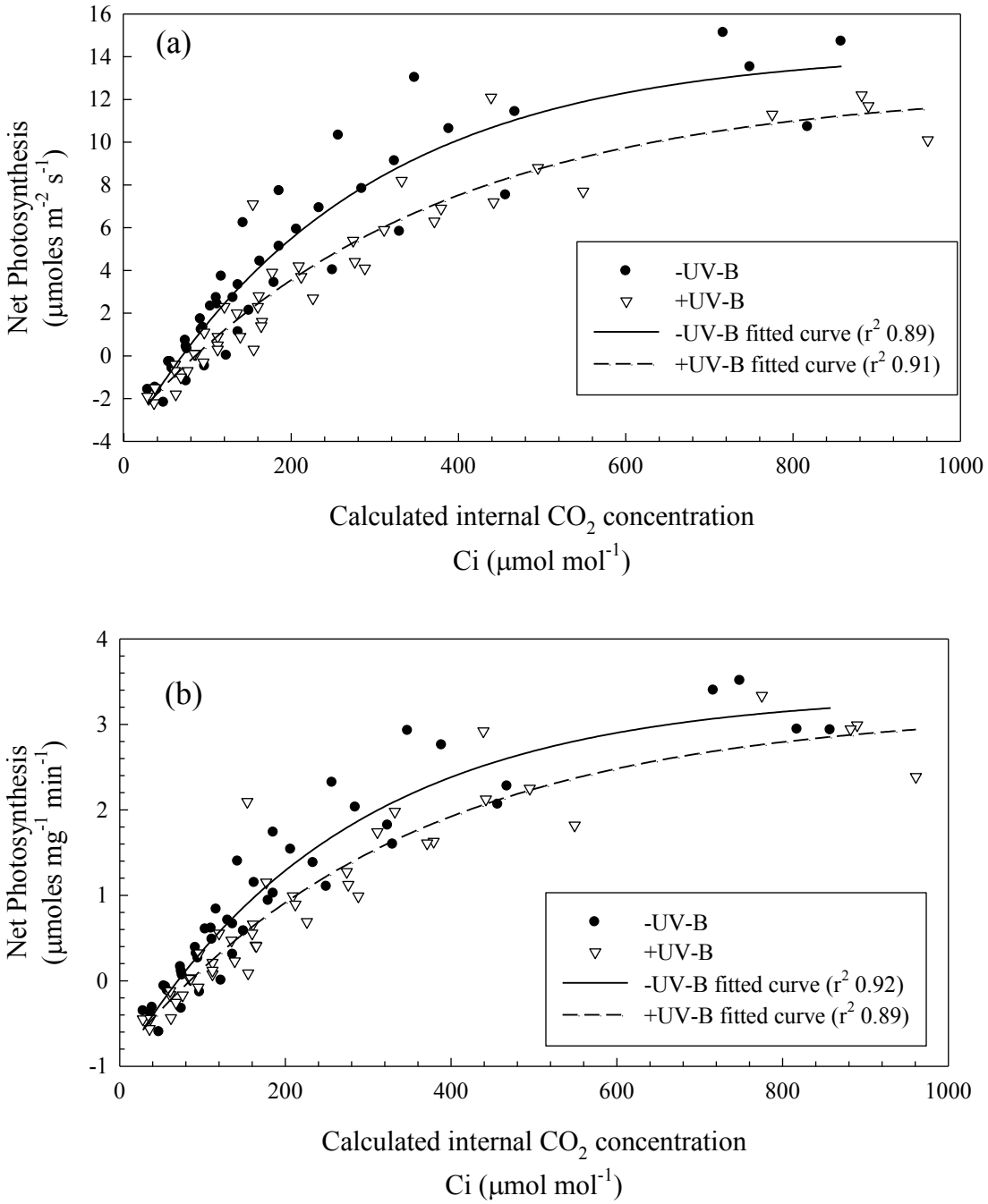


Figure 4.5 Effect of varying CO_2 concentration on the net CO_2 exchange (photosynthesis) of leaves from *Deschampsia antarctica* seedlings grown from germination either, exposed to UV-B (+UV-B), or excluded from UV-B (-UV-B) radiation until sampling (100 d). Data expressed either on a (a) unit area, or (b) total chorophyll mass basis. Mono-exponential function fitted to each data-set (n = 4).

There were no other consistent significant differences between the control and the UV-B-exposed regenerated plants with respect to A/c_i response. In fact, there was more variability between sampling dates in the measured parameters than between treatment and control (Figure 4.3).

Table 4.2 Summary of effect of increasing internal CO_2 concentration on net CO_2 uptake (A/c_i response) obtained from leaves of *Deschampsia antarctica* plants re-grown from samples taken from Signy I. Plants either exposed to, or excluded from, UV-B radiation. CO_2 compensation point analysed using Kruskal-Wallis test due to unequal variance. Brackets, ± S.E. * significant difference between control and UV-B-exposed on sampling date ($P < 0.05$). A_{max} maximal CO_2 saturated net assimilation rate; $V_{c,max}$ carboxylation efficiency. See text for details of analysis ($n \geq 3$).

Day	UV-B-exposed (UV) or excluded (C)	A_{max} (μmoles m^{-2} s^{-1})	CO_2 compensation point (c_i μmoles mol^{-1})	$[CO_2] = 0$ (μmoles m^{-2} s^{-1})	$V_{c,max}$ (μmoles m^{-2} s^{-1} / μmol mol^{-1})
0	Pre	13.4 ± 1.1	71.1 ± 5.9	-3.7 ± 0.2	0.052 ± 0.004
14	C	16.3 ± 1.1	52.8 ± 4.1	-3.9 ± 0.2 *	0.080 ± 0.007 *
	UV	13.2 ± 0.9	55.4 ± 1.7	-3.0 ± 0.3 *	0.058 ± 0.007 *
25	C	18.0 ± 0.7	33.5 ± 1.3	-2.8 ± 0.1	0.086 ± 0.004
	UV	15.3 ± 0.7	36.7 ± 1.3	-2.6 ± 0.2	0.073 ± 0.005
41	C	16.9 ± 0.5	37.6 ± 1.6	-3.8 ± 0.4	0.105 ± 0.008
	UV	19.5 ± 0.4	37.4 ± 0.5	-4.0 ± 0.1	0.110 ± 0.003
59	C	14.7 ± 0.4	35.1 ± 2.5	-2.2 ± 0.3	0.064 ± 0.004
	UV	12.8 ± 0.3	35.6 ± 1.6	-2.2 ± 0.2	0.063 ± 0.004

4.3.5 Chlorophyll content and xanthophyll cycle pigments

Total chlorophyll content in the leaves from regenerated plants varied between 0.7 and 1.0 μg mg^{-1} fresh mass. There was a significant ($P < 0.01$) rise in mean chlorophyll content after 3 days of UV-B exposure compared to the control (Figure 4.6). Subsequent sampling dates did not reveal any significant differences between UV-B-exposed and control ($P > 0.05$). However, there was some variability between sampling times. The seed grown material exhibited no significant difference (t-test, $P > 0.05$) when measured in chlorophyll content between control (2.00 ± 0.09 μg mg^{-1} fresh mass) and UV-B exposed (2.05 ± 0.10 μg mg^{-1} fresh mass).

A comparison of xanthophyll cycle carotenoids in the regenerated plants did not highlight any discernible difference between UV-B-exposed and control samples.

4.3.6 Phenolic content

The dates used for comparison of the regenerated plants (on account of technical limitations) were the following; fresh mass samples taken concurrently with tiller extracts after 3, 10, and 17 days; and dry mass samples taken on day 75. Moreover, equivalent quantitative result as reported below were also obtained on other dates, but sample size recovered ($n = 2$) was not sufficient for statistical analysis. However, despite this problem there was a significant, and consistent, quantitative alteration in flavonoid content between control and UV-B-exposed samples. There was no apparent *de novo* synthesis of novel compounds (Figure 4.7), but there was a change in the relative composition of the UV-B-exposed extracts regardless of sampling time or extraction method.

A preliminary inspection of chromatograms (peak absorption and overlaying of UV-B-exposed and excluded samples) resulted in six peaks being selected for quantitative analysis (Figure 4.7). The first peak of note, A; was consistently and significantly higher for both fresh mass (Figure 4.8, $P < 0.05$) and dry mass (Figure 4.9, t-test, $P < 0.01$) for the UV-B-exposed samples. Peak B was higher in all samples, though not always significantly. Of the other peaks analysed most were similar except peak E, which was consistently higher in the control samples, but only significantly different on one day 17.

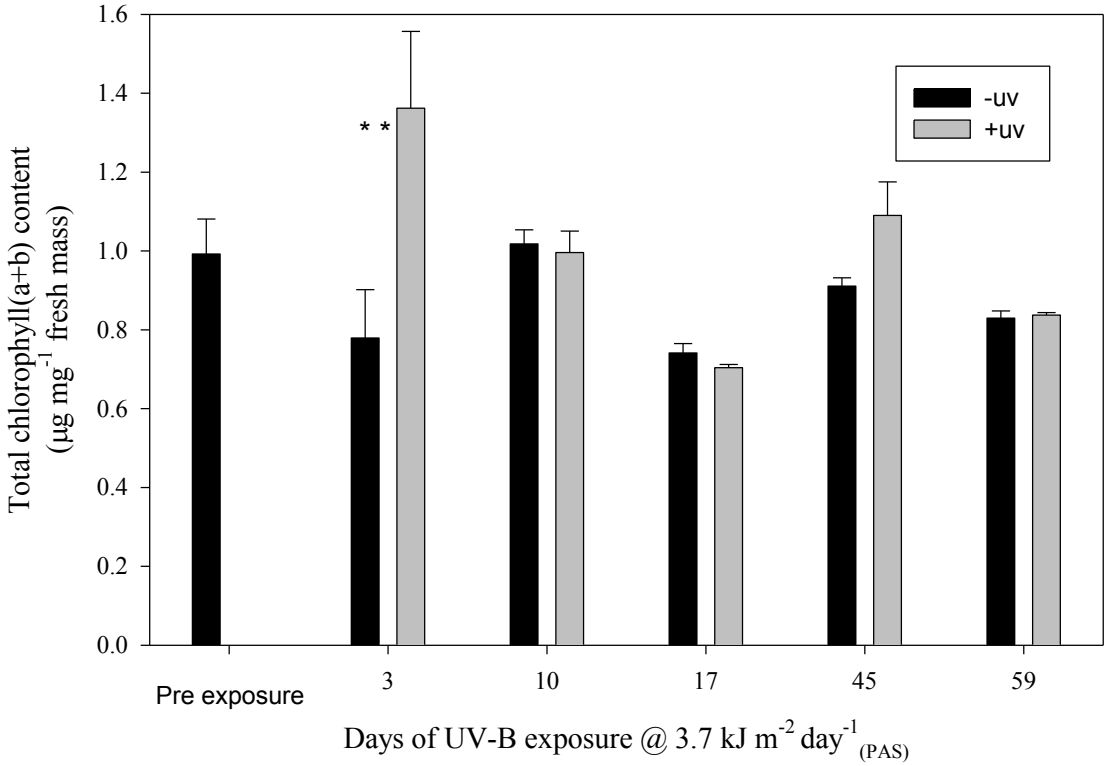


Figure 4.6 Chlorophyll content in UV-B exposed (+UV) and UV-B excluded (-UV) *Deschampsia antarctica* leaves exposed for up to 59 days. Samples taken 10 h into photoperiod.
Significant differences between exposed and excluded on each date indicated by * ($P < 0.05$) and * * ($P < 0.01$).
Mean ± S.E. (n = 3).

The seed-grown samples exhibited all the characteristic peaks found in the regenerated plants; including an increase in the UV-B-exposed sample of peak A compared to the excluded sample (ratio 1.27 exposed/ excluded). This would also confirm that there had not been de novo synthesis of unique flavonoid compounds, but an alteration in the balance in the relative amount of these substances. Interestingly, for the seed grown plants, there also appeared to be an overall increase in the relative amount of peak D in the UV-B-exposed sample (ratio of 1.57 UV-B-exposed/ UV-B-excluded).

The altered peaks A, B, and C, all had similar λ_{max} to that of the external standard orientin and peak B eluted at the same time as orientin. This would infer that peak B was orientin and that the other peaks were derivatives or closely related to orientin. Webby and Markham (1994) identified orientinisowertiajaponin (7-*O*-methylorientin) 2”-*O*-arabinopyranoside and orientin as dominant components, together with another; orientin 2”-*O*-β-arabinopyranoside. It is probable that peaks A and C were also orientin-like glycosolated flavones or derivatives of such.

Comparison of spectrophotometric data showed the UV-B-exposed samples had maintained a higher (though not significant) mean absorbance over the UV-B range than the controls. The broad absorbance maxima was found, as previously noted, to be 340 nm (Webby and Markham 1994).

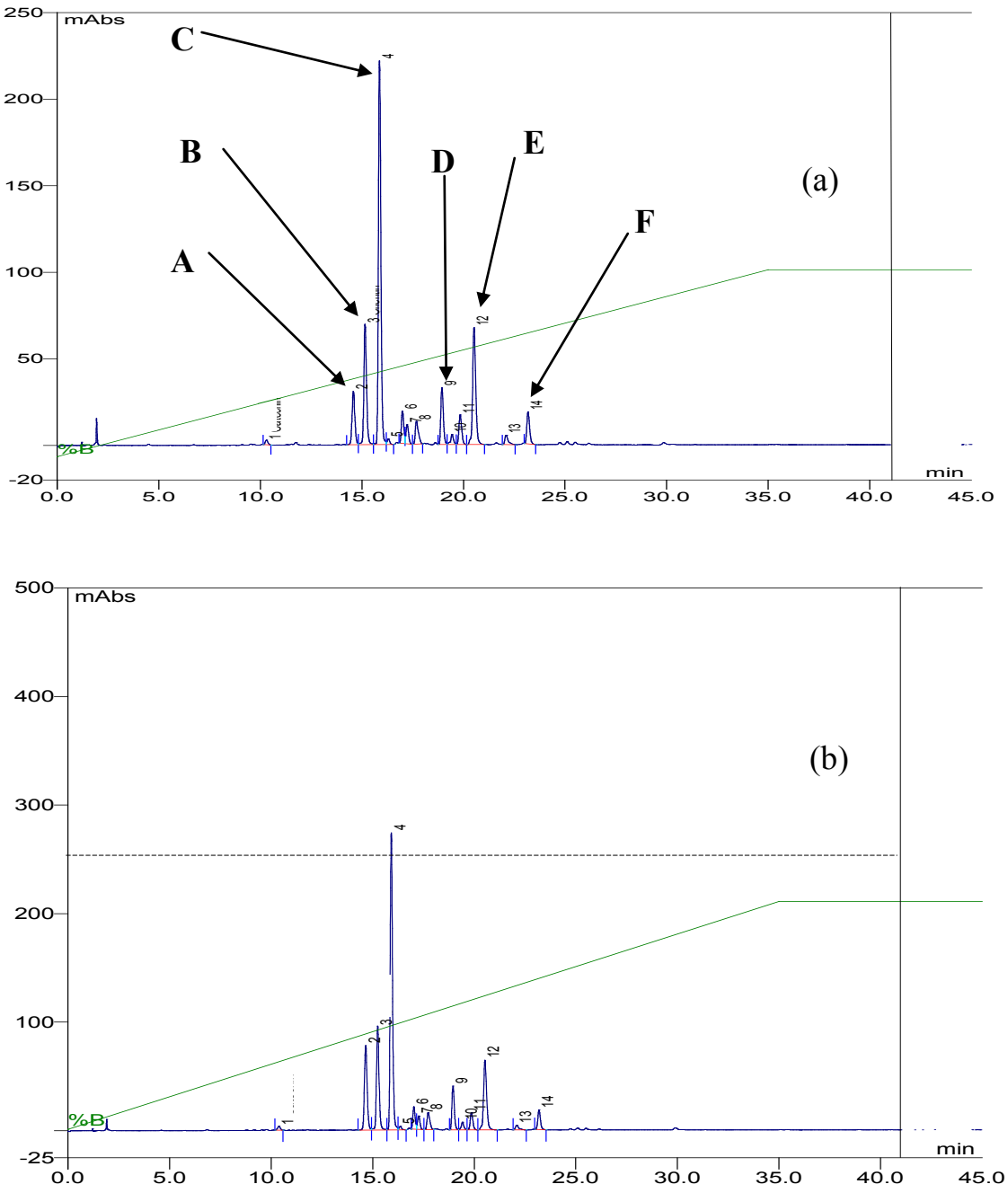


Figure 4.7 Representative chromatogram profiles of samples taken from regenerated tufts of *Deschampsia antarctica* leaves after exposure to UV-B radiation for 75 days. Extracts from similar dry mass of leaves. See text for explanation of peaks.
(a) Excluded from UV-B radiation (b) exposed to UV-B radiation.
Dashed line on (b) equivalent to maximum scale on the UV-B excluded sample.

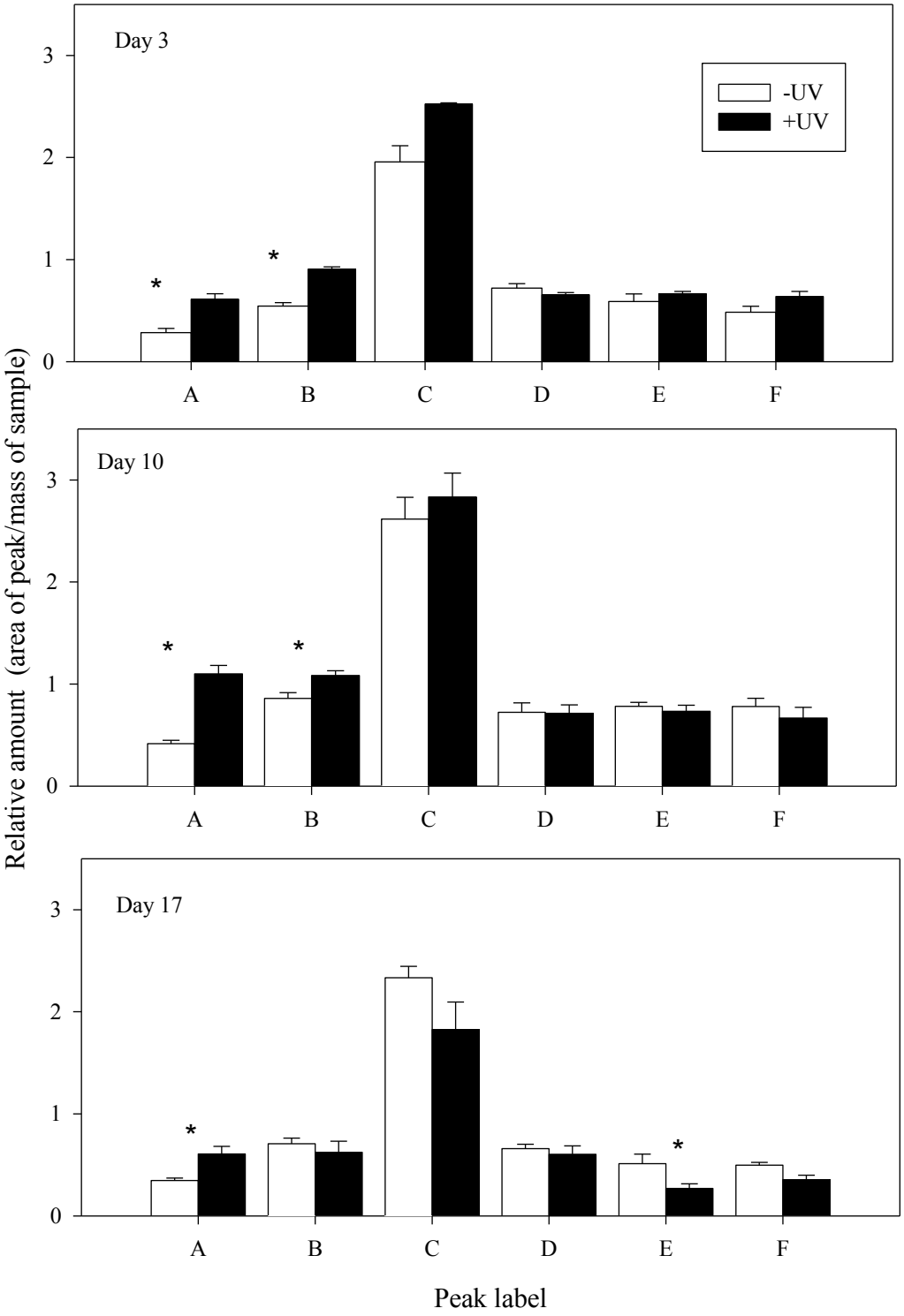


Figure 4.8 Relative amounts of selected peaks taken from chromatogram profiles of methanolic extracts obtained from *Deschampsia antarctica* leaves taken from re-grown tufts. Samples taken after 3, 10 and 17 days of exposure to, or exclusion from, UV-B radiation. Extracts from fresh mass. Mean + S.E. (n = 3). Significant difference between samples ($P < 0.05$) denoted by *.

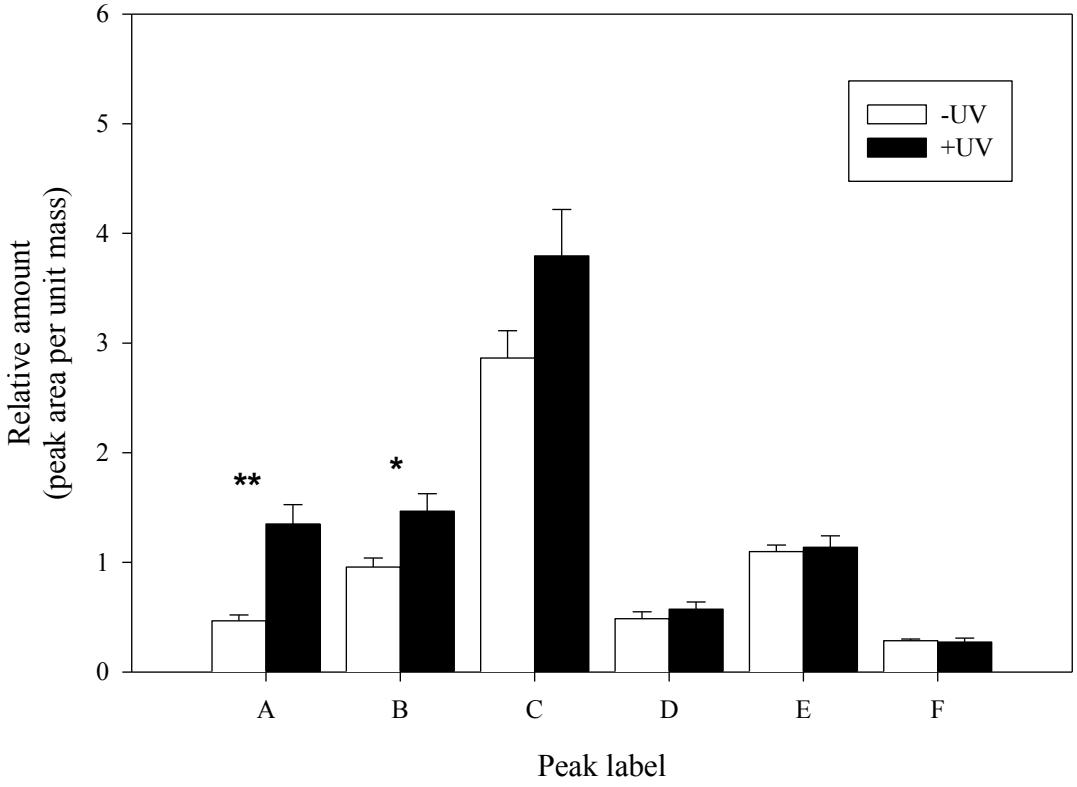


Figure 4.9 Relative amounts of selected peaks taken from chromatogram profiles of methanolic extracts obtained from dried samples of *Deschampsia antarctica* leaves taken from re-grown tufts. Samples taken after 75 days of exposure to (+UV), or exclusion from (-UV), UV-B radiation.
Mean + S.E. (n = 4).
Significant differences between samples ($P < 0.05$, $P < 0.01$) are denoted by * and * *.

4.3.7 Non-structural carbohydrates

The quantities of glucose, sucrose and fructose contained within the main leaf section (tiller) and base of plant (shoot) were determined. The presence of low quantities of inositol and raffinose was also confirmed within the shoots of both sets of plants.

Glucose and fructose concentration in both the leaves and shoots tended to decline during the photoperiod (Figure 4.10). In contrast, sucrose content (which was the greatest proportion of all the sugars) tended to increase during the day within the tillers. A similar pattern to this has been found in the field (Zúñiga *et al.* 1996). Whilst there were isolated significant differences on particular sampling times between control and UV-B-exposed samples, there was no consistent trend.

The concentration of glucose and fructose in the shoots were broadly similar to that found in tiller samples, whereas the amount of sucrose was usually much greater in the tillers than in the shoot samples. Such patterns would be expected, with triose phosphates being converted to sucrose (Zúñiga *et al.* 1996). Again, there was no obvious distinction between control and UV-B-exposed samples, except after day 59 when there were greater amounts of fructose and glucose in the UV-exposed tissue (Figure 4.10). This variability was probably a result of changes in plant developmental status. However, it is not possible to state whether this change/ delay was UV-B-induced.

A comparison between total soluble carbohydrate content (sucrose, glucose and fructose) in the shoots and tillers was also conducted. The only significant differences found were after day 59 when the UV-B-exposed shoots contained a higher mean content ($P < 0.05$), whilst in the pm sample the control shoots plants had a higher mean content ($P < 0.05$, Figure 4.11). The tillers only varied significantly after day 45 when the UV-B-exposed tillers contained a higher mean total ($P < 0.05$, (Figure 4.12).

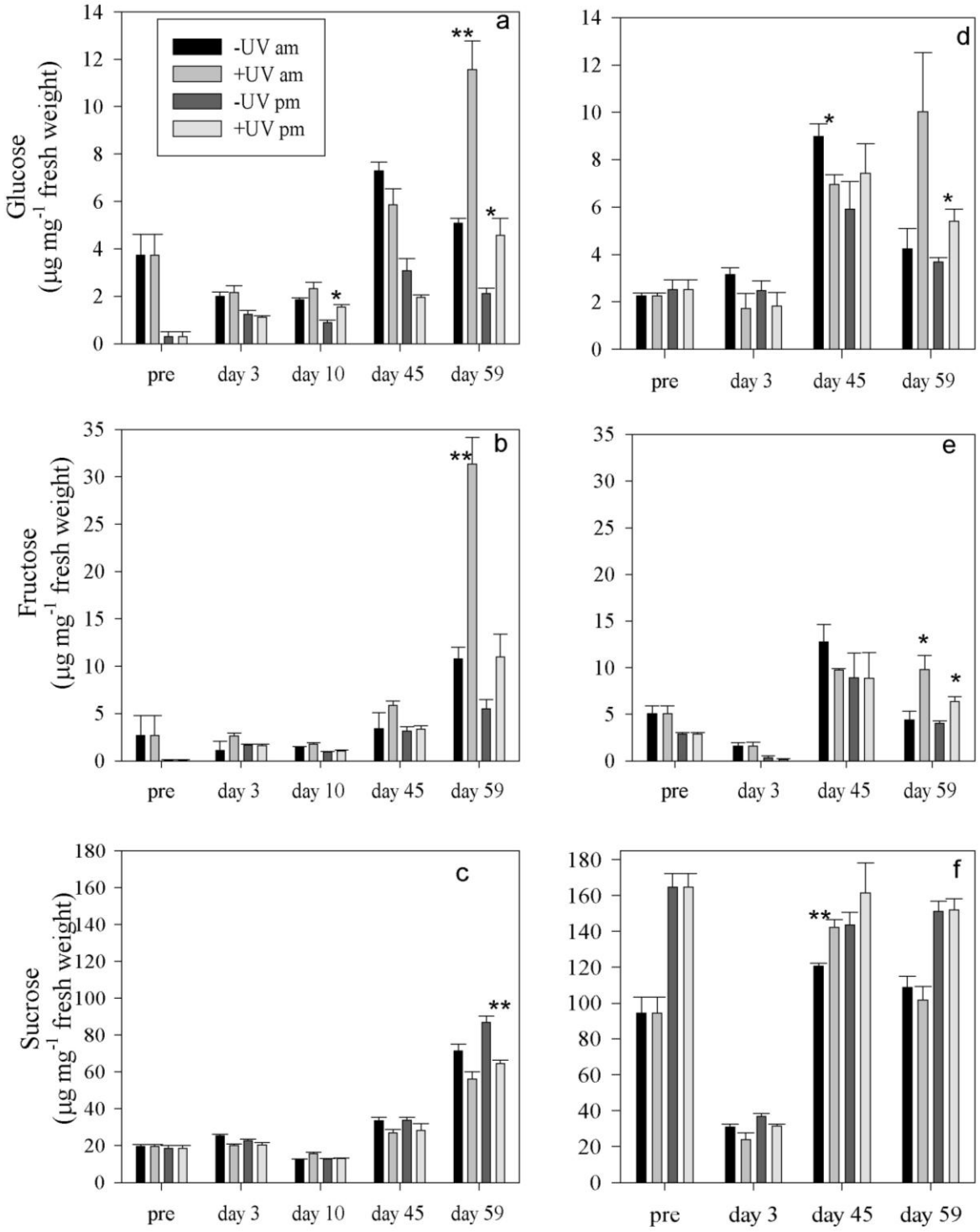


Figure 4.10 Diurnal variation of non-structural carbohydrates in *Deschampsia antarctica* either, exposed to (+UV), or excluded from (-UV) UV-B radiation for up to 59 days. Samples taken 1 h (am) and 10 h (pm) into photoperiod. Shoots (a – c), tillers (d – f).
Glucose (a & d), fructose (b & e), sucrose (c & f).
Significant differences between UV-B exposed and excluded at each sampling time denoted by * ($P < 0.05$) or * * ($P < 0.01$).
Mean + S. E. (n = 3).

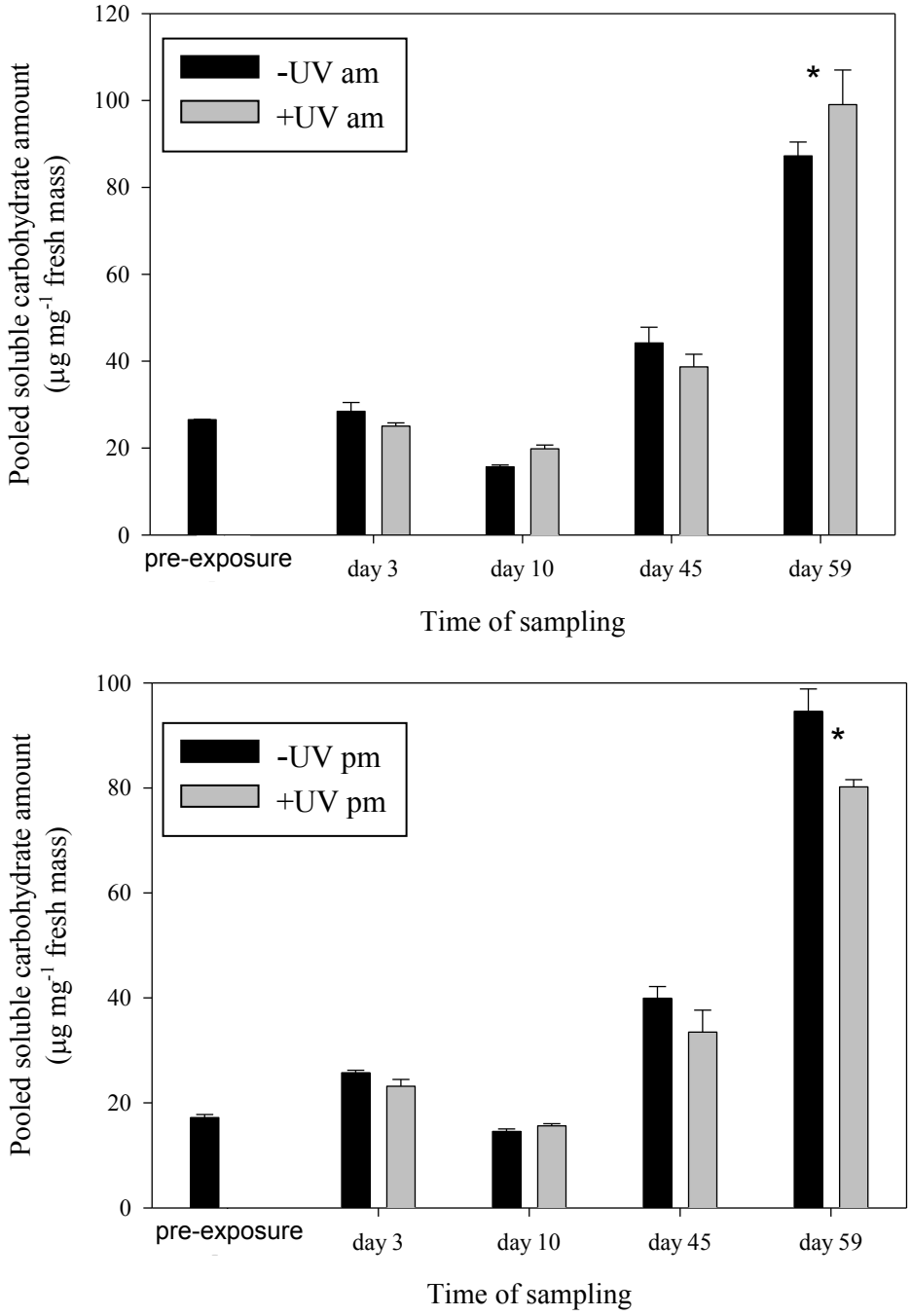


Figure 4.11 Diurnal variation in total amount of non-structural carbohydrates (glucose, fructose and sucrose) in *Deschampsia antarctica* shoots either, exposed to UV-B (+UV), or excluded from (-UV) UV-B radiation for up to 59 days.
Significant differences between exposed and excluded on each date are denoted by * ($P < 0.05$). Mean + S.E. (n = 3).

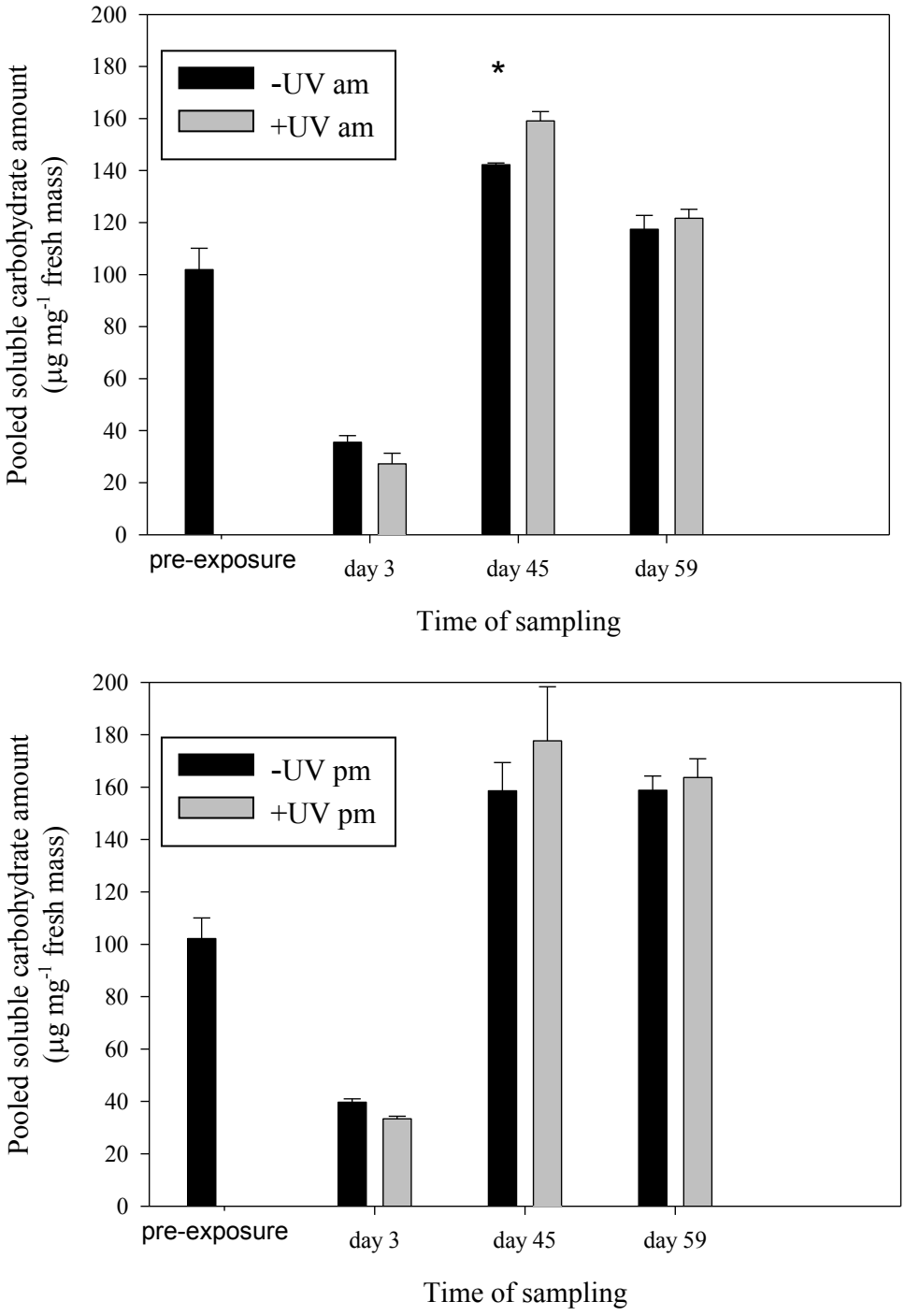


Figure 4.12 Diurnal variation in amount of total non-structural carbohydrates (glucose, fructose and sucrose) in *Deschampsia antarctica* tillers either, exposed to UV-B (+UV), or excluded from (-UV) UV-B radiation for up to 59 days.
Significant differences between exposed and excluded on each date are denoted by * ($P < 0.05$). Mean + S.E. ($n = 3$).

4.4 Discussion

This experiment has shown that exposure to UV-B radiation at environmentally relevant levels can lead to reduced leaf length, reduced carboxylation efficiency, increased flavonoid production, but no stimulation of new flavonoids. Some of these findings concur with other published reports, both field-based and growth chamber studies; alteration in morphology (Ruhland *et al.* 2005), but not alteration in F_{vm} (Lud *et al.* 2001) or response to varying PPFD (Rozema *et al.* 2001).

The distinct morphological differences in relation to UV-B exposure with the material grown from seed were not as strongly expressed in plants regenerated from stock material. The morphological differences found in this study were comparable with those reported in field studies by Ruhland and Day (2000) and similar growth chamber studies (Rozema *et al.* 2001). This shows that, whilst laboratory studies can be criticised (Allen *et al.* 1998), they can, as in this instance, also produce similar outcomes to field manipulations. Morphological responses have been noted in other vascular plants exposed to UV-B radiation by differing methods, for example *Pisum sativum* leaves irradiated in growth chambers (Gonzalez *et al.* 1998), exhibited smaller fully expanded surface area. Work on *Liquidambar styraciflua* (Dillenburg *et al.* 1995) and *Hordeum vulgare* (Liu *et al.* 1995), both found leaf elongation rate depressed by UV-B exposure (but not final leaf size) in field exposures and growth chamber work respectively. Work on sunflower seedlings (Ros and Tevini 1995), demonstrated an interaction between UV-B exposure and indole acetic acid (IAA) degradation, and a reduction in stem elongation. Changes in leaf area/ size as a result of UV-B exposure have been linked to inhibition of epidermal cell division (Gonzalez *et al.* 1998). However, Ruhland and Day (2000) could find no clear evidence of cell wall bound phenylpropanoids having an influence on alteration of leaf elongation in *D. antarctica*.

The alteration in the morphology as shown here did not affect the light dependent stage of photosynthesis, as measured by fluorescence parameters or gas exchange measurements at growth PPFD, or under light response curves. This would imply the efficiency of photon capture and thus PSII was unaffected. It may be expected that the A_{max} for the PPFD response should be lowered as a result of the decline in efficiency of Rubisco, but this was not the case. The PPFD response A_{max} may however, be quite variable and be altered by many factors; for example leaf age (Long and Hällgren 1993). Similar findings have also been reported in field experiments (as net

oxygen evolution as expressed on a leaf basis, Xiong and Day 2001, F_{vm}, Lud *et al.* 2001) and growth chamber work (CO_2 exchange, Rozema *et al.* 2001).

The extreme values recorded for WUE could have resulted from patchy stomatal opening that confounded the recording (Jansen and van den Noort 2000). Such changes in transpiration without any concomitant alteration in photosynthesis would be very unlikely. However, all other data, whilst being efficient for a C3 plant, are similar in comparison to other C3 plant published data (Yoshie 1986). Effects of UV-B on stomates have been previously described (Jansen and van den Noort 2000), but there is no reproducible evidence to support this in the work presented here.

The work reported here is the first A/c_i response on the hair grass under altered UV-B concurrent with some preliminary data in Montiel *et al.* (1999). The overall values of $V_{c,max}$ were higher than other published data for *D. antarctica* (e.g. 0.041 for whole canopy, Xiong *et al.* 1999). The seed-grown material exhibited a highly significant depression in $V_{c,max}$. As it was not possible to measure carboxylation characteristics of the plants prior to UV-B exposure, the (not unreasonable) assumption is that there was no substantial physiological difference between the emerging seedlings prior to alteration in UV-B exposure. The $V_{c,max}$ of the regenerated plants exposed to UV-B radiation was also lower, but this effect was not prolonged and recovered during the experiment. Decreases in $V_{c,max}$ would imply that UV-B exposure (particularly from germination) has led to a decrease in the efficiency of CO_2 assimilation. This would indicate that there had been a decline in the activity or efficiency of Rubisco. These effects, coupled with lowered net assimilation at ambient external CO_2 concentration, could reduce productivity of the seedlings. The variability of the A/c_i characteristics between the sampling dates of the regenerated plants (Figure 5.2) could also reflect different stages in development of the plants in the growth cabinet. Reductions in $V_{c,max}$ coincident with no alteration in F_{vm} have also been reported in other vascular plants (Nogués and Baker 1995, Keiller *et al.* 2003). Work on *Brassica napus* (Keiller *et al.* 2003), found that there was loss of protein rather than actual deactivation resulting in the observed lowered $V_{c,max}$, but no effect on PSII. Furthermore, it has been suggested that damage to PSII is secondary and only occurs at high UV-B fluence after there has been damage to Rubisco (Baker *et al.* 1997). The lowered A_{max} would signify limitations to regeneration of RuBP or non-cyclic electron transport (Long and Hällgren 1993). The lack of a fluorescence response in this study would indicate that the former would be the reason for this depression. Allen *et al.* (1997) found depression in $V_{c,max}$, A_{max} and F_{vm}

following exposure to 10 times the daily weighted UV-B irradiation used in this study; however, these exposures will probably never be attained under any realistic future ozone depletion scenario in the Antarctic.

The lack of sustained alteration in chlorophyll content was unsurprising and has also been reported by other authors (on a dry mass basis, Xiong and Day 2001). However, the same authors reported an increase when expressed as a function of area in the UV-B-exposed plants. The variability in chlorophyll content may have resulted from a number of factors. It was possible that (early) senescing leaves from the unexposed plants would have reduced chlorophyll content, but without obvious loss of colour. The use of fresh mass as the basis for calculation may have also contributed to this inconsistency. Furthermore, greening has been shown to be a stress response to ozone exposure. The lack of change in carotenoid content has also been reported in field manipulations of solar UV-B radiation on *D. antarctica* by Lud *et al.* (2001). This would suggest that either there is no role for this cycle in UV-B protection at the levels of UV-B exposure plants in this study were exposed to, or the xanthophyll system is operating below capacity.

The change in flavonoid composition in response to UV-B exposure was the same regardless of sample origin, namely an increase in proportion (and amount) of an orientin-like glycosolated flavone (peak A). Other work on the flavonoids in *D. antarctica* (van de Staaij *et al.* 2002, Ruhland *et al.* 2005), did identify orientin (and luteolin) in the samples, but could not find an alteration in either amount or relative composition in the flavonoids that were studied. There has been some conflict in the literature regarding the relative merits of the simple phenolics (especially cinnamic acids) and flavonoids in the screening of UV-B (Booij-James *et al.* 2000). The phenolics characterised in this study were probably glycosolated flavones, but other workers have found simple phenolics from *D. antarctica* in higher than expected concentrations, whereas UV-B absorbing soluble compounds (probably flavonoids) were present in lower amounts than anticipated (Ruhland and Day 2000). Moreover, there is evidence for the role of compounds from the phenylpropanoid pathway operating as free radical (oxygen species) scavengers rather than as a simple screening role (Grace and Logan 2000). Furthermore, flavonoids may be found in high concentrations without, or prior to, UV-B exposure, (van de Staaij *et al.* 2002). As purely a UV-B screening role, the alteration in the flavones described herein would probably be limited in the protection of photosynthetic apparatus. This would be in agreement with other workers (van de Staaij

et al. 2002, Ruhland *et al.* 2005). However, the consistent alteration in the relative amount of the orientin-like compound peak A is important; whilst it may not make a contribution to the overall protection from UV-B exposure, it could be used as a quantitative biochemical marker, or to estimate previous, and future, ozone depletion. A previous report on *D. antarctica* has noted no change in absorbance at 300 nm of bulk methanolic extracts (on a leaf area basis, Ruhland and Day 2000). However, the same group noted a significant increase in absorbance at 300 nm (on a leaf area and dry mass basis) as a result of UV-B exposure (Xiong and Day 2001).

The changes observed in the content of non-structural carbohydrates were inconclusive. This is not wholly unexpected because of the lack of change in photosynthetic activity recorded under ambient CO_2 concentration and at growth PPFD. Whilst there is some evidence of indirect effects of UV-B exposure on sugar concentration in pea (Mackerness *et al.* 1997), it was not confirmed by this study. The changes in content during the exposure period probably resulted from differing developmental stage or age of the leaves as the changes were shown in both the UV-B-exposed and the UV-B-excluded plants and part of plant be it shoot or tiller. The amount of sucrose was dominant, accounting for ca. 95% of the tiller and 70% of the shoot content respectively. The accumulation of sucrose could be impeded if there is a limitation in the efficiency of photosynthesis, as was shown at one time in the seed grown plants. Non-structural carbohydrates were not measured in the seedlings, so no link to lowered $V_{c,max}$ could be established. *D. antarctica* is known to accumulate high levels of sucrose and this has been linked to cold tolerance in the hair grass (Zuniga *et al.* 1996). The total amount between shoots and tillers did not vary greatly, but the composition did with tillers containing much more sucrose than the shoots. There would seem to have been a re-distribution or conversion of sucrose in the shoots, as the primary site of sucrose synthesis should have been the tillers.

The plants in this experiment were exposed to daily fluence of up to 4.89 kJ m^{-2} $_{(PAS)}$, which is of similar magnitude to that measured during the Austral spring/ summer on the Antarctic Peninsula (e.g. Rothera mean daily UV-B 4.1 kJ $m^{-2}{}_{(PAS)}$ Newsham *et al.* 2002). In contrast, UV-B doses mentioned in many papers have greatly exceeded these levels; 18 kJ m^{-2} $_{(PAS)}$ day^{-1} (Adamse and Britze 1992), and 32 kJ $m^{-2}day^{-1}$ $_{(PAS)}$ (Allen *et al.* 1997). This would imply that any responses noted in this study could occur in the field under naturally occurring UV-B exposure.

Exposure of the relatively mature re-grown tufts of *D. antarctica* to environmentally realistic levels of UV-B radiation can be expected to have few chronic effects as judged from this study. Studies by other workers broadly concur with this conclusion on the regenerated plants. However, the most distinct alterations were found in the seedlings grown without any prior developmental exposure to UV-B. Thus developmental stage would appear to be a critical factor in response to UV-B radiation. The regenerated material was re-grown from tufts, thus the plants were much older than that of the seed-grown material. No other published work on *D. antarctica* has utilised seed-grown material in UV-B manipulations. When extrapolated to the field, it would imply that the early development of the grass and the establishment of new colonies may be restricted by enhanced UV-B exposure on account of lowered photosynthetic efficiency. However, the seed samples came from Leonie I. on the Peninsula whilst the clonal tufts originated from Signy I. The hair grass is considered to have either originated from post-glacial migration or to be a member of a relic flora that has survived (and thus been isolated) for a greater period of time. In this respect it is possible that the differing response were due to different populations. Nevertheless, there are two pieces of evidence that would indicate that this is not the case; one is that the flavonoid content was comparable, inferring a lack of long term separation or diversity. The second is that Holderegger *et al.* (2003), working on the same two populations of hair grass from Signy I. and Leonie I., found very low genetic diversity between the two and thus reduced gene flow, indicating founder populations of a few individuals and establishment of the species predominantly by vegetative reproduction. The effects of UV-B exposure on leaf development need further investigation, and highlight a potential problem in extrapolating data recorded at differing developmental stages.

D. antarctica survives in the extreme Antarctic environment. The optimal temperature for photosynthesis is between 10 to 13^{o}C with ca. 30% of maximal rate occurring at 0^{o}C (Edwards and Smith 1988, Xiong *et al.* 1999). Also the A/c_i response of the grass is greatly affected by temperature (Xiong *et al.* 1999), with the least efficient response occurring at 25^{o}C. The hair grass is considered to have a narrow ecotypic range and as such will be expected to show little adaptation to environmental change (Edwards and Smith 1988). Furthermore, UV-B-induced alterations in ecosystems are thought to occur because of effects on competition for light in temperate species (Caldwell 1997). Therefore, the responses noted in the seed-grown material

warrant further investigation to fully understand the impact of enhanced UV-B on ecosystem dynamics. Finally, more work is needed to assess how much the hair grass will be affected by increases in UV-B levels in relation to possible increases in global CO_2 concentration and temperature. Biomass partitioning at differing CO_2 concentration has been shown to be altered in temperate species by enhanced UV-B exposure (Sullivan and Teramura 1994).

To conclude, *D. antarctica* does indeed respond to UV-B exposure in ways similar to other vascular plants, namely lowered carboxylation efficiency, shortened leaves and alterations in flavonoid content. However, developmental stage of the plant may determine the nature and degree of response.

CHAPTER 5

EFFECT OF DISCRETE WAVEBANDS OF UV RADIATION ON THE PHOTOSYNTHETIC COMPETENCE OF AN ALGA AND A LIVERWORT.

5.1 Introduction

The use of simple UV screening techniques in earlier experiments (Chapters 3 and 4) has shown differential sensitivity to UV-B radiation in those species tested. An effect on photosynthesis by way of decline in F_{vm} was clearly observed in the alga *Prasiola crispa* and the liverwort *Marchantia berteroana*. The fact that F_{vm} is altered by exposure to broad-band UV-B radiation does not necessarily mean that the concurrent increase in UV-B radiation as a result of ozone depletion will affect these sensitive species in the same way. Ozone depletion leads to a differential increase in specific wavebands within the UV-B range and very little alteration in other wavebands (see Chapter 1). To be able to state that the same effect would occur under ozone depletion it needs to be confirmed that the effects demonstrated are both wavelength dependent and caused by those wavelengths increased by ozone depletion. Furthermore, it should be possible to calculate the first action spectrum for a terrestrial Antarctic plant.

One way to refine the broad-band exclusion experiments is to carry out exposures over narrower wavebands. There are two main types of filter that are appropriate for such studies. Long-pass filters cut off wavelengths lower than a set value. These have advantages of being relatively inexpensive and (by using varying thicknesses) can cover a wide spectral range. Interference filters allow selective transmission of a narrow band of radiation, typically with a resolution of a 10 nm band for 50% maximal transmission. Clearly these are more selective, but have the disadvantage of being more expensive than long-pass filters.

Another major consideration in using filters is the choice of light source. A light source that is not continuous over the required spectral range will cause a discrepancy in the contribution of the transmitted light compared with that of the stated value of the filter. Thus, the actual peak transmission of an interference filter, with a nominal peak at 290 nm may be shifted to a higher or lower wavelength depending on the lamp characteristics (Holmes 1997). The actual peak wavelength can be determined by integrating the transmission properties of the filter with the lamp output.

The use of different lamps and filters will allow both monochromatic and crude polychromatic action spectra to be constructed. It is important to attempt to undertake both types of action spectra, as a monochromatic spectrum will not take into account any ameliorating, or detrimental effects, of background PPFD. This is an important consideration as UV-B radiation is always associated with the full solar spectrum. Awareness of this omission is needed to fully interpret such work, or to extrapolate findings to the field. Moreover, when utilising monochromatic action spectra, there will be negligible photosynthetic electron flow. Finally, photoreactivation will not be occurring under monochromatic radiation. A comparison between monochromatic and polychromatic irradiation will be of use in assessing weighting functions, as differing weighting functions forecast different (apparent) biological consequences as a result of ozone depletion (Madronich *et al.* 1995).

Much published work carried out on UV effectiveness/ action spectra has predominantly used monochromatic action spectra (e.g. PAS, Caldwell 1971), and more recently Flint and Caldwell (2003a), which was also validated with a field study (Flint and Caldwell 2003b). However, a number of polychromatic action spectra have also been reported. Thus Caldwell *et al.* (1986), using long pass filters, measured inhibition of photosynthesis (net CO_2 exchange) in *Rumex patientia*; with the action spectrum constructed by Rundel (1983). Furthermore, Quaite *et al.* (1992) produced an absolute action spectrum for DNA damage in alfalfa, which produced a broader activity (longer UV-A tail) than the PAS. Cooley et *al.* (2000) used the outdoor supplementation system as described by Holmes (1997) to create a polychromatic action spectra for growth responses in *Bellis perennis* L. and *Cynosurus cristatus* L. Bogenrieder (1982) used a series of interference filters, coupled with a xenon arc source, to determine action spectra on two higher plants; some workers however, consider this method to represent a monochromatic type rather than a true polychromatic spectrum (Coohill 1989). There

has been very little work regarding effectiveness/ action spectra on Antarctic species, with the only studies on marine phytoplankton such as Neale *et al.* (1994).

This series of experiments will try to determine the sensitivity of PSII photochemical efficiency (F_{vm}) to different wavebands of UV radiation in *Prasiola crispa* and *Marchantia berteroana*. They both have a flat, thallose-like structure; thus the measured UV fluence should be similar to that received by the plant (assuming no alteration in reflectivity as a consequence of exposure).

5.2 Materials and methods

5.2.1 Plant material

Prasiola crispa and *Marchantia berteroana* samples were obtained and maintained as described previously (Chapter 2). Cores (2 cm diameter) were taken from visually healthy green algal tissue and liverwort thalli, and acclimated on moist filter paper in the growth cabinet (see Chapter 2 for temperature and light regime) for 48 h prior to exposure under a UV-B opaque cloche.

5.2.2 Filter set-up

5.2.2.1 Long pass filters

The transmission properties of the filters are shown in Chapter 2. The protocols are as described in Chapter 2, with the following modifications. The plant material was exposed in the cabinet in 5 cm diameter Petri-dishes with blackened sides. The exposures were carried out within a 16/ 8 h day/ night cycle.

5.2.2.2 Interference filters

The lamp set-up and the filter transmission properties have been described previously (Chapter 2). Again, the basic cultivation protocols and equipment used are as described earlier with the exceptions noted below.

5.2.2.2a Monochromatic spectra

These exposures were of a preliminary nature to ascertain the potential of using the lamp set-up and the response of the plant material during such exposures. Exposures for *P. crispa* took place over a 10 day period, whilst those for *M. berteroana* were carried out over a 12 day period. All experiments used blackened Petri-dishes so as to exclude any stray light, and mounted on a revolving turn-table (a set-up previously used by M. G. Holmes *pers. comm.* June 1997) to allow for multiple samples to be exposed in a relatively short time period. Samples were exposed for the same time period, which meant a different cumulative total due to the emission properties of the lamp.

5.2.2.2b Polychromatic exposure

To determine the onset of a UV-B-induced change and to try to eliminate the variable of time dependent recovery, these experiments were carried out using shorter exposure periods (over successive days) than the monochromatic work. The samples were maintained in 2.5 cm diameter borosilicate glass vials, these have the typical transmission cut-off of ca. 310 nm and therefore negligible UV-B penetration. The diffuse light from the xenon lamp and the constant temperature room were used for supplemental light (around 50 μmoles m^{-2} s^{-1} PPFD, about one third of what the samples received in the growth cabinet). The samples were exposed to the same un-weighted fluence. This meant that, depending on filter transmission, the actual time under the lamp was variable, as a result of the non-linear emission characteristics of the lamp over the UV range. Samples were placed in the growth cabinet (16/ 8 h light/ dark period) at the end of the exposures for recovery under a UV-B opaque cloche. The maximum exposure is (regardless of wavelength) shown in Table 5.1.

5.2.3 Fluorescence measurements

Measurements of F_{vm} were made using an OS-100 fluorimeter. Three samples were used for each filter regime. Readings were taken following exposure (after a necessary 30 min dark adaptation). All other settings were as stated in Chapter 2.

Table 5.1 Summary of protocols for interference filter experiments

Species and protocol	Maximum exposure irrespective of wavelength (mJ cm^{-2})	Recovery test at end of exposure
Prasiola crispa M	400	No
Marchantia berteroana M	400	No
Prasiola crispa P	300	Yes (5 days)
Marchantia berteroana P	200	Yes (5 days)

M- monochromatic, P- polychromatic exposure

5.2.4 Construction of action spectra

Initially the data for F_{vm} were pooled together and plotted against un-weighted fluence. Subsequent manipulation involved only the work using interference filters. This was as a result of differential irradiance of the long-pass filters covering a broad waveband (see Chapter 2), thus allowing only an approximation.

To construct the action spectrum a linear regression was taken through the percentage depression of F_{vm} for each filter (the fluence–response). This was then plotted as depression of F_{vm} as a function of irradiance. Lastly the data were also plotted as a function of quantum effectiveness by calculating the fluence at each waveband (following Sutherland *et al.* 1994), then normalised to 1 at 300 nm for comparison with other published representative action spectra. The function ($y = 1/ (a + bx)$) was fitted to the quantum effectiveness data using Marquardt-Levenberg algorithm (from Sigmaplot 4.01) to allow de-convolution of the action spectra for use in comparison of ozone depletion scenarios. The calculated residuals from 290 to 380 nm at 1 nm intervals were then multiplied by the irradiance at each wavelength with data from McMurdo station, UV model (Fiscus and Booker 1994), and the Fi-totron™ cabinet respectively. The integrated irradiance was then calculated the using the trapezium rule. Radiation amplification factors (RAFs) were calculated following Madronich *et al.* (1995), using the power rule for large ozone changes. This was carried out over the range of the PAS (280 to 313 nm) and for the UV-B range (280 to 320 nm), which is substantially altered by ozone depletion. Only the polychromatic action spectrum was used as a weighting function for ozone depletion. This was because the monochromatic exposures were carried out over a longer timescale, and would have allowed for a greater chance of

recovery from UV exposure.

The model of Fiscus and Booker (1994) was used with the following settings; 1000 mB atmosphere, no aerosol, 50% relative humidity, rural, snow-cover.

5.3 Results

5.3.1 long-pass filters

5.3.1.1 *Prasiola crispa*

Changes in F_{vm} were related to both wavelength and dose received (Figure 5.1 a-d). There were three distinct groupings at 360 nm, 335 nm, and 320 to 305 nm. This division is almost certainly related to the choice of wavelengths at the energy applied. The decline in F_{vm} was similar to that shown previously (Chapter 3). The data for samples exposed to UV-B radiation were averaged and pooled over their respective exposure (Figure 5.2) and showed an approximate curvilinear relationship with a flattening of response on increasing exposure. However at lower exposure range (< 5 kJ m^{-2}) there was a similar response regardless of data weighting.

The samples showed a biphasic recovery following exposure, with samples (except 305 nm) returning to values that were not significantly different from that after 2 h UV exposure (Figure 5.3a). The fall in F_{vm} was consistent with the product of a fall in F_m and a rise in F_o (Figure 5.3b). Recovery was largely related to a return of F_o to pre-exposure values (Figure 5.3b). The F_{vm} values were plotted against the differential exposure of UV-B radiation following Holmes (1997, Figure 5.4). It can be seen that at lower exposures of un-weighted UV-B radiation there was an approximate curvilinear relationship, while increased exposure led to a decline in F_{vm} depression rate.

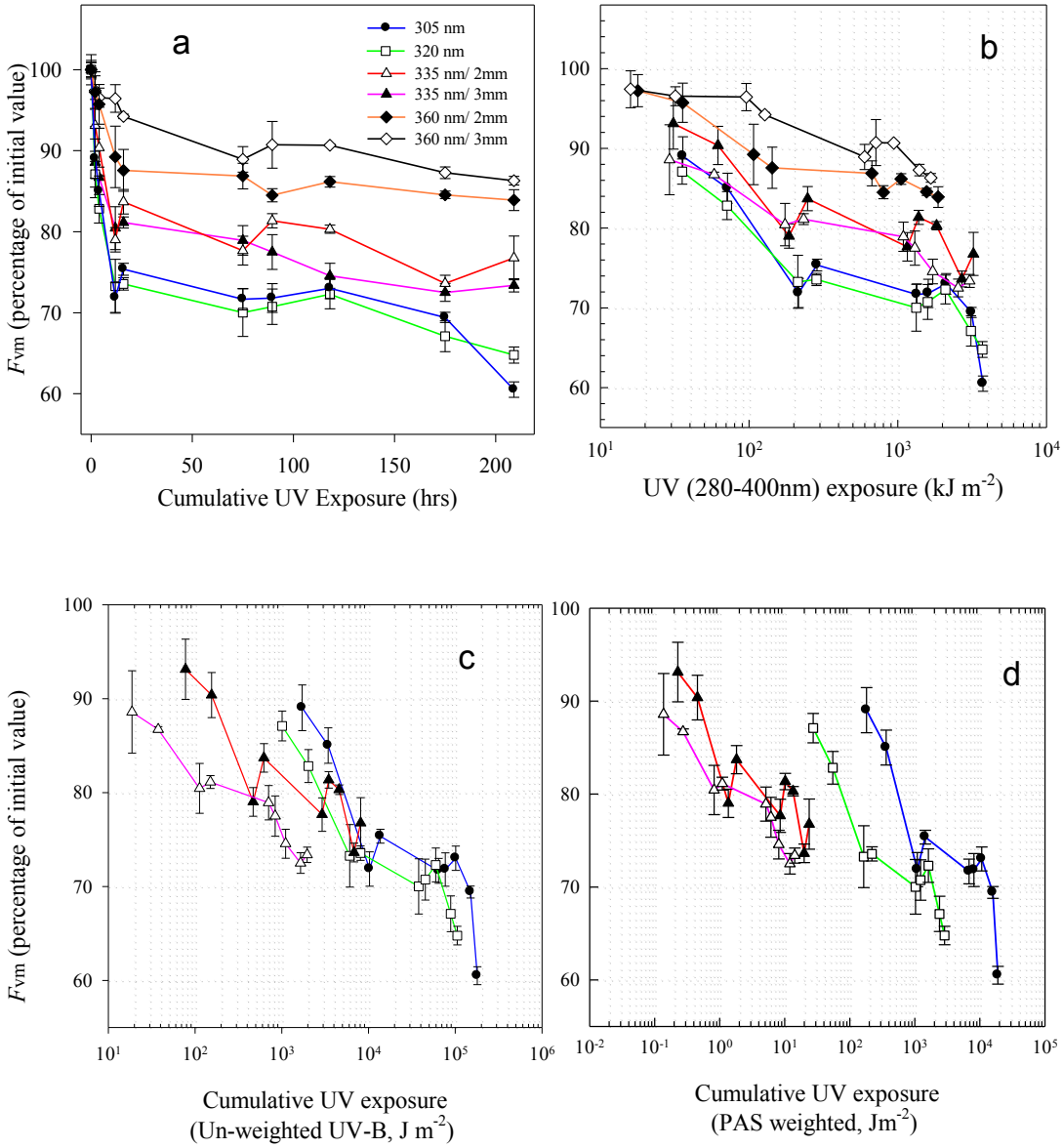


Figure 5.1 Effect of exposure to different UV wavebands using long-pass filters on the photosynthetic competence (F_{vm}) of the alga *Prasiola crispa*. Plants exposed for up to 209 h.
(a) Total hours exposed.
(b) Unweighted UV radiation.
(c) UV-B (280 to 320 nm) radiation.
(d) PAS weighted function.
All data expressed as a percentage of initial values.
See (a) for legend. Figures (c) and (d) no 360 nm data is included (not in UV-B range). Mean ± S.E. (n = 3).

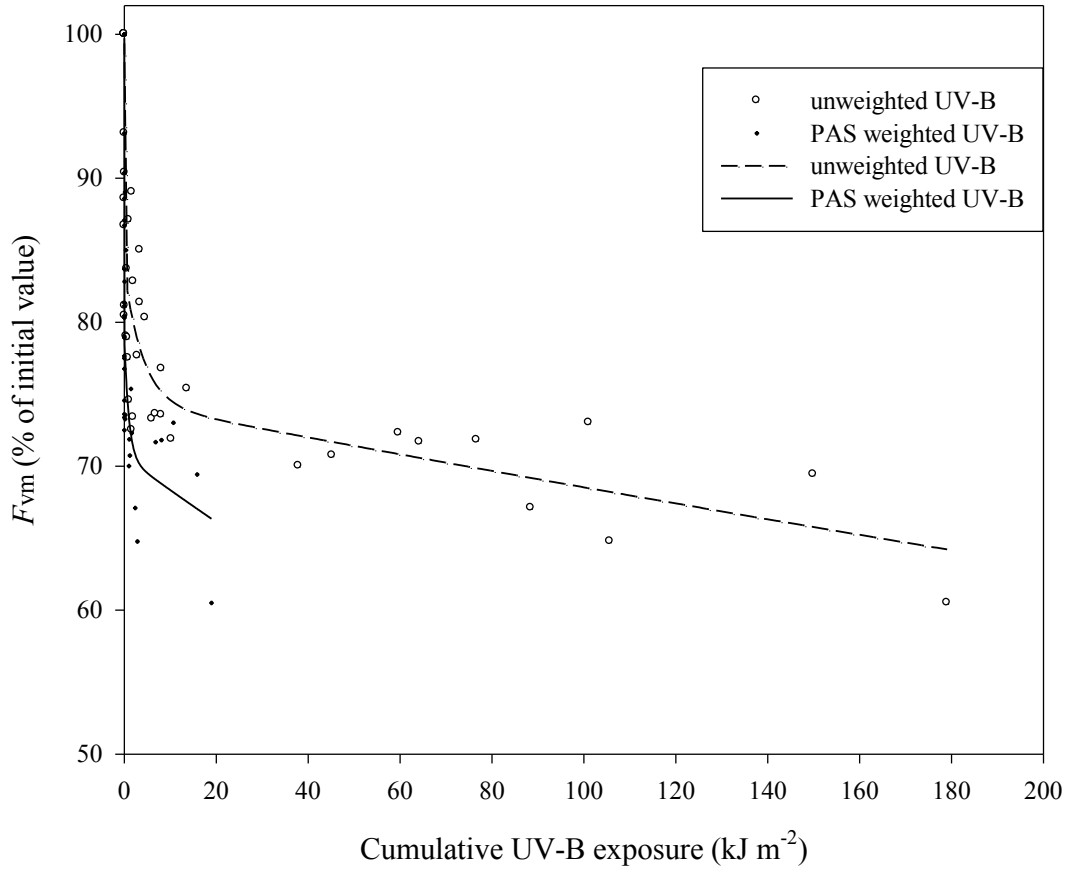


Figure 5.2 Effect of UV-B exposure on photosynthetic competence (Fvm) of the alga *Prasiola crispa*. Comparison between unweighted and the same data weighted against Caldwell's plant action spectrum (PAS). Data pooled from results in Figure 5.1c.
Mono-exponential decay function fitted to each data-set (r^2 unweighted 0.84, PAS weighted 0.82).

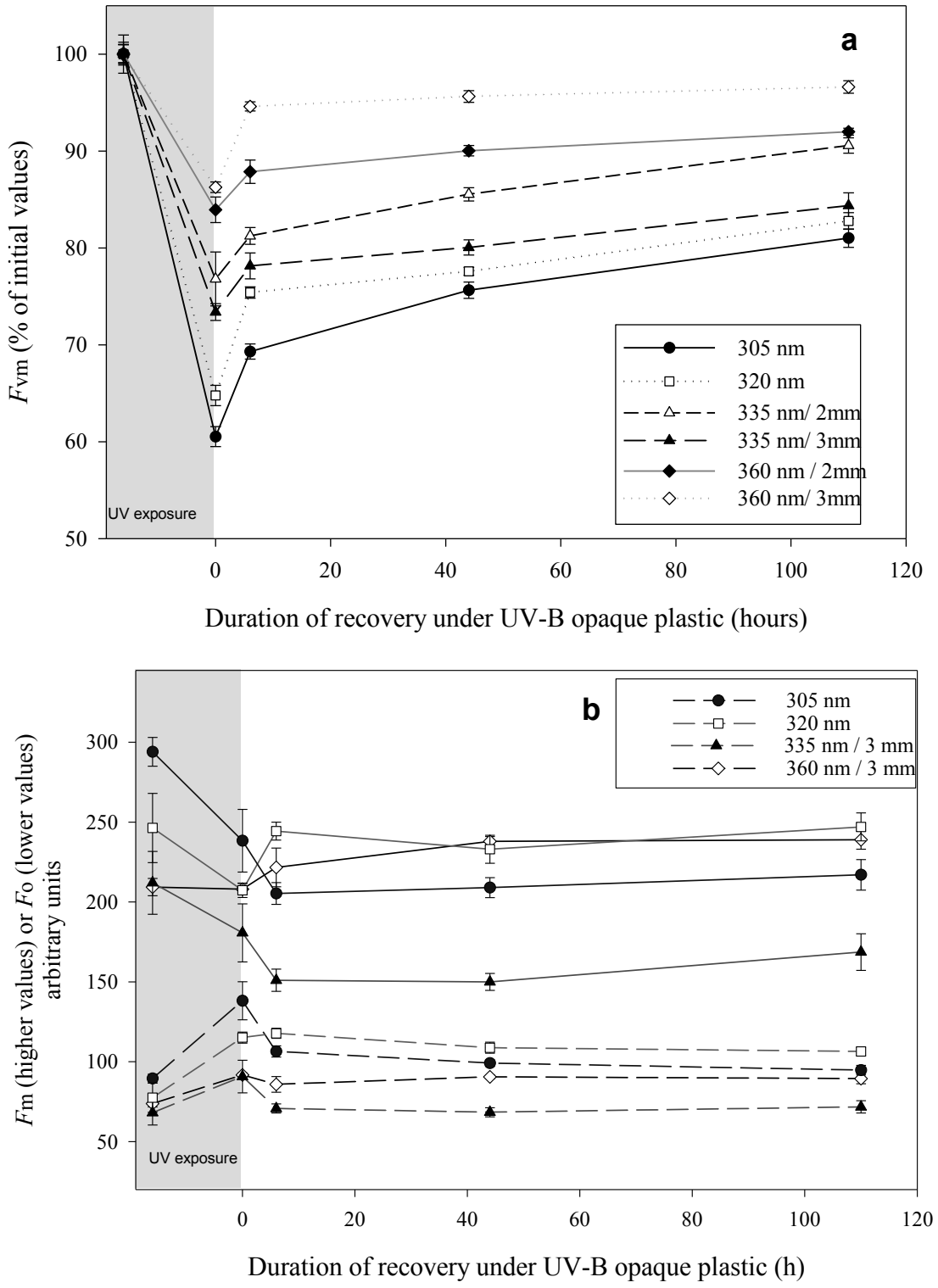


Figure 5.3 Measurement of photosynthetic competence (F_{vm}) obtained from the alga *Prasiola crispa* placed under UV-B opaque plastic following exposure to UV-B radiation for 209 h.
(a) F_{vm} and recovery (all samples).
(b) F_m and F_o recovery (not all samples shown).
Mean ± S.E. (n = 3).

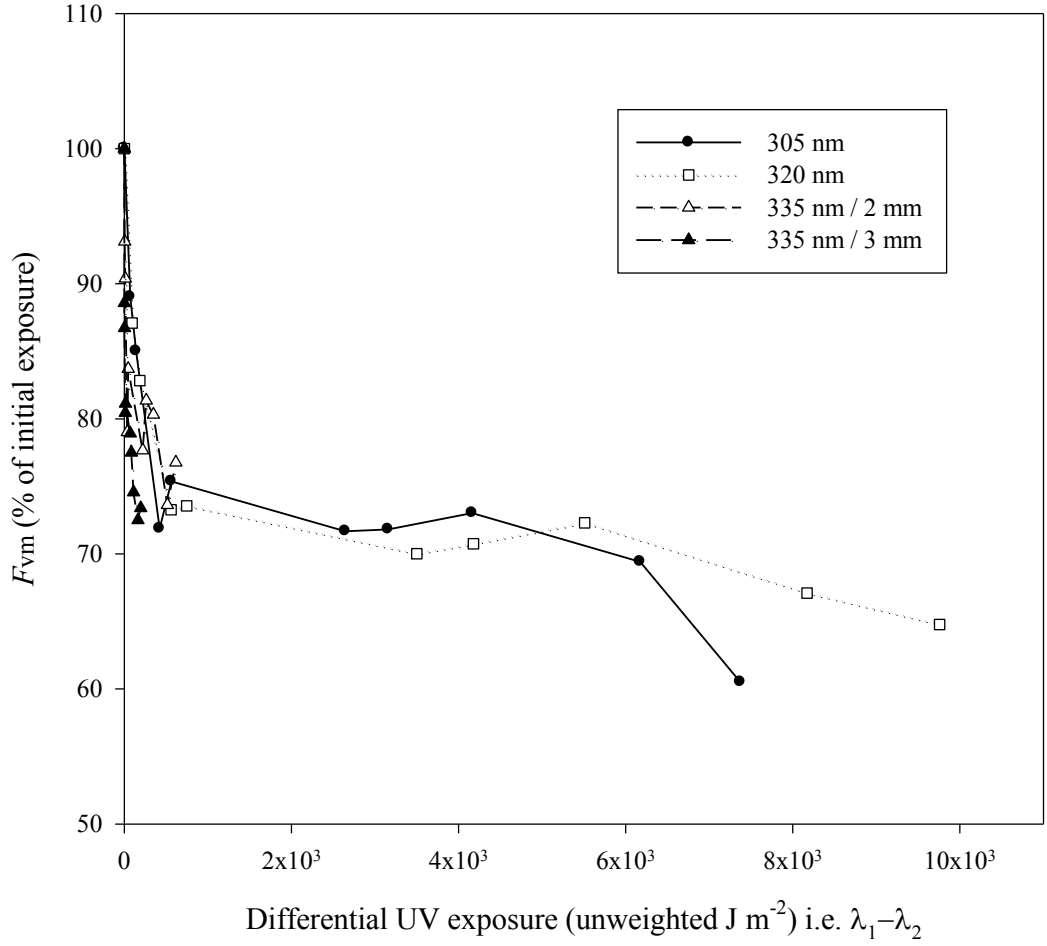


Figure 5.4 Effect of differential UV exposure on F_{vm} depression obtained fr
F_{v}

Figure 5.4 Effect of differential UV exposure on F_{vm} depression obtained from the alga *Prasiola crispa*. Mean values shown for each waveband. F_{vm} depression at longer wavelength subtracted from the lower wavelength.

5.3.2 Interference filters (i) Monochromatic spectra

5.3.2.1 *Prasiola crispa*

Samples exposed to < 310 nm radiation showed a significant, albeit small, fall in F_{vm} compared with those exposed to longer wavelength radiation. The data were normalised to the control value for that time to remove variation at each sampling time (Figure 5.5a). The linear regression data are summarised in Table 5.2.

Table 5.2 Linear regression and re-calculated quantum effectiveness data for depression of F_{vm} in *Prasiola crispa* exposed to monochromatic UV radiation (see text for details).

Filter wavelength (nm)	Gradient from linear regression	r^2 for linear regression	Quantum effectiveness (quanta m^{-2} $x10^{-22}$)	Relative quantum effectiveness
290	0.0354	0.81	24.2	1.67
300	0.0219	0.85	14.5	1
310	0.0049	0.32	3.14	0.217
320	0.0041	0.39	2.53	0.175
360	0.0023	0.18	1.27	0.0875

There was a trend for a decline in the rate of F_{vm} depression as wavelength increased. However, there was some variability at the higher wavelengths that resulted in a less reliable regression fit.

5.3.2.2 *Marchantia berteroana*

The F_{vm} of the sample exposed to 290 nm radiation declined much more rapidly in comparison with those exposed to longer wavelengths over an equivalent un-weighted exposure (Figure 5.5b). In this instance the fall in F_{vm} could be attributed to a drop in F_m values, rather than a rise in F_o (F_m fell from a typical value of 300 to ca. 200 units). Exposure to 300 nm radiation also caused a clear fall in photochemical efficiency. The depression in F_{vm} lessened with increasing wavelength. The results are summarised in Table 5.3. It should be noted that the samples exposed to longer wavebands received a much greater irradiance despite having an equivalent time for each exposure.

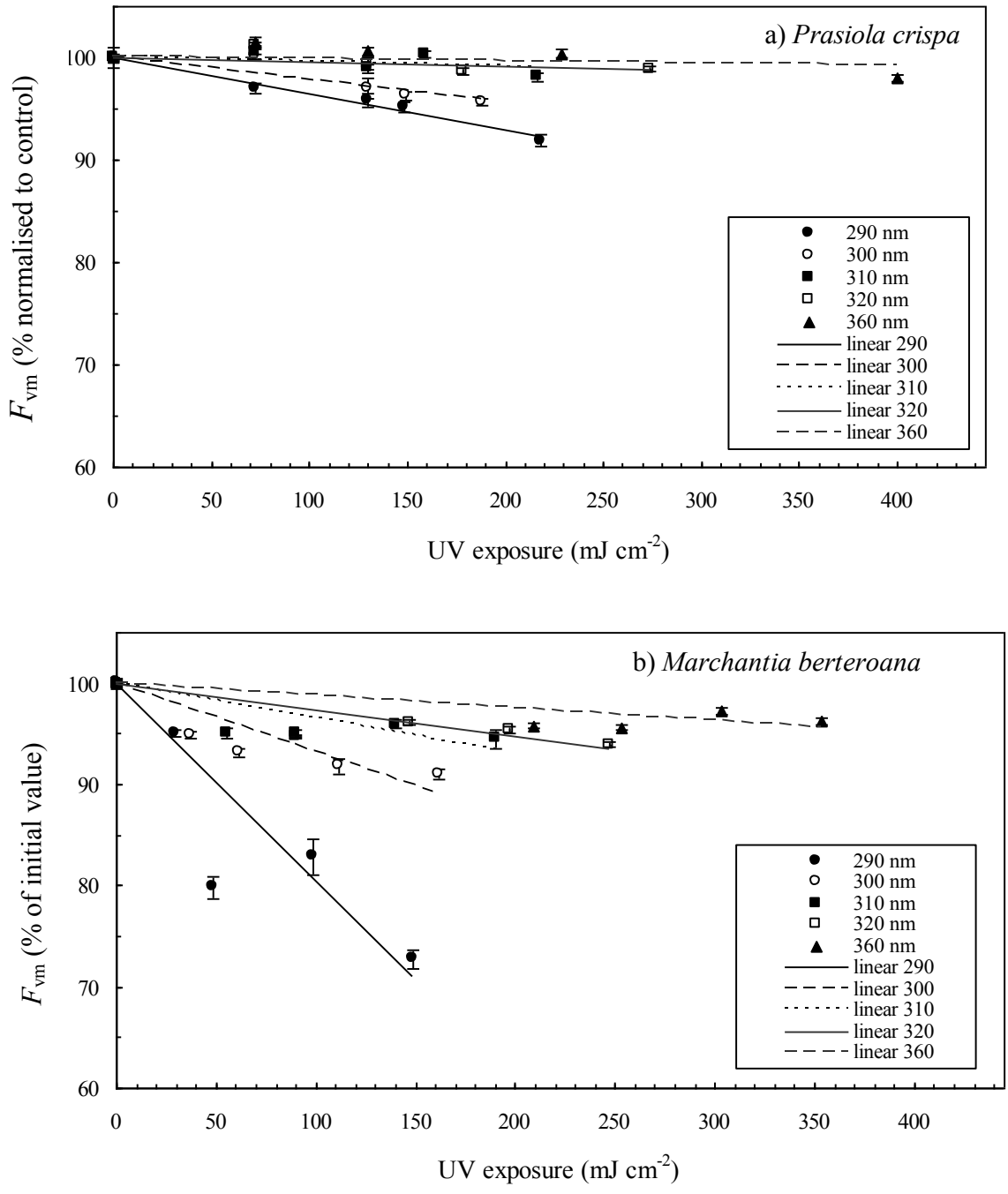


Figure 5.5 Effect of applying discrete wavebands of UV radiation on the photosynthetic competence of (a) *Prasiola crispa* and (b) *Marchantia berteroana*. Linear regression applied to each data-set (see text for details). Mean ± S.E. (n = 3).

Table 5.3 Linear regression and re-calculated quantum effectiveness data for depression of F_{vm} in *Marchantia berteroana* exposed to monochromatic UV radiation (see text for details).

Filter wavelength (nm)	Gradient from linear regression	r^2 for linear regression	Quantum effectiveness (quanta m^{-2} $x10^{-22}$)	Relative quantum effectiveness
290	0.1960	0.76	134	2.97
300	0.0681	0.65	45.1	1
310	0.0350	0.26	22.4	0.497
320	0.0265	0.6	16.5	0.365
360	0.0128	0.58	7.06	0.157

By the end of the experiment there was a distinct colour change (browning and purple discoloration) in the thallus of the 290 nm sample. An attempted extraction of this pigment did not prove successful. However, a methanolic extract of the samples did indicate the presence of substances absorbing strongly at ca. 300 nm. It is possible that the chlorophyll had been bleached or destroyed thus allowing other previously masked pigmentation to be visible.

5.3.3 Interference filters (ii): Monochromatic exposure with concurrent exposure to 50 μmoles m^{-2} s^{-1} PPFD (+PPFD)

5.3.3.1 *Prasiola crispa*

There was a range of responses depending on filter type (290 nm, 300 to 320 nm, and 360 to 380 nm, Figure 5.6a). A marked decrease in F_{vm} was noted in samples exposed to 290 nm radiation, with values falling to below 60% of initial value after 150 mJ cm^{-2} cumulative exposure (Figure 5.6a). The drop in F_{vm} was initially as a result of a fall in F_m which was accompanied by, then exceeded by, a rise in F_o on a percentage basis, which ended 50% greater than the original value (Figure 5.7a). The 300 nm sample showed a steady and significant decline in F_{vm} after 150 mJ cm^{-2} exposure (except for reading after 210 mJ cm^{-2}), which was mainly a result of a rise in F_o. F_{vm} values for both the 310 and 320 nm samples decreased slightly (though significantly) after 180 mJ cm^{-2} exposure. All of the other samples exhibited F_{vm} values after 260 mJ cm^{-2} similar to those recorded after 60 mJ cm^{-2} exposure. The regression data are summarised in Table 5.4. There was a good fit for most of the regressions except those for the 360 nm wavelength. The F_{vm} values of most samples showed some recovery to within 10% of the original ratio after they were returned to a UV-B-excluded environment. There was however no recovery of F_{vm} in the 290 nm sample.

Table 5.4 Linear regression and re-calculated quantum effectiveness data for depression of F_{vm} in *Prasiola crispa* exposed to monochromatic UV radiation concurrent with 50 μmoles m^{-2} s^{-1} PPFD.

Filter wavelength (nm)	Gradient from linear regression	r^2 for linear regression	Quantum effectiveness (quanta m^{-2} $x10^{-22}$)	Relative quantum effectiveness
290	0.210	0.89	144	5.53
300	0.0393	0.83	26.0	1
310	0.0373	0.84	23.9	0.919
320	0.0269	0.90	16.7	0.642
360	0.0056	0.27	3.19	0.119
380	0.0080	0.71	4.18	0.161

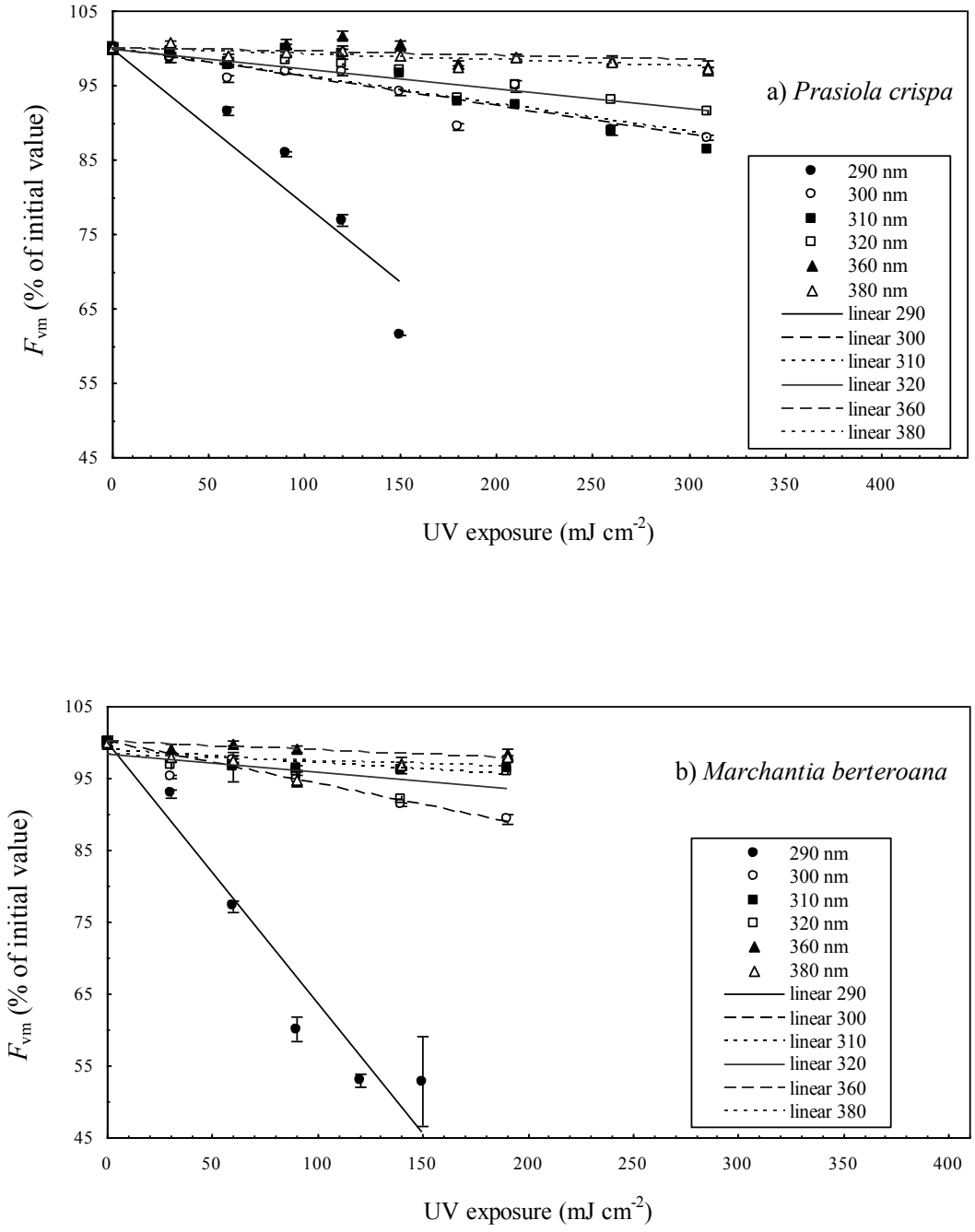


Figure 5.6 Effect of applying discrete wavebands of UV radiation concurrent with 50 µmoles m^{-2} s^{-1} PPFD on the photosynthetic competence of (a) *Prasiola crispa* and (b) *Marchantia berteroana*. Linear regression applied to each data-set (see text for details). Mean ± S.E. (n = 3).

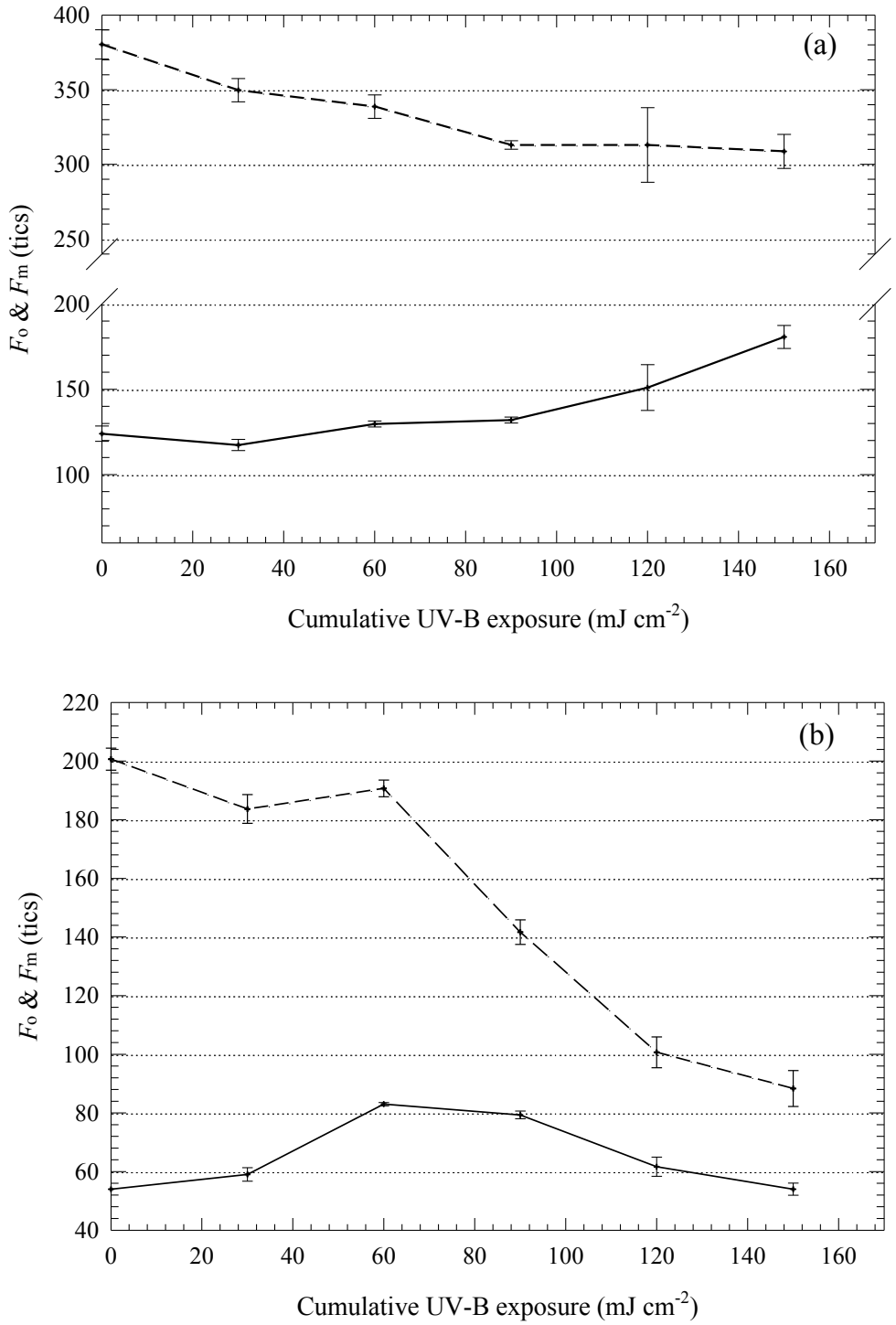


Figure 5.7 Effect of exposure to 290 nm radiation applied together with 50 μmoles m^{-2} s^{-1} PPFD on the dark-adapted values, minimal fluorescence (*F*o, solid line) and maximal fluorescence (*F*m, broken line).
See text for full details.
(a) *Prasiola crispa,* (b) *Marchantia berteroana*.
Mean ± S.E.

5.3.3.2 *Marchantia berteroana*

There was a distinct fall in F_{vm} of the 290 nm sample after the first exposure of 30 mJ cm^{-2}, dropping by approximately 5% of its original value (Figure 5.6b). This had dropped to < 60% of the initial value by 120 mJ cm^{-2} cumulative exposure. The 300 nm sample started to decline after 90 mJ cm^{-2} exposure. Whilst there were significant differences between the other sample exposures (≥ 310 nm), none of the other F_{vm} measurements dropped to below 90% of initial values. The results for the linear regression and the quantum effectiveness are shown below (Table 5.5). Most of the linear regressions yielded a reasonable fit except those where the response to UV exposure was small.

Table 5.5 Linear regression and re-calculated quantum effectiveness data for depression of F_{vm} in *Marchantia berteroana* exposed to monochromatic UV radiation concurrent with 50 μmoles m^{-2} s^{-1} PPFD.

Filter wavelength (nm)	Gradient from linear regression	r^2 for linear regression	Quantum effectiveness (quanta m^{-2} $x10^{-22}$)	Relative quantum effectiveness
290	0.3620	0.94	248	6.39
300	0.0585	0.86	38.7	1
310	0.0178	0.63	11.4	0.295
320	0.0254	0.49	15.8	0.407
360	0.0122	0.57	6.73	0.174
380	0.0094	0.16	4.91	0.127

The change in F_{vm} at 290 nm was largely the result of a drop in F_m, though there was a slight transient contribution by rising F_o. Thus F_m fell from a typical value of ca. 200 to under 100 units (Figure 5.7b). The drop in F_{vm} in the 300 nm sample was also partially a result of a fall in F_m, but was mainly accounted for by the rise in F_o (data not shown).

The damage done at 290 nm was partially reversible during the recovery period (returning to 62% of the starting ratio). This recovery was different in magnitude to that for the 300 nm sample (and the other samples), as F_{vm} recovered to within 5% of the original ratio after five days.

5.4 Construction and comparison of action spectra

The data for *Prasiola crispa* from the above sections (long-pass and interference filters) showed a general trend for greater depression of F_{vm} and increasing fluence i.e. damage was cumulative. There was a good relationship between the two lowest wavebands and depression of F_{vm}, and would indicate a broad UV-B response regardless of exposure protocol. Furthermore, there was a flattening of the depression after very large exposures (Figure 5.8). It is important to note the differential irradiance (Chapter 2 Figure 2.4), this shows that the main difference between the two long-pass filters in the growth cabinet was a peak wavelength at 312 nm. There would have been the possibility of repair processes being undertaken because of the timescale involved. Moreover, if the PAS weighted exposure for the long-pass is noted (Chapter 2, Figure 2.5) then the large differential between the two is not evidenced by effect on F_{vm} depression particularly at larger exposures. However, the long-pass filter measurements are not fully comparable to those of the interference filters on account of the differing methods used in measuring irradiance.

The combination of the sets of interference filter data yielded a reasonable relationship between the 300 nm exposure regardless of the species or presence of light. However, there was a clear distinction between the monochromatic exposure and the +PPFD exposures at 290 nm.

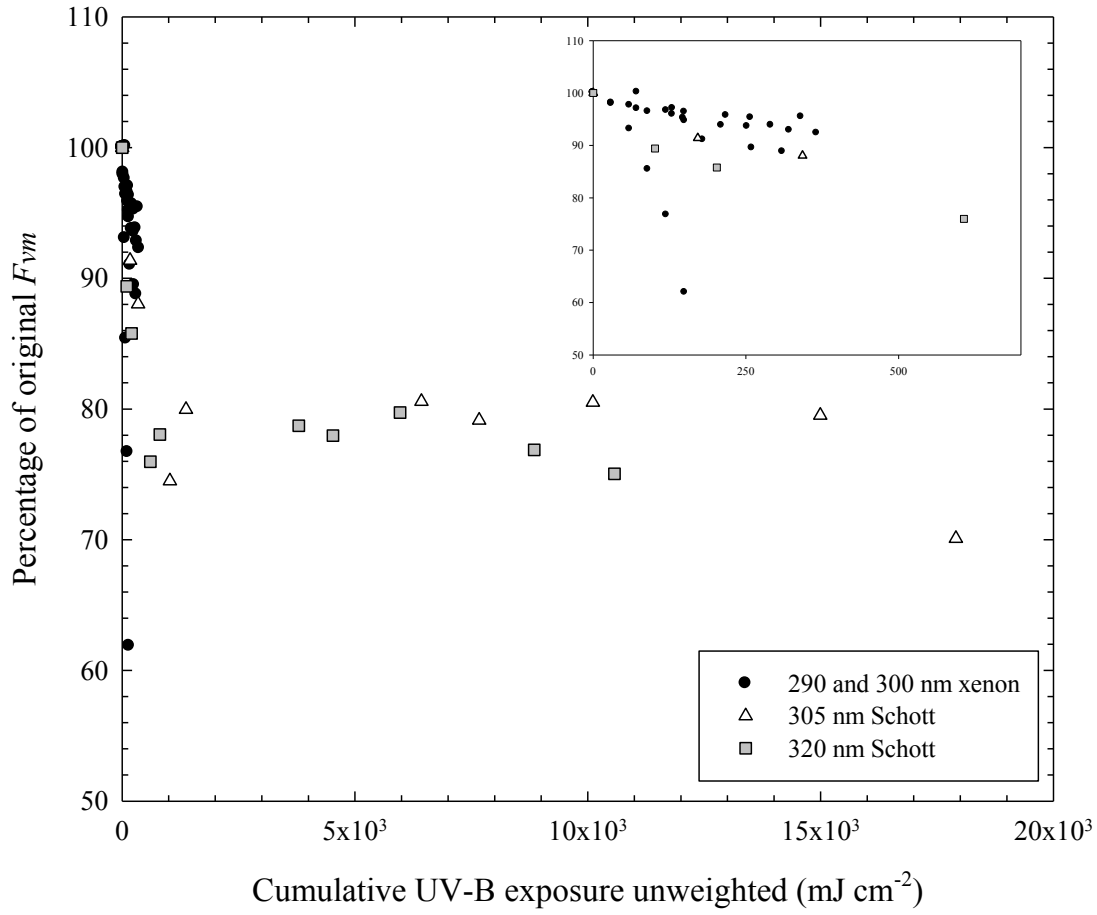


Figure 5.8 Combined data for all three UV exposures on the photosynthetic competence (Fvm) of the alga *Prasiola crispa*; long-pass filters (Schott), interference filters (xenon; dark and +PPFD). Inset shows detail for first 700 mJ cm^{-2}.
Mean values shown.

The quantum effectiveness calculated from all interference filter exposures are shown (Figure 5.9) and normalised to 1 at 300 nm (Figure 5.10). There was a similar depression in F_{vm} regardless of species or treatment above 300 nm. However, < 300 nm there was a marked difference with the +PPFD exposures leading to a greater efficacy of this waveband. The mean quantum effectiveness for the exposures are shown in Figures 5.9 and 5.10. The increased damage under the +PPFD exposure is again highlighted.

The +PPFD and monochromatic action spectrum are shown against other published action spectra, all normalised to 1 at 300 nm (Figure 5.11). The action spectrum of Rundel (1983) was based on the inhibition of photosynthesis (net CO_2 exchange) in *Rumex patientia*. Steinmüller's action spectrum (Tevini and Steinmüller 1988, cited in Coohill 1994) was constructed using a variety of plant photo responses. The action spectrum of Setlow (1974) was a carcinogenesis model based on DNA damage in a variety of organisms. Lastly, Quaite *et al.* (1992) relates to DNA damage in alfalfa seedlings. The two mean Antarctic action spectra show some similarities with other published spectra. Interestingly, the monochromatic spectrum is most similar to the PAS, which was itself based on monochromatic exposures, but has a UV-A tail that is absent in the PAS. The +PPFD spectrum follows a similar pattern to that of Rundel (1983), but maintains a higher relative effectiveness. However, it rises much more steeply < 300 nm than any other plant-based action spectrum shown.

The +PPFD action spectrum was used to calculate the change in biological effective UV-B radiation due to ozone depletion. This was carried out using irradiance recorded in the Antarctic (McMurdo Station, Figure 5.12), and from a model ozone depletion scenario (Figure 5.13). The +PPFD spectrum displays greater sensitivity over the 300 to 313 nm waveband in comparison to the PAS, but the PAS rises more steeply between 310 and 300 nm. The integrated irradiances and RAFs are shown below (Table 5.6).

Table 5.6 Calculated irradiance and radiation amplification factors taken from spectral irradiance data shown in Figures 5.12 (a, McMurdo), 5.13 (b, model ozone depletion), and 5.14 (c, 'Fi-totron'™ growth cabinet). For details on calculation see text. All irradiance data W m^{-2}, radiation amplification factors (RAF) dimensionless. RAFs calculated for < 313 nm and < 320 nm.

a)

McMurdo Station	PAS (313 nm)	+PPFD (313 nm)	+PPFD (320 nm)
2 November 1998 (170DU)	0.21	0.45	0.96
14 November 1998 (299 DU)	0.05	0.15	0.52
RAF	2.46	1.89	1.09

b)

Model ozone Depletion	PAS (313 nm)	+PPFD (313 nm)	+PPFD (320 nm)
170 DU	0.15	0.36	0.91
300 DU	0.04	0.13	0.51
RAF	2.24	1.78	1.03

c)

'Fi-totron'™ cabinet	PAS (313 nm)	+PPFD (313 nm)	+PPFD (320 nm)
'OX/02'	0.085	0.15	0.23

For both the Antarctic data and the model depletion scenario, the biologically effective radiation was much greater when calculated using the +PPFD spectrum compared to that for the PAS under ozone depletion. The effect of wavelengths above 313 nm is highlighted when the +PPFD weighting is calculated to 320 nm. This led to a large fall in RAF, and thus the potential deleterious effect of ozone depletion. However, the PAS realises a greater relative enhancement than the +PPFD function as a result of ozone depletion because of its steeper rise in effectiveness from 310 to 300 nm. The lower RAF of the +PPFD would indicate that *P. crispa* and *M. berteroana* would both be less susceptible to ozone depletion than would be expected using the PAS.

Weighting of UV radiation in the growth cabinet (Figure 5.14) again highlights the importance of the < 300 nm waveband. Calculation of the weighted radiation for the +PPFD spectrum < 290 nm gave values that were over 100 times more effective than that shown for the PAS (Figure 5.14). The lack of effectiveness of the UV-A band is shown in the +PPFD spectrum and its omission from the PAS. The calculation of irradiance matched that calculated using the spectroradiometer software (see Chapter 2). The +PPFD spectrum gave rise to a greater dose in the cabinet than that calculated for the PAS. A 12 h +PPFD weighted irradiance would be 6.48 kJ m^{-2} compared to 3.67 kJ m^{-2} $_{(PAS)}$. The instantaneous +PPFD weighted irradiance (to 313 nm) calculated for the growth cabinet was the same as that recorded at McMurdo station under 300 DU ozone (Table 5.6a), but was three times less than that under 170 DU ozone. This contrasts to the PAS weighted irradiance, which was 2.5 times lower than under 170 DU. This would infer that the calculated UV weighted exposures used in the growth cabinet, when based on the PAS, would be under-estimating the UV weighted exposure for a given ozone depletion scenario.

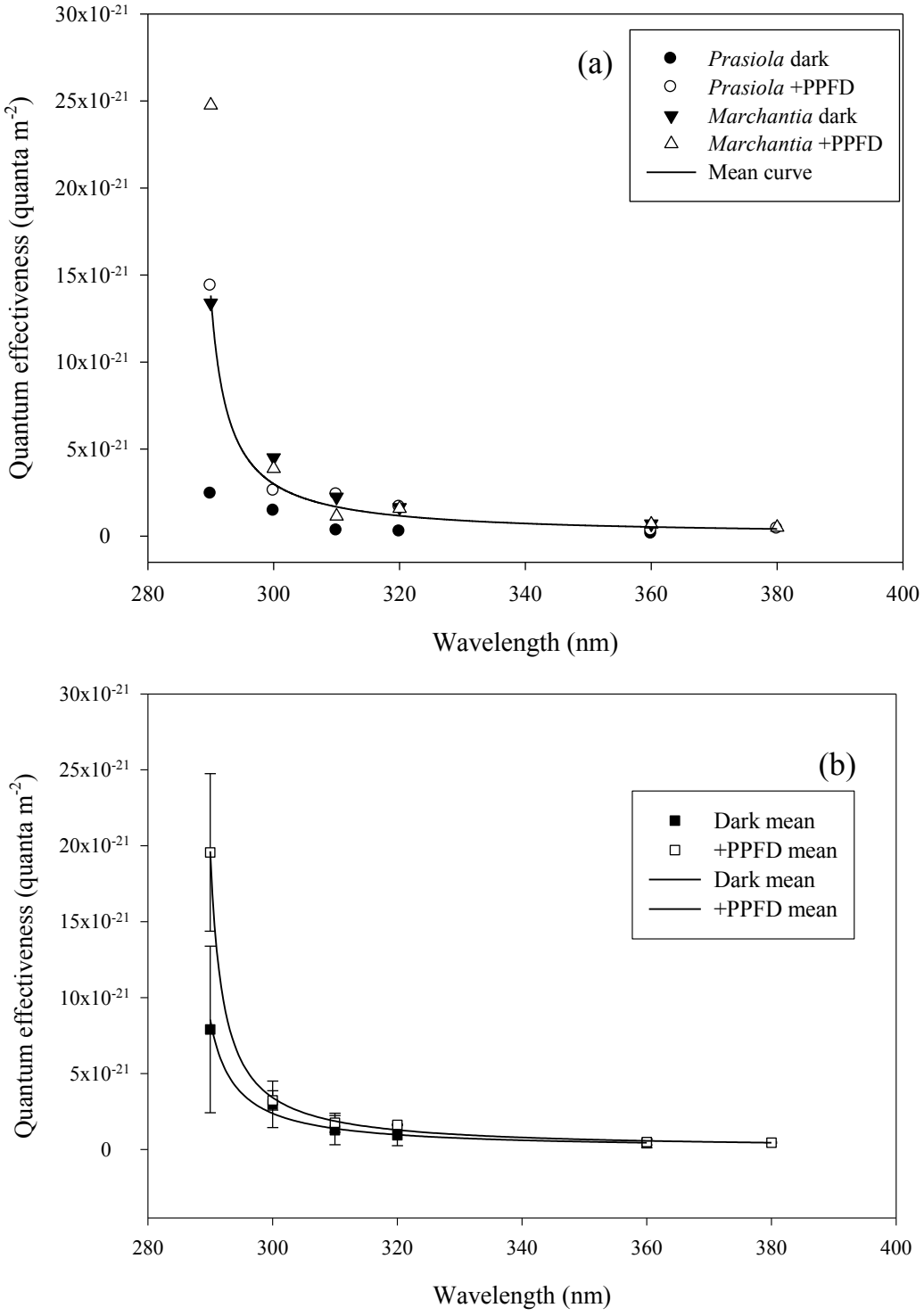


Figure 5.9 Plot of quantum effectiveness for depression of the dark-adapted fluorescence ratio F_{vm}. Data obtained from the alga *Prasiola crispa* and the liverwort *Marchantia berteroana*.
(a) Individual quantum effectiveness and mean curve.
(b) Mean quantum effectiveness for dark and +PPFD exposed.
Mean ± S.E., curves fitted ($y = 1/(a + bx)$).
(r^2; mean = 1, Dark mean = 0.97, +PPFD mean = 1).

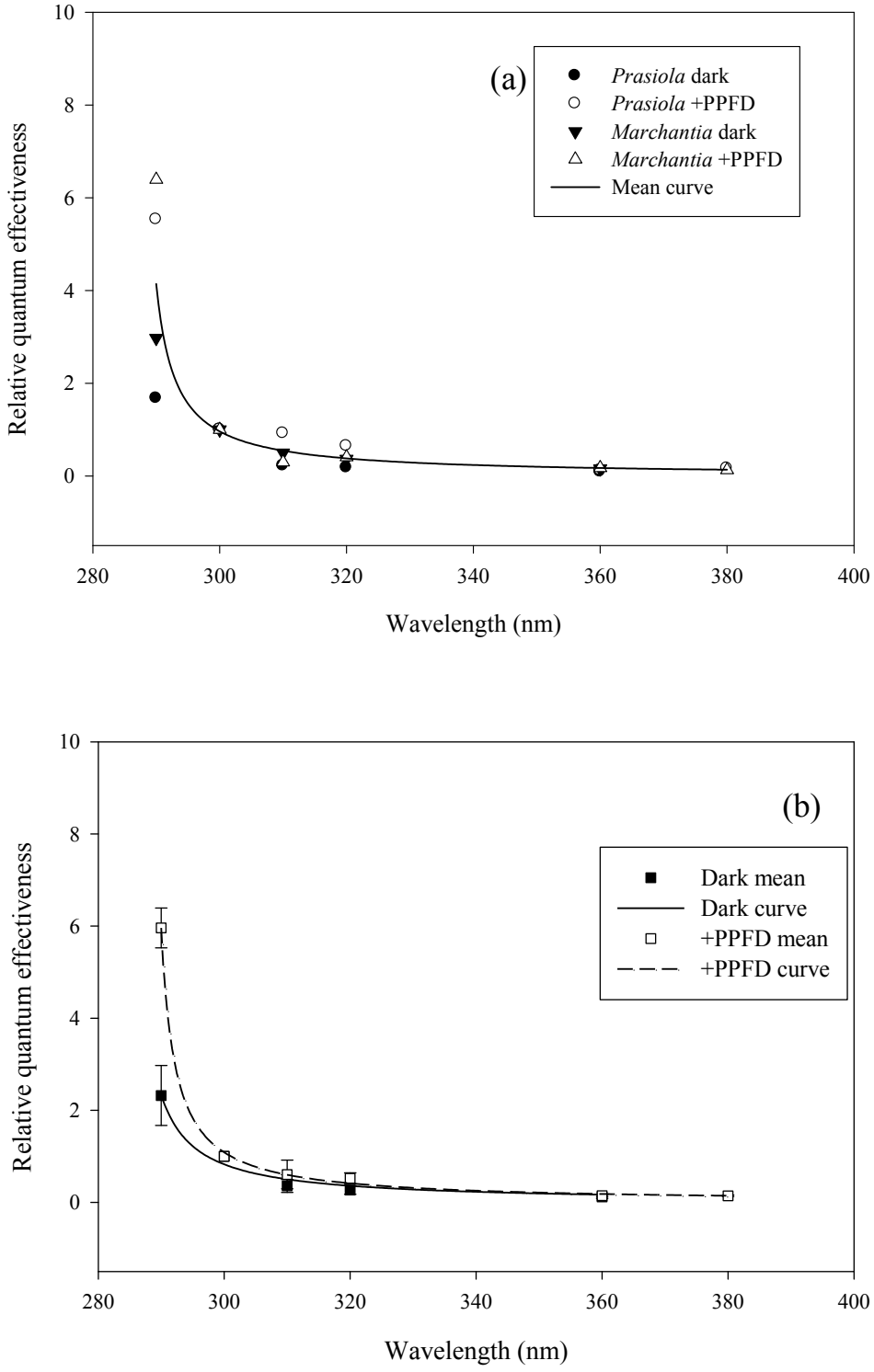


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Figure 5.10 Plot of relative quantum effectiveness for depression of the dark-adapted fluorescence ratio F_{vm} normalised to 1 at 300 nm. Data as for Figure 5.9.
(a) Individual data and mean curve.
(b) Mean quantum effectiveness for dark and +PPFD exposed. Mean ± S.E., curves fitted ($y = 1/(a + bx)$) (r^2; mean = 1, Dark mean = 0.98, +PPFD mean = 1).

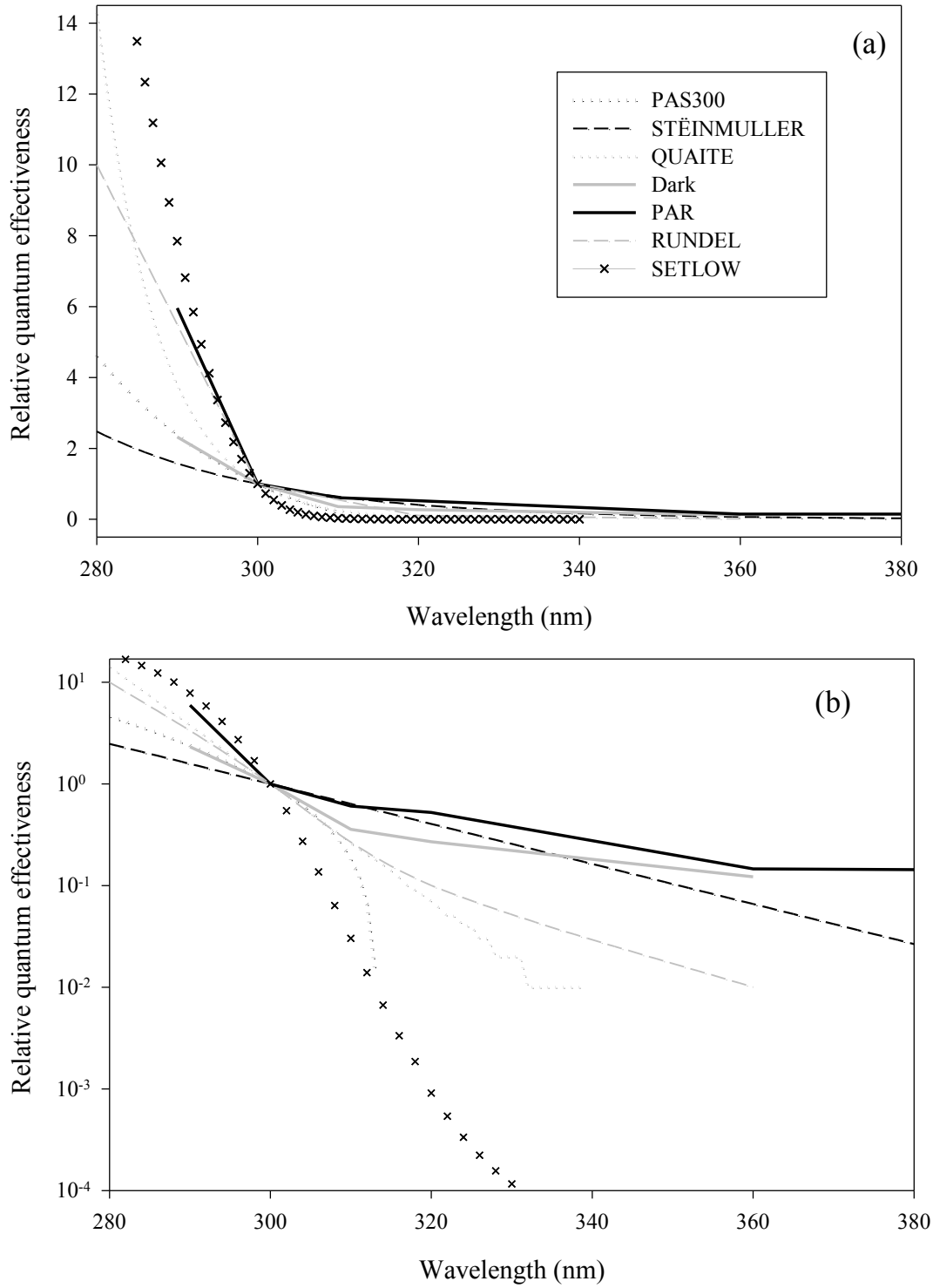

Figure 5.11 Comparison between the monochromatic (dark) and +PPFD action spectra. Also plotted other selected action spectra (all normalised to 1 at 300 nm).
(a) Linear plot. (b) semi-logarthmic plot.
See text for full details of other action spectra .

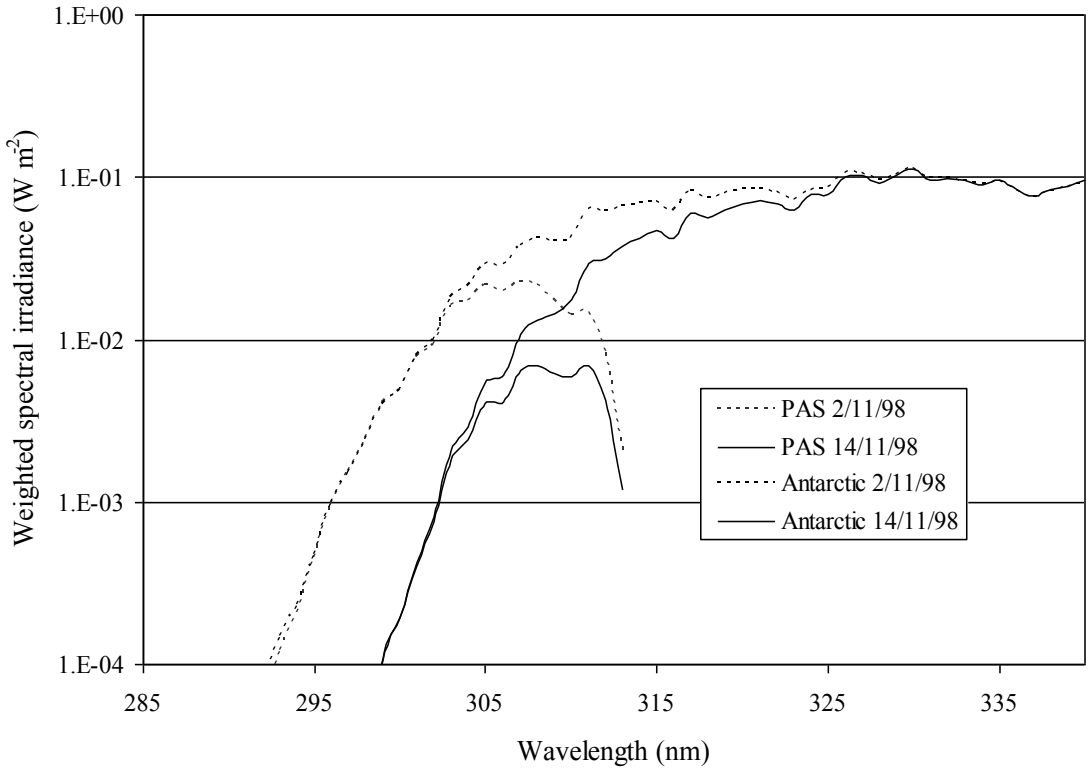

Figure 5.12 Effect of action spectra on actual ozone depletion at McMurdo Station during peak ozone depletion (02 November 1998, 170 DU) and shortly after (14 November 1998, 299 DU). UV radiation data from Figure 1.4. The Antarctic spectrum derived from the exposures concurrent with PPFD (Antarctic) and the action spectrum of Caldwell (PAS). All spectra normalised to 1 at 300 nm. UV radiation data taken at similar times on each date.

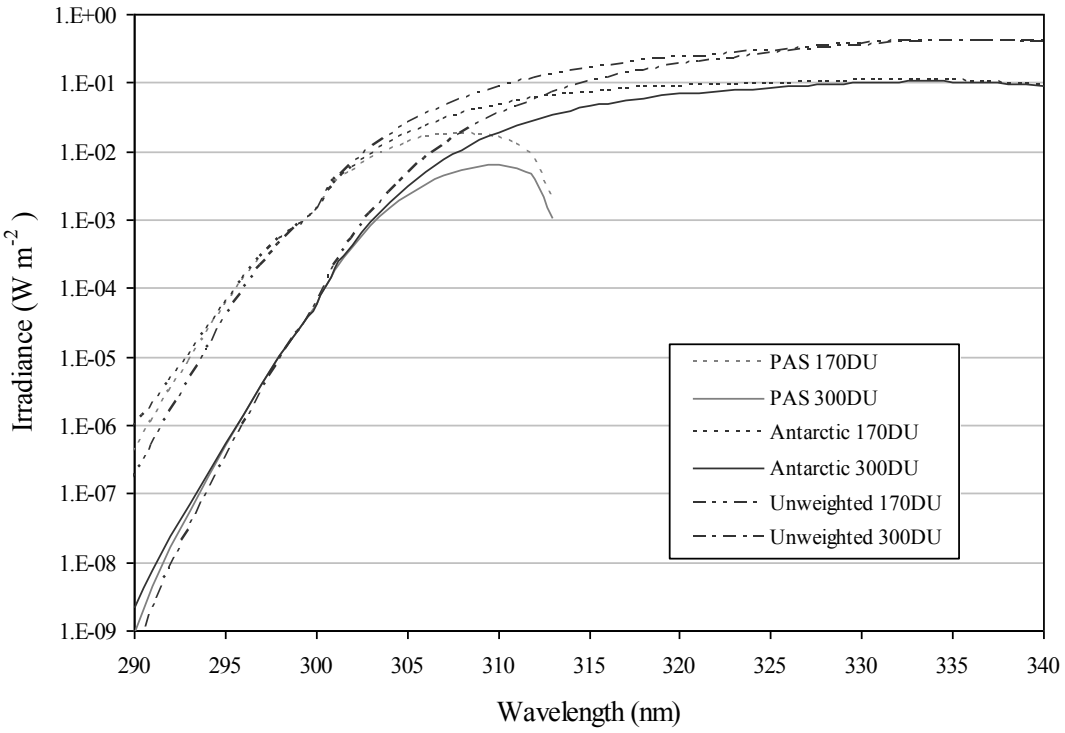

Figure 5.13 Effect of action spectra on model ozone depletion.
The Antarctic spectrum derived from the exposures concurrent with PPFD (Antarctic) and the action spectrum of Caldwell (PAS). All spectra normalised to 1 at 300 nm. UV radiation data calculated for 70^{o} latitude, using the computer program of Fiscus and Booker (1994). See text for details.

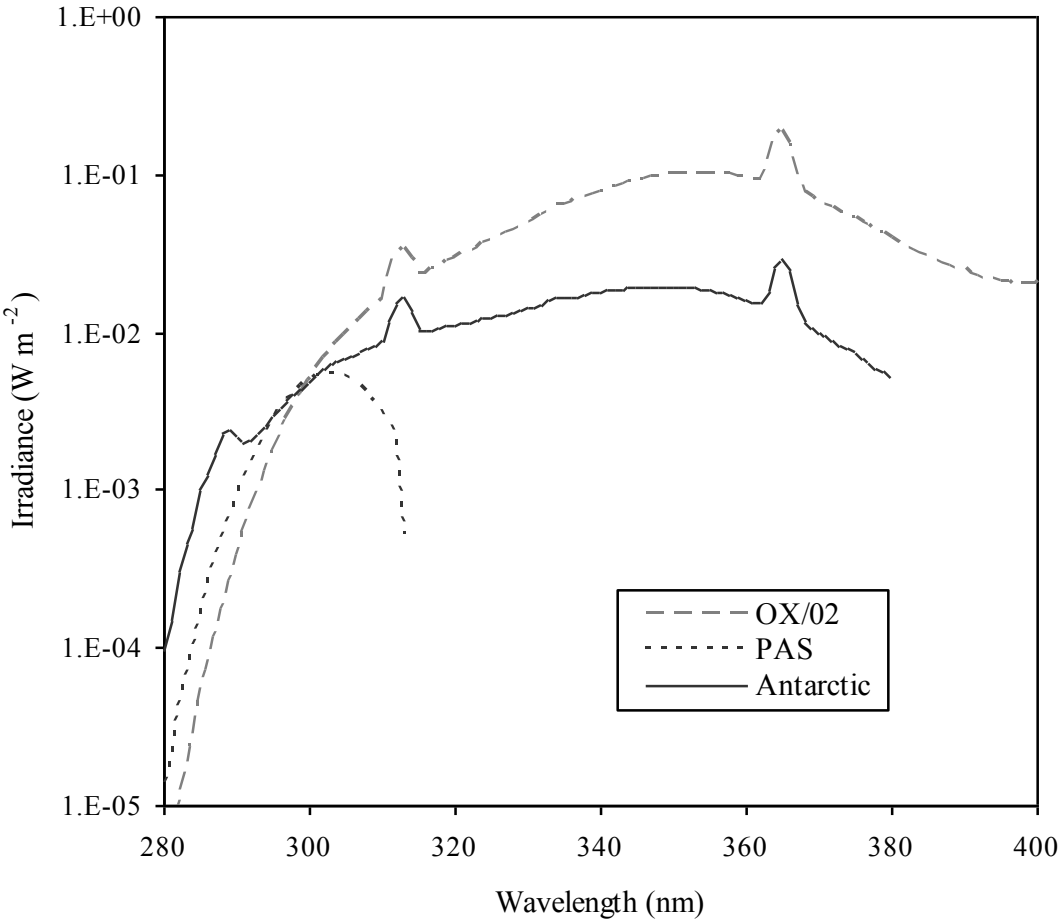

Figure 5.14 Effect of action spectra on irradiation in the 'Fi-totron'™ growth cabinet used for UV-B exposures. The Antarctic spectrum derived from the exposures concurrent with PPFD (Antarctic) and the action spectrum of Caldwell (PAS). All spectra normalised to 1 at 300 nm. Un-weighted UV radiation data recorded under the UV-B transparent plastic 'OX/02' at approximate growth height is shown for comparison.

5.5 Discussion

This study has shown a clear relationship between depression in F_{vm} (and therefore potential photochemical efficiency), and the wavelength of UV radiation received. Use of long-pass filters showed a clear correlation between F_{vm} depression and the PAS function (Figure 5.1c). However, at much greater exposures the rate of loss of F_{vm} is reduced. This may have resulted from repair of the UV-B-induced damage or a change in proportion of effectively shielded photosystems. The fall in F_{vm} resulted from both a loss in F_m and then an initial rise in F_o. This would infer that there may have been multiple causes for the loss of F_{vm} and differs from the earlier work on *P. crispa* (Chapter 3) where loss was predominantly due to F_o. The un-weighted exposures carried out with the long-pass filters were of a similar magnitude to earlier work reported in this thesis (and lower than most published work e.g. Post and Larkum 1993).

Results using interference filters without background PPFD confirmed that depression in F_{vm} was a wavelength dependent (and not solely energy received) response for both species investigated. Furthermore, as the samples were only exposed to discrete wavebands of UV radiation, it is possible to state that short wavelength UV-B is most effective at depressing F_{vm}. Such effects would be expected as they have been noted previously in a wide range of plant material (see Chapter 1). Irradiation of both *P. crispa* and *M. berteroana* at 290 nm initially caused a fall in F_{vm}. The drop in F_{vm} was generally greater in *M. berteroana*. This fall could be attributed to changes in both F_m and F_o, however, the rise in F_o was more marked in *P. crispa* than in *M. berteroana*. The lowered F_{vm} at higher wavebands mainly resulted from the rise in F_o and this would imply that there were different mechanisms of UV-B-induced damage/ repair occurring in the hepatic and the alga. Moreover, there may have been differential penetration of UV-B to sensitive tissues within each plant (due to localisation of protective pigments, Reuber *et al.* 1996). Work on pea has also demonstrated an increase in F_o and a decline in F_m (Strid *et al.* 1996), and a further study on photoinhibition has also described the same response (Rintamäki *et al.* 1994). A rise in F_o is usually related to inactivation of PSII reaction centres and D1 damage (Krause and Weis 1991, Rintamäki *et al.* 1994). Rintamäki *et al.* (1994) related rises in F_o to reversible damage, such as D1 turnover. Moreover, Tevini *et al.* (1988) attributed a UV-B-induced rise in F_o to possible antennae chlorophyll damage. UV-B radiation has been shown to enhance photoinhibitory damage in Antarctic marine phytoplankton (Smith *et al.* 1992), and to be an additive

effect in a photoinhibition sensitive *Brassica napus* mutant (Olsson *et al.* 2000). However, some workers have suggested that UV-B and photoinhibitory damage may be the result of two separate mechanisms (e.g. Jansen *et al.* 1996). Later work (Hideg *et al.* 2000) has differentiated donor/ acceptor side effects. Here, UV-B (and chilling) was involved in donor side damage, whilst photoinhibition was linked to acceptor side damage. Until resolution of the inter-species differences between alterations in F_o and F_m have been fully explained, it is difficult to state what mechanisms are responsible for the loss in photosynthetic competence observed in this study.

It may be expected that, when exposed to background PPFD, PSII is already functioning and undergoing repair processes, hence any additional stress will be greater in the dark than with the exposures coupled with light. However, this was not the case when the results with or without background PPFD are compared. This was particularly notable at lower wavelengths of UV-B, where both species exposed to 290 nm radiation together with background PPFD experienced a greater loss of F_{vm}. The loss in F_{vm} at 300 nm was of a similar magnitude, regardless of supplementation of PPFD. This would suggest that the presence of visible light can increase the susceptibility of the target(s) of UV-B without interacting with other less sensitive sites. Studies on temperate plant species have found that increased levels of PAR can ameliorate the effects of UV-B radiation (e.g. Adamse and Britz 1992). However, the monochromatic exposures were carried out over a longer timescale; these intervals would have allowed for increased repair or recovery of PSII, and could help explain these apparently confounding results.

The post UV-B exposure period in the long-pass filter study on the alga would appear to have allowed for repair/ recovery as F_{vm} values increased throughout. However, the work using interference filters with concurrent PPFD showed mixed results. At 290 nm there was no recovery, but rather a continued loss of F_{vm} in *P. crispa*. Whilst *M. berteroana* seemed to be more sensitive to UV-B radiation, this effect would appear to be more elastic, in that the fall in F_{vm} was at least partially reversible. Again, this would indicate different targets or mechanisms of UV-B-induced damage.

The appearance of the purple pigment in *M. berteroana* was directly related to UV-B exposure, as it only occurred under the 290 nm interference filter and appearance of the pigment during the long-pass filter study occurred first under the 305 nm filter. Chlorophyll bleaching could have been one reason for its appearance. A number of compounds have been identified in *M. berteroana* (Chapter 3) that could have contributed to this colouration. The browning that occurred was peripheral so should

not have been important regarding the fluorescence measurement. These measurements were focused on the central area of tissue and thus omitted the dead material.

Action spectrum construction yielded a very good fit considering the limited data. The addition of PPFD would appear to substantially enhance the UV-B-induced damage at 290 nm, especially when the measurements used to construct the action spectrum (fluorescence emission from PSII) are related to a process that relies on PPFD. The action spectrum produced herein can be used to compare and recalculate weighted previous work on Antarctic ozone depletion. However, the action spectra produced here was constructed without using all the tests that ideally should be conducted (e.g. reciprocity, Holmes 1997).

Comparison with other published action spectra demonstrate similarities to the one produced here. Work by Holmes (1997) and that on *Cynosurus cristatus* (Cooley *et al.* 2000) showed a similar 'shoulder' around 320 to 330 nm. Moreover, Cen and Björn (1994) found a levelling off of response to ultraweak luminescence in *Brassica napus* at ca. 320 to 340 nm. Overall effectiveness was analogous to that of Rundel (1983). Work by Shinkle *et al.* (2004) found differential response of hypocotyl growth with differing responses for 280 to 300 nm and 300 to 320 nm; concluding involvement of different photoreceptors, and thus would have implications in using broadband exposures. Flint and Caldwell (2003a) produced an action spectrum similar to that of PAS but with a flattened extension into the UV-A range. This spectrum has already been used for weighting (e.g. Boelen *et al.* 2006). Lastly Yao *et al.* (2006) produced similar action spectra to the PAS except with the conclusion that monocotyledonous plants were more sensitive to UV-A than dicotyledonous plants. The flattening of effect above 320 nm should have little effect as regards consequence of ozone depletion due to minimal alteration in received UV radiation as there is only weak absorption by ozone at this waveband.

Importantly, increased effectiveness of the +PPFD action spectrum may well impact on the prediction of the consequences of ozone depletion. The +PPFD action spectrum gives greater weighting to those wavelengths that would be most affected by ozone depletion compared to the PAS, and results in a greater effective dose than the PAS. This was shown when the +PPFD spectrum was used on actual measurements taken at McMurdo station. However, the contrasting RAFs highlight the steeper gradient of the PAS from 310 to 300 nm, thus greatly increasing the effect of ozone depletion as calculated using the PAS weighting compared with the +PPFD spectrum. These

differences lead to three important considerations. The relative enhancement of the +PPFD spectrum under ozone depletion is lower, thus leading to less deleterious prognosis than that would be expected if based on the PAS. However the ambient UV-B irradiance prior to ozone depletion is probably more damaging to these plants than would be anticipated if weighted using the PAS. Lastly, any UV enhancements carried out using lamp-based systems based on exposures calculated using the PAS would under-estimate the ozone depletion calculated for that exposure compared to that based on the +PPFD function.

To conclude, damage to PSII efficiency was wavelength dependent with decreasing wavelengths of UV-B radiation having an increased effect on F_{vm}. Supplementary PPFD enhanced F_{vm} depression, but may be related to duration of interval between exposures. However, the exact target/ mechanism of the UV-B-induced damage is still unclear. The +PPFD action spectrum produced here yielded a higher absolute biologically effective exposure than the PAS, but the weighted irradiance was less altered by ozone depletion than the PAS.

CHAPTER 6
GENERAL DISCUSSION

6.1 Introduction

The findings of this thesis are discussed in the same order as the stated aims in Chapter 1 and are summarised in Figure 6.1. The simple effect of exposure to, or exclusion from, UV-B radiation on the non-vascular plants and the hair grass is first examined. The resolution of the UV-B effect into those narrow wavebands that were responsible is then considered. The implications of the experimental findings of the thesis are scrutinised next; firstly whether the findings of this work would impact on the plants *in situ*, and secondly, the effect that enhanced UV-B radiation could have had in the past on the colonisation and development of the Antarctic vegetation. The problems that can arise when undertaking experimental work altering UV-B exposure are given attention and, lastly, what future work still needs to be carried out to fully elucidate the effects of enhanced UV-B radiation on the Antarctic flora as a result of ozone depletion.

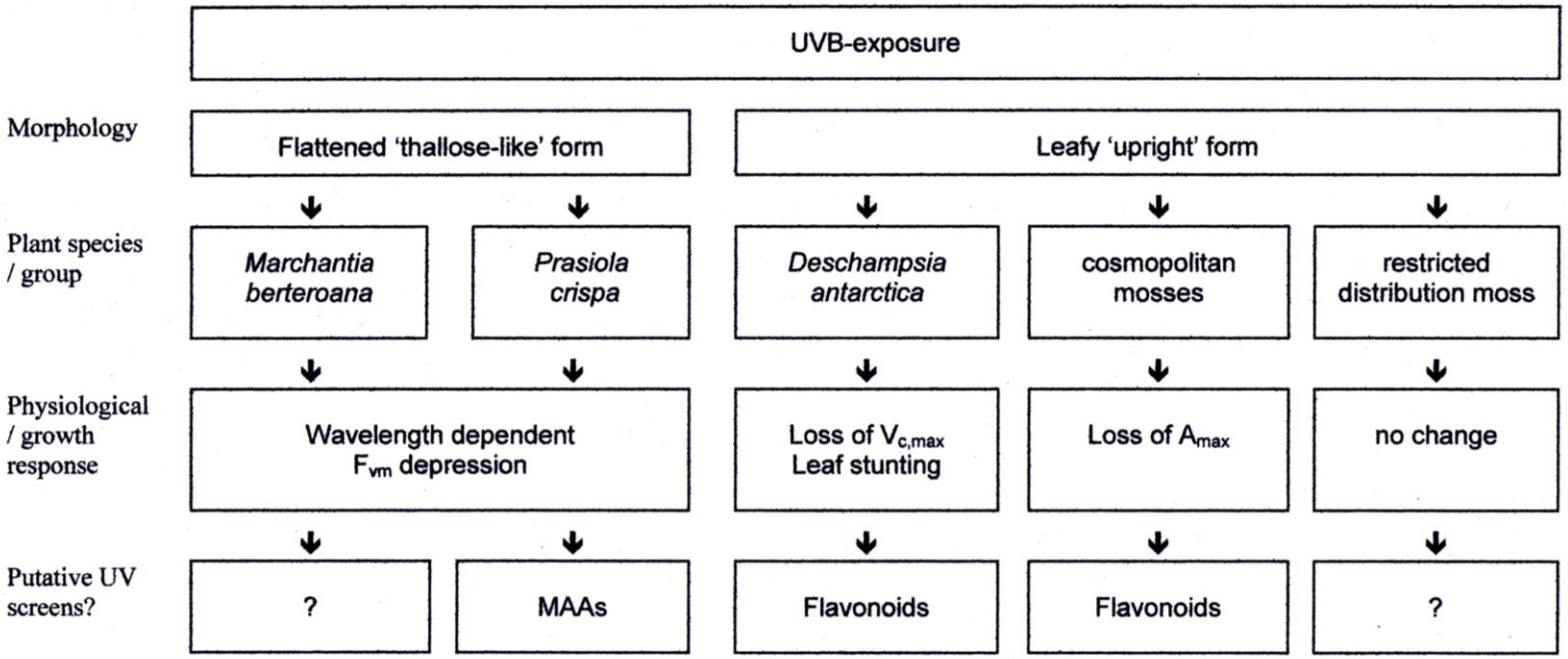

Figure 6.1 Flowchart summarising the findings of the thesis.

6.2 Effect of UV-B on cryptogams

Work on the cryptogams showed a range of responses to UV-B radiation, with those having a sheet-like morphology being most susceptible to damage. Where a depression in F_{vm} was noted, the causes, changes in F_m and F_o, varied between different species, indicating different targets, or sensitivities, though was not conclusive. This showed some parallels with a photoinhibitory-like response (Rintamäki *et al.* 1994). Other studies have shown that UV-B exposure protects against photoinhibition in aquatic plants (e.g. Hanelt *et al.* 2006), while Schlensog *et al.* (2003) noted that fully hydrated Antarctic cryptogams, when naturally exposed to high light levels were tolerant of this high PAR by way of the xanthophyll cycle. Loss of F_{vm} could potentially lead to changes in overall photosynthetic efficiency, although none was noted under the conditions by which photosynthesis was recorded in this study, i.e. saturating CO_2 concentration, particularly for *P. crispa* and *M. berteroana*. Thus loss of F_{vm} was also recoverable once UV-B exposure was terminated. This would suggest that whatever part of the photosystem was being affected; any damage was quickly reversible. Changes in photosynthetic activity, measured by net oxygen exchange, were ambiguous and could not imply any substantial effects. Similar findings have been reported elsewhere (Rozema *et al.* 2001, Xiong and Day 2001, Lud *et al.* 2003). The loss of A_{max} however, could have been caused by damage to the light independent stage, which this study could not adequately model. Furthermore, the light response curves were recorded under saturating CO_2 concentration, thus (at saturating PPFD) the lowered A_{max} should not be limited by effects on light interception.

Prevention of UV-B damage (and thus protection of photosynthetic apparatus) by way of UV-absorbing compounds was apparent in some species, yet the metabolic cost of protective compound production remains unknown. The evidence of a role for the flavonoid production, as well as that for MAAs, needs to be extended to verify any potential screening effect, while, the protective strategy employed by the mosses not shown to have extractable flavonoids needs elucidating. With regard to the latter, some studies on Antarctic mosses have implicated xanthophyll cycle carotenoids (Robinson *et al.* 2005), unidentified methanol-soluble UV-B screening pigments (Newsham 2003), and cell wall-bound screening compounds (Clarke and Robinson 2008) as providing protection from UV-B radiation. There has been some additional evidence indicating a role for MAAs protecting algae against UV-B (Lee and Shiu 2009), indeed Abhishek *et*

al. (2006) noted a similar response of MAA accumulation to either UV-B or, a saline immersion.

6.3 Effect of UV-B on *Deschampsia antarctica*

The developmental history appeared to be an important UV-B exposure response variable for *D. antarctica*. Seed-grown plants exhibited the most pronounced loss of leaf area and $V_{c,max}$, which should result in lower overall productivity since assimilation has an area component, and $V_{c,max}$ was lower at ambient CO_2 concentration. However, no such loss in net assimilation on an area basis was recorded as a result of UV-B exposure in the mature plants; as reported elsewhere (Xiong and Day 2001, Rozema *et al.* 2001). These productivity differences could potentially impact on the grass extending its range south along the Antarctic Peninsula. Other workers have noted differential responses to UV-B exposure at different developmental stages; Reifenrath and Müller (2007) for example, found lower levels of flavonoids in young leaves of *Sinapis alba* compared to older leaves, whilst Pradhan *et al.* (2008) reported an alteration in photosynthesis in primary leaves from wheat seedlings of differing ages. Loss of leaf area has also been shown to contribute to a decrease in field biomass of *D. antarctica* (Xiong and Day 2001). While leaf area loss would infer a lower requirement for UV-B screening compounds, the mechanism responsible for stunting leaf growth is unknown. Increased production of the orientin-like compound will not obviously act as a bulk UV-B screen sufficient for *D. antarctica* to tolerate enhanced UV-B radiation. Again, the specific role of orientin-like compounds remains unclear. The similarity between the flavonoid samples from clonal plants sourced from Signy I. and those taken from the individual plants grown in the UK from seed collected from Leonie I. would imply that there is little difference in UV-B response between different populations of the hair grass separated by 2,000+ km. There is little genetic variation between the two populations (Holderegger *et al.* 2003), together with a broad equivalence between the results described herein and other published work on field-based studies in the Antarctic Peninsula (Huiskes *et al.* 2001, Lud *et al.* 2001, Xiong and Day 2001, Ruhland *et al.* 2005). These minor variations include changes in phenolic compounds, alteration in leaf morphology, insensitivity of F_{vm} response and light response A_{max}.

In addition to the direct effects on the photosynthetic activity there may be additional indirect effects on the overall survival and species interaction. The smaller

leaves produced by *D. antarctica* would impact on its productivity during the early spring when productivity is considered to be most sensitive (Edwards and Smith 1988) and coincides with the increased UV-B radiation associated with ozone thinning. Loss of leaf area would also impact sward area and density. For example *D. antarctica* is often associated with moss cushions (Longton 1988), where reduced leaf area would result in reduced shading, leading to a potential change in the grass-moss ecosystem. Additional changes in phenolic content noted in a number of species studied that could potentially affect decomposition rates, nutrient cycling and soil formation. Work on Arctic ecosystems (Gehrke 1998), has shown potential UV-B-induced changes, though more recent work (Rozema *et al.* 2006), indicates little effect of seven years enhanced UV-B exposure. However, any response reported for Arctic terrestrial vegetation needs to be translated to the equivalent Antarctic ecosystem with some caution (Rozema *et al.* 2006).

Given the lack of any observable short-term effect on PSII, a change in emphasis to other aspects of vascular plant physiology has been suggested, with a focus on development and biochemistry (Allen *et al.* 1998). This includes such effects at ambient rather than extreme UV-B exposure (Paul and Gwynn-Jones 2003), with emphasis on more longer term study (Aphalo 2003, Rozema *et al.* 2005). Such approaches are appropriate for *D. antarctica* where the most substantial effects are only reported for morphology and $V_{c,max}$.

6.4 Wavelength dependent response

Work using the long-pass and interference filters unequivocally demonstrated that loss of F_{vm} (and thus PSII damage) in two cryptogams was both UV-B dependent and most depressed by those wavelengths that are most altered by ozone depletion. The work on de-convoluting the general effect on the alga and liverwort to those most effective wavebands did show increase in sensitivity of F_{vm} on exposure to lower UV-B wavebands; particularly those wavebands most affected by ozone depletion, i.e. a greater effect of the < 300 nm waveband. Thus increased UV-B radiation caused by ozone depletion can be expected to depress the photochemical efficiency in these two cryptogams. Furthermore, there also appeared to be enhanced damage with concomitant exposure to PPFD. The +PPFD action spectrum was broadly similar to some other published action spectra (Setlow 1974, Rundel 1983, Flint and Caldwell 2003a), but

rises more steeply in the UV-B wavelengths that will be enhanced by ozone loss compared with other action spectra, in particular the PAS and Quaite *et al.* (1992); though the PAS has a steeper slope between 300 and 310 nm. This was shown to have potential consequences on the biological weighting of the spectroradiometric data from the Antarctic, which are commonly weighted with the PAS, including the UV-B exposures reported in this thesis. The +PPFD action spectrum should give rise to a higher biologically effective dose under a normal ozone layer (300 DU) compared to the PAS. This differential effect would make pre-ozone thinning levels of UV-B more damaging than would have been expected using the PAS; requiring a re-evaluation of studies that used PAS-weighted exposures. However, the potential deleterious effect of ozone depletion will not be as great as that calculated using the PAS. Furthermore, the plants would be experiencing more damage to PSII under normal levels of ozone than would be expected from using the PAS, as a result of the higher sensitivity of the +PPFD function. Thus the potential impact on photosynthesis in those cryptogams studied could be greater than anticipated in vascular plants, or than originally thought when based on calculations using the PAS. In addition, any lamp-based studies using the PAS would under-estimate the effective UV-B exposure for ozone depletion compared to +PPFD action spectrum. This study has demonstrated problems in constructing future depletion models until appropriate action spectra have been constructed for representative Antarctic species. As such there may be discrepancies between the responses shown here and previously published action spectra, nevertheless the problems regarding interpretation and reliance of RAFs should be noted (Coohill 1994). There are limitations with the work on action spectra such as lack of repetition, reciprocity and no proof of linearity of response. Furthermore, previous work has shown that the effects of UV-B can be ameliorated under high PAR, and that the combination of UV-B and low PAR is particularly damaging (Adamse and Britz 1992). This could account for these reported observations. Furthermore, the action spectra produced (based on F_{vm} depression) was not supported with any alteration in gas-exchange measurements (Chapter 3) which were taken under saturating conditions of CO_2 and thus may have masked any detrimental effect. Moreover, the action spectra cannot be directly linked to loss of photosynthetic productivity in the Antarctic, nor can they be related to DNA damage spectra like that of Quaite *et al.* (1992). Nevertheless, there has (to date) been no other UV-B action spectra attempted on terrestrial Antarctic vegetation, so these provide, at the very least, comparative data. Other polychromatic

action spectra on growth responses in temperate meadow species have shown differential effects on different species with, in this case, one being more effected by UV-A than the other (Cooley *et al.* 2000). There is also the problem with studies that use one particular weighting function e.g. Setlow (1974) for DNA damage which may not then be valid if photosynthesis is also investigated by way of F_{vm} (Lud *et al.* 2001). Weighting functions will probably need to be both species-specific and response-specific (Caldwell *et al.* 2007), until there are long-term studies on growth effects such as dry mass accumulation, which should be an integral of possible multiplicit UV-B effects.

The +PPFD action spectrum showed ambient UV-B exposure to have a greater damaging effect than the PAS, but to be relatively less effective under ozone depletion. This has potentially important consequences on interpretation of the effect of Austral spring ozone thinning. Attempts to predict potential effects of ozone depletion on the Antarctic vegetation were not fully investigated, yet these preliminary studies highlight potentially more damaging consequences than the PAS would elicit under non-depleted ozone and in comparative lamp-based studies. The presence of other limiting Antarctic factors could well make interpretation of these findings, and other published work, difficult. The confounding, ameliorating factors of temperature, nutrient status and CO_2 concentration for example, would all be expected to impact on any UV-B response.

6.5 Potential consequences of UV-B response

All of the plants studied survive under, at least temporary, conditions of extreme water stress. The features of vascular plants that can assist in water conservation, including a thick waxy cuticle, narrow curled leaves, could also assist in the shielding from UV-B radiation (Holmes and Keiller 2002). A study on conifers has shown that UV-B exposure at ambient levels may confer protection from drought stress (Poulson *et al.* 2002), whilst Gitz *et al.* (2005) noted an increase in water use efficiency in some soybean cultivars after UV-B exposure. However, this is obviously not the case for (the poikilohydric) cryptogams, which lack a cuticle and tend to lose physiological activity under desiccation (Longton 1988). This factor could render cryptogams more susceptible to UV-B radiation than the hair grass i.e. the hair grass must have mechanisms to cope with water stress that could also assist in UV-B resistance. Differences in response between the cryptogams could lead to changes in their relative

abundance and distribution. The additional energy requirement for production of flavonoid screening compounds to protect the sensitive species, *B. argenteum* and *C. purpureus* from enhanced UV-B exposure may not make the higher photosynthetic rate exhibited by both species as advantageous as it would appear. It could provide the more resistant species, such as *S. glaciale*, with an opportunity to compete in areas where they would not normally occur. Moreover, desiccation and UV-B tolerance may well be linked in mosses (Takács *et al.* 1999) but again, confounding factors make it difficult to extrapolate possible UV-B effects. Furthermore, with regards to putative UV-B absorbing compounds, many studies report on a crude methanolic extraction screened from 280 to 360 nm (e.g. Newsham *et al.* 2002, Dunn and Robinson 2006). It is not possible to distinguish between a relative change in proportion of relevant compounds, nor is it possible to state that these compounds would even screen against UV-B radiation *in vivo* or, if they are located in an appropriate layer of the plant to affect such screening. An increase in concentration of UV-B absorbing compounds could also be related to PAR and/ or UV-A. In this regard, Fedina *et al.* (2006) found a decrease in UV-B inducible and UV-B absorbing compounds after a saline treatment and thus considered such compounds to be produced as a consequence of stress, and not necessarily as a response to protect against UV-B. The work presented in this thesis does show flavonoid compounds that would be expected to act as screens and that there could be a change in relative composition without an alteration in bulk amount. Also the pigment production in *M. berteroana*, whilst being linked to UV-B exposure would not be expected to be effective as a screen against UV-B radiation, but would protect against excess PAR (Snell *et al*. 2009). For this particular case it could be that increased UV-B exposure is tolerated by improved protection against photoinhibition thereby lessening the burden on repair of PSII as a result of photoinhibition. Furthermore, the relative composition of UV-B screening compounds may be affected by soil nutrient status (de la Rosa *et al*. 2001). Finally, it has also been noted (Lappalainen *et al.* 2008), that a number of UV-B absorbing compounds are reported on a mass of tissue rather than on area basis; this again could have important consequences regarding the efficacy of such potentially important UV-B absorbing screens.

6.6 Potential effect of UV-B on the origin and distribution of Antarctic plants

The differing mechanisms of UV-B response between the organisms could also result from colonisation of or whether these plants were maintained in isolated refugia. Recent work (Convey *et al.* 2008) has suggested that some Antarctic species were not post-glacial or post Pleistocene colonists, but survived glacial periods in ice-free refugia. If this were so then it could explain the different response to UV-B in the mosses which in Victoria Land could have originated from South America (e.g. *S. glaciale*) or have a cosmopolitan distribution (e.g. *B.* a*rgenteum*). Work on one endemic and three cosmopolitan mosses on the Antarctic continent (Dunn and Robinson 2006), could only find UV-B exposure to adversely affect the endemic moss *G. antarctici*, which did not contain appreciable amounts of UV-B absorbing compounds. This would infer that, along with *S. glaciale* which was found not to contain UV-absorbing compounds in the work presented in this thesis, there are two possibilities; these species of restricted distribution were isolated on the continent and thus cannot, unlike the more adaptive cosmopolitan species, respond to raised UV-B levels that are result of ozone depletion. The alternative is that they have become highly specialised to cope with the Antarctic environment, particularly when UV-B levels were higher than at present, and thus do not respond to the recent ozone thinning. UV-B levels would have been, until ozone thinning, lower than over more northerly locations, thus any isolated species could be expected to have had long term exposure to a lower level of UV-B exposure than those cosmopolitan mosses. However, Leavitt *et al.* (2003) suggested that past UV-B levels may have been greater than experienced under current ozone depletion. If this is confirmed then the relic species would have experienced the higher level of UV-B, but the route taken to protect against UV-B differed from the production of flavonoids that was found in other mosses. The carotenoids observed in *C. purpureus* and *G. antarctici* (Lovelock and Robinson 2002) would offer an alternative protection from UV-B though leading to lower productivity rates. UV-B exposure has been considered to be important in somatic mutation amongst mosses (Chown and Convey 2007), thus any protection from UV-B would be as a secondary consequence to other stress survival mechanisms as in the case of *S. glaciale*, a moss of limited distribution, but which appears to be tolerant to UV-B exposure.

D. antarctica has been considered to have originated from a migration event during the last interglacial period (Mosyakin *et al.* 2007). Based on this, it would be expected that the hair grass would be adapted to a higher UV-B environment as confirmed in the mature clonal tufts interpreted in a number of other studies (e.g. Rozema *et al.* 2001). The seedlings however were more responsive in spite of probably being genetically very similar to the clonal tufts, inferring that the plants show an inducible adaptation over their developmental lifecycle rather than be a genetically predetermined and constitutive one. The plants grown in this study were only limited by UV-B exposure and possibly by low PAR. Extrapolation to the field situation needs to be done with care, but it would be anticipated that any non-stress UV-B effect would be more responsive to other limiting conditions such as water or nitrogen, or indeed a combined synergistic response.

6.7 Small-scale laboratory work vs long-term field study

Possible criticisms of this thesis start with the source of the plant material. Comparison between results at Edmonson Point, and UK studies on the collected material demonstrate that there was no physiological damage to the plants as a result of transportation; hence the maintenance of this regenerated material was acceptable. The cryptogams obtained from Edmonson Point were, where possible, collected in a naturally frozen state for transport to UK. Maximal net O_2 evolution rates measured in the UK were slightly higher if expressed as a function of dry mass. However, this is probably as a result of the more favourable conditions in the growth cabinet in terms of sustained optimal temperature. The UK cultured mosses also kept their same Antarctic ranking with regard to net assimilation; with *B. argenteum* exhibiting the highest maximal rate. The grass plants were either regenerated clones from tufts brought back as 'cool stow' (0 to 4^{o}C), or grown from seed. The seed-grown plants did not experience this 'transportation stress'.

The radiation and temperature environment of the growth cabinets are important variables as it was not logistically possible to fully simulate the *in situ* Antarctic conditions. Thus criticisms can be levelled at the fact that the UV-B, UV-A and PAR ratios were not identical to those experienced by plants in the field. However, all of the critical responses described above have occurred at moderate UV-B fluence, which could be experienced in the natural environment. Thus mean daily UV-B radiation,

recorded at the Antarctic Peninsula, of 4.1 kJ m^{-2} (PAS) (Newsham *et al.* 2002) is comparable to 4.89 kJ m^{-2} (PAS), for 16 h exposure in the growth cabinet. Furthermore, low levels of PAR are thought to increase UV-B-induced damage (Adamse and Britz 1992), so any UV-B tolerant organisms found in this study should also be tolerant under Antarctic conditions of (relatively) high PAR.

The lack of UV-A radiation experienced under the UV-B opaque ('VE') cloche could also have altered the results in these studies. The UV-A boundary is however, purely arbitrary, so any photo-receptor or process that may require 320 to 400 nm radiation will most likely have radiation responses tail at < 320 nm and > 400 nm. Accordingly, there have been a number of studies that show no effect (Jackson and Seppelt 1997), an ameliorating effect (Quesada *et al.* 1995), an additive effect (Day *et al.* 1999), or an enhanced effect (Newsham *et al.* 1996), of UV-A radiation. In this regard, Day *et al.* (1999) showed the decrease in leaf length in *D. antarctica* was a component of both UV-B and UV-A exposure. However, another report from the same group utilising only UV-B alteration (Xiong and Day 2001), produced results equivalent to those of this thesis. Problems with use in a field situation with regard to temperature and water availability have already been mentioned, as have limitations regarding UV spectrum. However, since no single lamp and filter combination will adequately simulate ozone depletion, cloches can (as here) be used as a starting point, to further elucidate any 'UV effect' to the causative waveband. If that waveband is unaltered by ozone depletion then there should be no resultant effect on the species, or process, in question. Even with more appropriate controls, errors in appropriate weighting of UV-B exposure can still occur (Holmes 1997).

The relatively high temperatures used in the growth cabinet were also regularly attained on the growth surface of mosses in measurements taken during the fieldwork (Appendix 2) and in other studies (e.g. Newsham *et al.* 2002). It was not however feasible in the growth chambers to achieve lower temperatures (or freeze-thaw events) as a result of ice build up in the cabinet. Again, any alteration of photosynthetic activity resulting from UV-B exposure in the cabinet should be valid in the field, as the environmental conditions will be more extreme, and thus potentially more damaging.

6.8 Future work

It is unlikely that the area and depth of ozone thinning will decrease in the near future, and this work has shown that the concomitant increase in UV-B radiation could further limit the photosynthetic activity of the plants from the cold and frigid continental Antarctic. Future work needs to focus on a number of areas. Firstly, at a physiological level, with regards to the light dependent stage of photosynthesis; cause of F_{vm} depression needs explanation. The work by Rodrigues *et al.* (2006) would indicate that PSII is still a primary target of UV-B and thus depression in F_{vm} may still be expected. The conclusions from the growth cabinet data certainly need to be extrapolated to the Antarctic where soil nutrient status and condition, temperature range (including freeze-thaw cycles), and ambient PAR (and photoinhibitory events) can be achieved. The confounding effects of increased temperature and increased CO_2 levels need to be integrated; and this has been attempted by some workers (Tegelberg *et al.* 2008). This area requires urgent attention with respect to Antarctic species. There are a number of possible reasons for alteration in F_{vm}, and the lack of corroboration from the gas exchange data need fuller explanation. Furthermore, the similarity between UV-B responses in some species and photoinhibition need to be fully assessed. Lastly the variability in mechanism of F_{vm} depression and the recovery from UV-B exposure warrant investigation. The lowered $V_{c,max}$ in the hair grass needs to be extrapolated to whole plant studies to see if this could be part of the reason for reduced leaf length in this thesis and longer term studies (Day *et al.* 2001). A fuller understanding of the alteration in the leaf morphology and the effect on biomass is still needed, notwithstanding the work by Ruhland *et al.* (2005). Identification of the putative UV-B absorbing compounds needs to be carried out for cryptogams and to show, in the field, that these compounds are produced in response to decreased ozone levels. Moreover, with those plants found to be insensitive to UV-B exposure, the tolerance mechanisms need elucidation.

The metabolic cost of UV-B exposure needs fuller investigation; not just under ozone depletion, but also under normal ozone levels. There has been little work carried out in this aspect regardless of any studies on terrestrial Antarctic vegetation, notwithstanding the recent work of Snell *et al.* (2009). The impact of nutrient status is particularly pertinent. The soil on the continent is nutrient poor and thus response to UV-B may not be as extreme as shown in growth cabinet where nutrient limitation

should have been avoided. Again this needs to be de-convoluted. There has been some work on non-Antarctic species (de la Rosa *et al.* 2001), where an increase in nutrients caused a decrease in UV-B response by way of lowered accumulation of UV-B absorbing compounds. Riquelme *et al.* (2007) however reported an **increase** in UV-B absorbing compounds under conditions of **both** UV-B exposure **and** nitrogen deficiency. The work by Snell *et al.* (2009) estimated the production of a photoprotective anthocyanidin accounted for 1.85% of carbon fixation although the authors noted that, whilst the proportion may be small, its cumulative effect in the restrictive Antarctic environment may be greater.

Plant developmental stage can alter the UV-B response. The published work on *D. antarctica*, (e.g. Day *et al.* 2001, van de Staaij *et al.* 2002), did not use seedlings. Thus it is of interest to develop this aspect with longer term studies such have been reported in the Arctic (Rozema *et al.* 2006). Secondly these studies could be used to establish the history of ozone depletion as has been attempted using moss samples in both Finland (Huttunen *et al.* 2005) and, more recently, in the Antarctic (Ryan *et al.* 2009). This could be attempted using *C. purpureus* or *D. antarctica* harvested from herbarium samples currently housed by the various Antarctic research institutions.

The +PPFD action spectrum presented in this thesis again highlights the need for specific UV-B action spectra for a particular system as it is only really suitable to study specific plants and their individual or specific physiological response; so would be used with reservation on the hair grass as a result of the lack of a response of F_{vm}. If not however refuted, then it may lead to a re-appraisal of previous studies on certain cryptogams and changes in the calculation of biological effective doses in lamp-based supplementation systems. The alternative is to further increase the number of field studies carried out under actual ozone depletion in the Antarctic as exemplified by Newsham (2003) and Dunn and Robinson (2006). Furthermore, Newsham and Robinson (2009) also noted that a differing degree of response to UV-B between exposure protocols may have been as a result of using inappropriate weighting functions.

There is also the problem of possible increases in global atmospheric CO_2 concentration. The effects of increased CO_2 concentration have been shown to be altered by exposure to UV-B radiation (Sullivan and Teramura 1994), and CO_2 could itself have a more pronounced effect on the vegetation than the subtle effects of altered UV-B fluence. The consequence of lowered curvature of the A/c_i curve (Chapter 5) in *D.*

antarctica could, as previously mentioned, have implications with regards increases in global CO_2 concentration. Ambient CO_2 concentration is photosynthesis limiting, so plants would be able to photosynthesise at higher rates, and could overcome any UV-B-induced depression in $V_{c,max}$. In addition, the interactions between all three factors, UV-B, temperature and CO_2 concentration have yet to be investigated in the terrestrial Antarctic ecosystem.

The origin and spread of terrestrial Antarctic plants is subject to much debate and speculation (Convey *et al.* 2008); UV-B could have been a limiting factor with respect to dispersal and adaptation by, for example, screening DNA from UV-B exposure, a potential determining factor (Chown and Convey 2007), and needs to be evaluated. Moreover, the differing strategies of the bryophytes need to be fully ascertained and whether this is related to UV-B levels in the Antarctic or used to re-construct past levels of UV-B radiation. In this respect, it is important to elucidate the effects of UV-B exposure as a stress component in the context of whether the responses are inducible (facultative) or constitutive (Bradshaw and Hardwick 1989). In the case of the mosses it would be anticipated that relic species would exhibit a constitutive rather than an inducible response to UV-B which would be expected from cosmopolitan species.

Lastly, more research is needed to investigate the short-term diurnal effects of UV-B exposure as a stress component of ambient solar exposure regardless of ozone thinning (Paul and Gwynn-Jones 2003). Sullivan *et al.* (2007) noted diurnal changes in UV-B screening compounds, moreover, in an Antarctic context; Lud *et al.* (2001) also reported diurnal variation in CPD formation.

6.8 Concluding remarks

This series of experiments has shown that there are species-specific responses to UV-B exposure and that these responses depend on exposure wavelength and duration. This has also been found by other workers with no one typical response; there has been differing response to wavelength (Shinkle *et al.* 2004), and of species (Dunn and Robinson 2006). The experiments have also shown that UV-B radiation has the potential to act as a limiting influence on the growth and development of certain Antarctic species. It has also been demonstrated how rapidly the studied plants can respond to altered UV-B exposure. Moreover, it is important to produce species- and

response-specific weighting functions until (if possible) such a universal action spectrum can be constructed (Caldwell *et al*. 2007).

Early spring ozone depletion has been occurring for at least 20 years, therefore all the species in this study have had prior exposure, and thus a number of seasons in which to adapt to enhanced UV-B radiation. It is vital to determine what happened in the intervening years and the effect of pre ozone 'hole' UV-B radiation on terrestrial Antarctic vegetation before any definitive conclusions can be drawn.

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Proof

Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic

Pedro Montiel, Andrew Smith & Don Keiller

The effects of UV-B exclusion and enhancement of solar radiation on photosynthesis of the two phanerogams which occur in the maritime Antarctic (*Deschampsia antarctica* and *Colobanthus quitensis*), and the moss *Sanionia uncinata* were investigated. Data on air temperature and solar radiation illustrate a drastic seasonal variation. Daily O_3 column mean values and UV-B measured at ground level document the occurrence of the O "hole" in the spring of 1997, with a concomitant increase in UV-B. The grass, *D. antarctica*, exhibited a broad temperature optimum for photosynthesis between 10–25°C while photosynthesis did not saturate even at high irradiance. The high water use efficiencies measured in the grass may be one of the features explaining the presence of this species in the maritime Antarctic. The net photosynthesis response to intercellular CO_2 (A/c_i) for *D. antarctica* was typical of a C_3 plant. Exposure to a biologically effective UV-B irradiance of 0.74 W m^{-2} did not result in any significant change in either the maximum rate of photosynthesis at saturating CO_2 and light, or in the initial carboxylation efficiency of Rubisco. ($V_{c.max}$). Furthermore while ambient (or enhanced) solar UV-B did not affect photochemical yield, measured in the field, of *C. quitensis* and *D. antarctica*, UV-B enhancement did affect negatively photochemical yield in *S. uncinata*. In *D. antarctica* plants, exposure to UV-B at low irradiances elicited increased flavonoid synthesis. The observed effects of UV-B enhancement on the moss (decreased photochemical yield) and the grass (increase in flavonoids) require further, separate investigation.

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Summer climatic conditions in the Antarctic, together with the isolation from more northerly land-masses, restrict the vegetation in the maritime Antarctic to mosses, lichens, algae, cyanobacteria and to two vascular species: the pearlwort *Colobanthus quitensis* and the grass *Deschampsia antarctica* (Smith 1984). Evidence of climatic change in the Antarctic Peninsula includes an upward trend in summer air temperatures since the late 1940s (Smith 1994). Mean annual air temperatures have also increased by 0.022 to 0.067°C per year (King 1994). Since the mid-1970s there has been a marked thinning of the stratospheric ozone layer over the polar regions (Farman et al. 1985), which has continued throughout the 1980s and 1990s (Jones & Shanklin 1995). Climate change is likely to lead to shifts in species, communities and relative abundance of polar vegetation (McGraw & Fetcher 1992). Without detailed knowledge of species physiology and ecosystem properties, such shifts will be difficult to predict.

Monitoring populations of both Antarctic vascular species over a 27 year period has revealed a significant increase in numbers of individuals and populations at two separate localities in the maritime Antarctic (Fowbert & Smith 1994). In *D. antarctica* no specific adaptations to the Antarctic environment are evident in terms of reproductive strategies (Convey 1996), or the fatty acid composition of phospholipids and galactolipids in leaves and roots (Zuñiga et al. 1994). However, an inverse relationship of chloroplast to cell area index and temperature has been reported

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for *D. antarctica* along a climatic and latitudinal gradient (Jellings et al. 1983). More significantly, one previous study (Edwards & Smith 1988) suggests that photosynthetic rates in both species approach 30% of their maximum at 0°C. A much larger number of studies on the cryptogamic vegetation of the region exist, including photosynthetic measurements in both lichens (reviewed by Schroeter et al. 1997) and mosses (e.g. Davey & Rothery 1996).

Cellular responses to extreme fluctuations in solar radiation, temperature and water status are likely to be critical to plant competitive balance (Larcher 1995). In Antarctic terrestrial biota these relationships remain largely unexplored. The biological effects of UV-B on vegetation can be direct or indirect; direct effects include DNA photodamage and physiological effects (reviewed by Caldwell et al. 1995). Although direct damage of Photosystem II has been widely documented (Bornman 1991; Nedunchezhian & Kulandaivelu 1997), inhibition of photosynthesis by UV-B is more likely to be linked to CO_2 fixation (Baker et al. 1997). Photoprotective responses to UVR include structural modifications and increased synthesis of UV-B absorbing compounds (Caldwell et al. 1995; Rozema et al. 1997).

Research into the biological effects of increased UV-B in the Antarctic has focused mainly on marine ecosystems (see Karentz 1994; Goes et al. 1994; Riegger & Robinson 1997; Neale et al. 1998), with relatively few studies on photoprotective pigments of terrestrial macro-algae, mosses and cyanobacteria (e.g. Post 1990; Garcia-Pichel & Castenholz 1991; Post & Larkum 1993). The present study defined photosynthetic responses in *D. antarctica* and assessed whether the two vascular plants, and the moss *Sanionia uncinata*, were responsive to manipulation of the solar environment (chiefly UV-B exclusion and enhancement) under ambient conditions at Léonie Island.

Methods

Monitoring of solar radiation: At Rothera Station (67°34′07″S, 68°07′30″W), in the south-western Antarctic Peninsula, a Bentham DM 150 scanning spectroradiometer has measured spectral global irradiance since 1997. Measurements are made from 280 to 600 nm with a step size of 0.5 nm and a resolution of 1 nm. The same instrument is used to calibrate UV-A and UV-B sensors (Delta-T Devices Ltd., Cambridge, UK) and irradiance (Photosynthetically Active Radiation, PAR, 400–700 nm) quantum sensors (Skye Instruments Ltd., Powys, UK) which are part of a year-round, automated station at Léonie Island (67°36′S, 68°20′W), 9 km south-west of Rothera Station.

Field experimentation: All field experimentation was carried out on Léonie Island. The species selected included the two vascular plants (*D. antarctica*, *C. quitensis*) and the moss *S. uncinata*. These species are found co-existing in similar locations, are widespread along the Antarctic Peninsula and provide a representative contrast between vascular and cryptogamic life strategies. Plants were transplanted to a north-facing terrace, dominated by grass swards. Following acclimation (10 days), plastic screens were positioned to affect changes in solar UV radiation. The screens (Du Pont Polyesters Group, Middlesborough, UK) used included UV transparent (Perspex OXO-2), UV opaque (Perspex VE) and UV-B opaque but UV-A transparent (Melinex). UV-B enhancement (around 30% of background solar levels) was provided by a UV-B lamp (313 nm maximum, Cole-Palmer Instrument Company, London, UK) fitted with Sanalux glass panels to absorb UV radiation below 280 nm. The UV-B lamp was positioned in such a way so as to provide uniform UV-B enhancement over the test area, whilst not shading any plant material from sunlight. This enhancement (square-wave addition) was given for 5 h day^{-1} around solar noon (13.00 local time). The UV-B lamp (115 VAC, 60 Hz) was switched on only when irradiance exceeded 300 μmoles m^{-2} s^{-1}. Such an arrangement allowed a total of five contrasting treatments, namely ambient (direct sun), UV transparent, UV exclusion, UV-B exclusion only and UV-B enhancement.

Controlled environment experiments: Plants (*D. antarctica*) were collected at Signy (60°43′S, 45°38′W) and Léonie islands and transported to the BAS headquarters (Cambridge, UK) where they were acclimated in growth chambers prior to experiments. Chamber temperatures were 15°C/7°C for daily 16 h day/8 h night cycles, respectively. These conditions were used throughout, with plants kept inside growth incubators under modified covers made from the same UV

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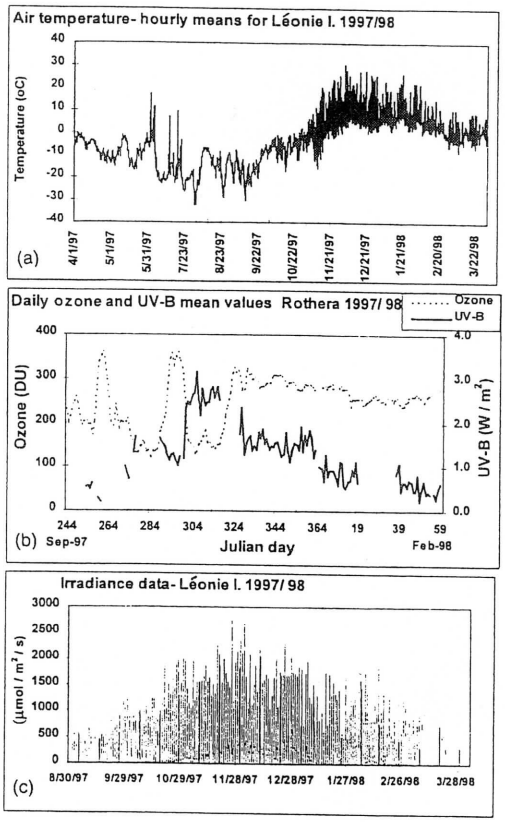

Fig. 1. Seasonal fluctuation in temperature, ozone and solar radiation at Rothera Station and Léonie I., 1997–98. (a) Air temperatures (20 cm above ground) are hourly average of readings taken every 10 min (April 1997–March 1998). (b) Ozone column values and UV-B irradiance fluences (derived from scans from the Bentham spectroradiometer) are daily means (September 1997–March 1998). (c) Irradiance (photosynthetically active radiation, PAR) are values (10 min intervals) from a quantum sensor (400–700 nm) (September 1997–March 1998).

transparent and opaque acrylic plastics as employed in the field studies. Typical irradiance in the growth cabinets was 150 µmol m^{-2} s^{-1}, at plant level, while UV-B and UV-A was maintained at around 0.4 and 5.1 W m^{-2}, respectively, with a daily weighed ("generalized plant action spectrum" by Caldwell [1971]; parametrized by Thimijan et al. [1978]) dose of 4.89$_{BE}$ kJ m^{-2} day^{-1} for a 16 h day^{-1} cycle. Although the total irradiance in the growth cabinets was low compared to peak irradiance in the field (Fig. 1c), the ratio of UV-B/UV-A/PAR was similar to that measured in the field, in the absence of significant ozone depletion. It must be noted that in the Antarctic the high seasonal variation in irradiance (Fig 1; see also Davey & Rothery 1996) combines with daily variation; high UV-B irradiances can occur at low irradiances during ozone hole events in early Spring (Webb 1997).

Photosynthetic gas exchange for D. antarctica: A Ciras-1 infra-red gas analyser (PP Systems Ltd., Herts., UK) was used, together with a microprocessor controlled cuvette which allowed complete control of leaf micro-environment variables (CO_2, temperature, irradiance and humidity). The Ciras-1 system allows simultaneous recording of photosynthetic and transpiration rates. Only the grass proved suitable for the automated cuvette. Water use efficiency (WUE) was calculated from CO_2 and H_2O exchange rates (Nobel 1991). Steady-state photosynthetic rate (measured after 7–8 min. equilibration for temperature and light response curves) was determined at an air flow rate of 300 ml min^{-1}, 345 ppm CO_2, (unless A/c_i curves were collected) and 80% relative humidity (5–6 mB). Attached *D. antarctica* leaves were used throughout. Temperature response curves (expressed as % of maximum rate achieved per plant, so as to eliminate interplant variation caused by different leaf mass), were obtained (10–15 February 1998) from eight plants from three contrasting habitats: full sun (up to 1800 µmol m^{-2} s^{-1}), partial sun (up to 1200 µmol m^{-2} s^{-1}) and shade (up to 250 µmol m^{-2} s^{-1}). Irradiance was maintained at 1500 µmol m^{-2} s^{-1} while cuvette and leaf temperature was increased stepwise from 2°C to 30°C and decreased similarly from 30°C to 2°C. Light response curves were also measured using plants from the same contrasting environments; irradiance was increased stepwise from 0–1500 µmol m^{-2} s^{-1}, following a 15 min dark acclimation, whilst leaf temperature was maintained between 15°C and 20°C.

Photochemical efficiency: Chlorophyll fluorescence was used to monitor PSII photochemistry in undisturbed plants exposed to the different UV treatments. Measurements were made in the morning and again towards the end of the day period in order to evaluate any possible interaction between UV treatments and extended exposure to high irradiance. A portable OS100 modulated chlorophyll fluorometer (Opti-Sciences Inc, MA, USA) was used to obtain

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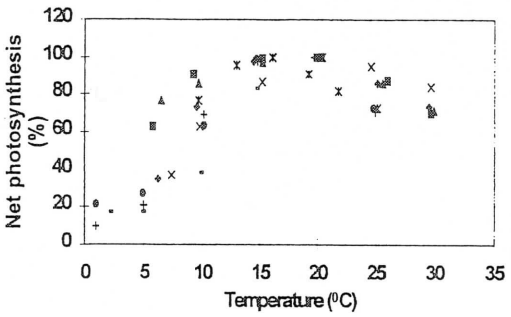

Fig. 2. Temperature response curves for net photosynthesis (% of maximum rate achieved per plant;10–15 February 1998) in *D. antarctica* plants (N = 8) from contrasting habitats, i.e. full sun-exposed, partial sun-exposed and shade. Irradiance was kept at 1500 µmol m^{-2} s^{-1} while cuvette and leaf temperature was increased stepwise from 2°C to 30°C and decreased from 30°C down to 2°C. The different symbols represent measurements for replicate plants.

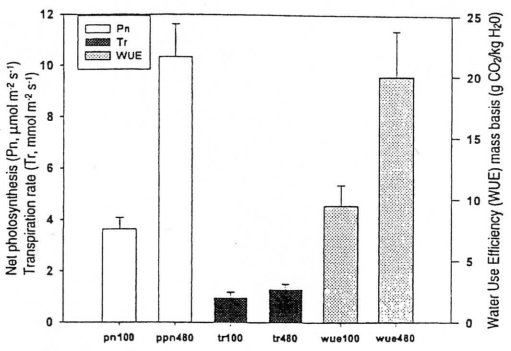

Fig. 4. Water Use Efficiency (WUE) for *D. antarctica* at two irradiance levels (100 and 480 µmol m^{-2} s^{-1}) determined from net photosynthesis and transpiration data. Plants were collected from field locations and acclimated to growth cabinets. Growth cabinet conditions: temperatures of 15°C/7°C for daily 16 h day/ 8 h night cycles, respectively.

steady-state yield values from undisturbed plants under field conditions. The fluorometer was used with a modified, 65° open-body cuvette guide and an irradiance (PAR) sensor. Transplanted plants were "tagged" so that positioning of the fluorometer fiber optic tip on the same spots (4–5 per treatment) was reproducible throughout the experiments. Relative amplitude of the modulated light was set at 60 (vascular plants) and 70 (the moss) with a 0.8 s pulse duration.

Total flavonoid analysis by HPLC: Gradient HPLC analysis of flavonoids was performed on a Prodigy ODS3 column (Phenomenex, Cheshire, UK), at 30°C with diode array detection. Plant tissue was ground in a pestle and mortar with cold 50% aqueous methanol containing 0.5% (v/v) glacial acetic acid, after which the extracts were centrifuged and passed through 0.45 µm filters. Mobile phases included ammonium dihydrogen phosphate (pH 2.5) and absolute acetonitrile (Lunte 1987).

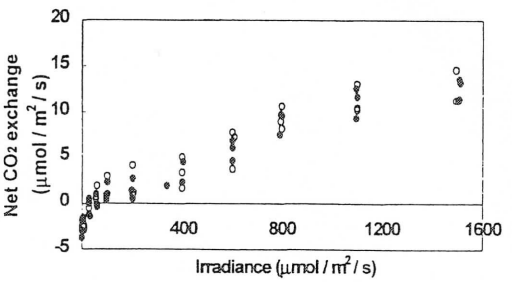

Fig. 3. Light response curves for *D. antarctica* plants from contrasting habitats (open circles = full sun-exposed; filled circles = shade). Irradiance was increased stepwise from 0–1500 µmol m^{-2} s^{-1} (dark acclimation for 15 min). Leaf (and cuvette) temperature were kept between 15°C and 20°C.

Results

Data on air temperature (20 cm above ground) and solar radiation illustrate a drastic seasonal variation (Figs. 1a, c). Daily O_3 column mean values and UV-B (averaged from the Bentham scans) illustrate the occurrence of the O_3 "hole" in the spring of 1997, with a concomitant increase in UV-B during November 1997 (Fig. 1b).

Photosynthesis measurements showed that *D. antarctica* had a broad temperature optimum (approximately 90% of the maximum) between 10–25°C (Fig. 2).

Photosynthetic rate response to irradiance revealed no saturation at high irradiance even in plants from shaded environments. Similarly there was very little difference in light compensation points between plants collected from the contrasting habitats (Fig. 3).

Apparent water use efficiency (WUE) was very high, with values ranging between 62 and 123 mol H_2O per mol CO_2; typical values for C_3 species are between 300–500 mol H_2O per mol. Such efficiency, if confirmed in the natural environment, may partially explain the capacity of this species to

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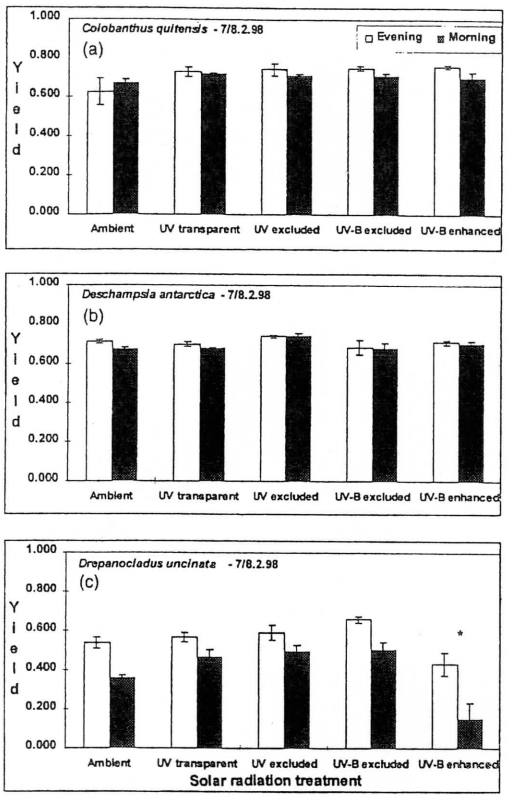

Fig. 5. Steady-state yield fluorescence parameter determined in the natural environment for (a) *C. quitensis*, (b) *D. antarctica* and (c) *S. uncinata*, following 7 days exposure to ambient conditions, total UV radiation exclusion, selective UV-B exclusion and UV-B enhancement (7–8 February 1998). Means ± 1 SE are given. Measurements were carried out in the morning (10.00 h, filled bars) and evening (19.30 h, open bars). Statistical significance (*t*-test, one-tail) is indicated (* $P < 0.5$) for the comparison of treatments vs ambient control.

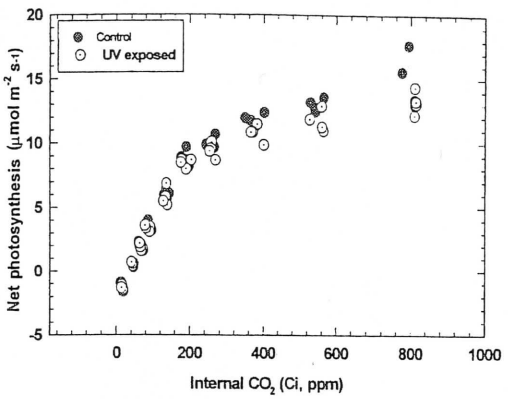

Fig. 6. A/c_i response curves (net photosynthesis response to intercellular CO_2 concentration) for *D. antarctica* plants, maintained in controlled environment cabinets, and acclimated to UV-B exclusion or exposure (biologically effective irradiance of 0.74 W m^{-2} UV-B). Steady-state photosynthesis (measured after 7–8 min equilibration) was determined at an air flow rate of 300 ml min^{-1}, 345 μl l^{-1} CO_2, and 80% relative humidity (5–6 mB). Attached *D. antarctica* leaves were used throughout.

survive in the cold semi-desert conditions of the maritime Antarctic (Fig. 4).

Chlorophyll fluorescence is used routinely as an intrinsic probe of photosynthetic function and as a screening tool for environmental stress tolerance, e.g. low temperatures (Öquist & Huner 1993), dehydration (Casper et al. 1993), and UV-B (Vassiliev et al. 1994). For both phanerogams the data suggest that following an initial transient reduction in PSII yield, induced by UV-B enhancement (data not shown), there was no significant change in PSII efficiency (Figs. 5a, b). In contrast, a significant and sustained decrease in photochemical yield was recorded for the moss *S. uncinata* when exposed to enhanced UV-B (Fig. 5c).

UV-B enhancement had little or no effect on the net assimilation response to intercellular CO_2 concentration (A/c_i) in *D. antarctica* (Fig. 6).

HPLC analysis of *D. antarctica* shoots kept in controlled environments at low irradiances showed that total flavonoid were increased by exposure to UV-B, with some flavonoid species showing marked increases (peaks 1, 2, 3 and 7 in the overlay chromatograms; Fig. 7). The identity of these flavonoids requires further investigation.

Discussion

D. antarctica exhibited a typical C_3-type response to temperature with a broad optimum. Optimal temperatures for net CO_2 uptake are usually between 20°C to 35°C for C_3 plants (Nobel 1991); thus the lower optimum for *D. antarctica* could be regarded as a specific adaptation. A wide optimum has the advantage that large daily fluctuations in temperature result in only small changes in photosynthetic rate (Larcher 1995). The data also confirmed that *D. antarctica* can sustain net photosynthesis (15–25% of maximum rate) at temperatures approaching 0°C (Edwards & Smith 1988). The response of net photosynthesis to irradiance indicate that light saturation did not occur at high irradiance even when using plants

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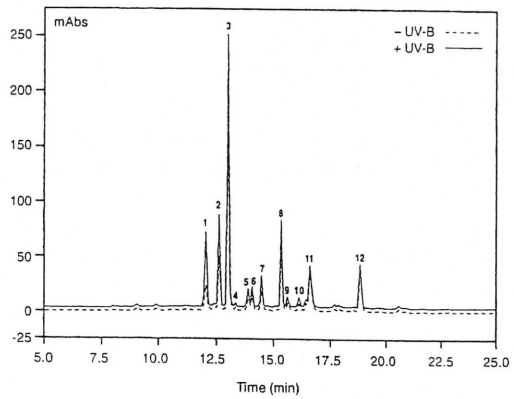

Fig. 7. Deschampsia antarctica. Overlay of chromatograms from two flavonoid extracts from tillers grown under conditions of low irradiances and where UV-B was excluded (−UV-B) or allowed through (+UV-B) for 17 days. Plants were grown at 15°C/7°C for 16 h day/8 h dark daily cycles. Extracts were prepared using similar amounts of leaf material (around 20 mg fresh weight) and injection of 60 µl from equal dilution volumes.

from shade habitats. A high saturation point may allow fuller exploitation of the high irradiance levels experienced in the region during the growing season (Fig. 1c), and may also serve as a protective mechanism against photoinhibition (Long & Humpries 1994).

The gas exchange data, A/c_i response curves in particular (Figs. 2, 6), confirm that *D. antarctica* uses C_3-type photosynthesis (Sage 1994). However instantaneous water use efficiency (WUE) values were markedly higher than expected for a C_3 grass. Comparable WUE values have been reported in both C_3 and CAM succulents, from the Southern Namib desert, suggesting that some C_3 plants can achieve high WUE values (Eller & Ferrari 1997). It is hypothesized that the high WUE of the hair-grass may play an important role in the successful performance of the plant in an environment where free water is restricted.

The finding that UV-B exposure did not effect PSII photochemical yield in either vascular species (Fig. 5a and Fig. 5b) concurs with the emerging consensus that PSII damage is only manifested at high and unrealistic UV-B exposures (Allen, McKee et al. 1997; Allen, Nogués et al. 1998). Similarly, exposure to a biologically effective UV-B irradiance of 0.74 W m^{-2} (Caldwell-weighted), at a relatively low irradiance in growth cabinets, did not result in any significant change in either the maximum rate of photosynthesis at saturating CO_2 and light (J_{max}), or in $V_{c.max}$. In contrast, Baker et al. (1997) reported significant reductions in both these parameters at UV-B irradiances of 0.63 W m^{-2}. The responses of the vascular plants, in terms of photosynthesis and photoprotective pigments, to exclusion/enhancement of solar UV-B, indicates that current UV-B levels, experienced by *C. quitensis* and *D. antarctica* during the growing season, may not constitute a direct threat to photosynthetic activity. Furthermore, because of snow cover these species are unlikely to experience the elevated UV-B levels occurring during the spring O_3 depletion event (Fig. 1c). The negative effect of UV-B enhancement on photochemical yield in the green moss *S. uncinata* requires further evaluation (Fig. 5c).

This study has shown that exposure to enhanced solar UV-B irradiance elicited increased flavonoid production in *D. antarctica* (Fig. 7), thus sequestering energetic resources. Vegetation of the maritime Antarctic are slow growing, and subjected to numerous abiotic stresses, thus photoassimilate allocation may prove critical to survival. In Antarctic ecosystems particular attention should be paid to indirect plant responses to enhanced solar UV-B radiation; these are likely to affect competitive balance in species at the limits of their survival, with possible implications to biodiversity within ecosystems (Caldwell et al. 1995). It has been suggested that the current trend towards warmer growing seasons in the region will result in increased colonisation by vascular plants (Fowbert & Smith 1994). The results of this study support this hypothesis, as photosynthesis in these species appears to be well-adapted to current levels of solar irradiance and UV radiation.

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BIOTEX FIELD REPORT

December 1995 - February 1996

PHOTOSYNTHETIC RESPONSE OF PLANTS TO AMBIENT AND CONTROLLED CONDITIONS AT EDMONSON POINT AND SURROUNDING AREA, VICTORIA LAND, ANTARCTICA

A. E. SMITH

Participants

United Kingdom
Sandor Ertz (BAS; plant biochemistry, photo protective pigments)
Nicholas Russell (Wye College, University of London; microbial biochemistry)
Ronald Lewis-Smith (BAS; plant ecology, bryophytes, lichens)
Andrew Smith (Anglia Polytechnic University; plant physiology)
David Wynn-Williams (co-leader, BAS; microbial ecology)

Italy
Roberto Bargagli (co-leader, Universita di Siena; pedology, soil chemistry)
Franco Bersan (Universita di Trieste; fungi and lichens)
Paolo Cavacini (Universita di Roma; algal ecology)
Francesco Frati (Universita di Siena; *Collembola* population ecology and genetics)

United States of America
Diana Freckman (Colorado State University, Fort Collins; nematode ecology)

Maps of the region covered and the localities visited are provided at the end of this report (see Appendix 1 & 2)

A. E. Smith (BAS Report No. BIOTEX/1995/NT4)

PREFACE

This work was undertaken as part of my PhD research ("*Effect of enhanced UV-B radiation on photosynthetic metabolism in Antarctic plants*") being carried out at Anglia Polytechnic University (APU), Cambridge. Originally, my project was to be laboratory-based using plant material provided by the British Antarctic Survey (BAS), Cambridge. However, about eight months after the commencement of my research, and two months before the BIOTEX 1 expedition to Victoria Land, Antarctica, was due to start, two plant physiologists from Australia and New Zealand withdrew. So as to not lose this component of this international research programme, the co-ordinators of BIOTEX gave me the chance to participate at very short notice, and APU very kindly provided me with a return airfare to New Zealand. Since this would allow me a unique opportunity to carry out field research related to my project, I willingly accepted. Part of my brief was to field test, evaluate and report on some recently developed equipment purchased from PP Systems by BAS, and which will be used in planned future Antarctic research. Many of the problems encountered with this equipment were due largely to the fact that there was insufficient time, between acquiring it and my departure to the Antarctic, to test it (about forty-eight hours were available between arrival of the prototype equipment and the deadline for delivery to the shipping office). It was appreciated, both by myself and BAS, that there was a considerable risk in undertaking this work with untested equipment, but I was confident and determined I would succeed. However, for this reason, not all the data obtained with this equipment may be suitable for inclusion in my PhD. A separate report has been produced (see Appendix 1) for PP systems with recommendations for modifications and improvements for future use, especially with Antarctic plants.

Summary of aims and objectives of A. E. Smith field programme

Aim: To examine, by means of oxygen evolution, carbon assimilation and chlorophyll *a* fluorescence, the photosynthetic response of selected terrestrial plants to ambient and controlled conditions at Edmonson Point and surrounding areas of Northern Victoria Land, continental Antarctica.

Objectives:

1. Evaluation of Field Analytical Equipment

(a). Assessment of the efficacy of the newly purchased portable infra-red gas analyser (IRGA), "CIRAS" (Combined Infra Red Analysis System, PP Systems Ltd, Hitchin, UK) in both general field use and, specifically, in operation with four types of recently constructed sample chambers (cuvettes).

(b). Evaluation of a portable modulated fluorimeter "OS-100" (Opti-Science, USA), both as stand-alone field equipment and in combination with the IRGA.

(c). Evaluation of a combined Hansatech oxygen electrode/fluorescence system in studies with selected mosses.

2. Experimental Physiology

(a). *In situ* and laboratory experiments.

To obtain gross physiological data for a variety of plants present at Edmonson Point and surrounding localities, both by *in situ* use of the IRGA and fluorimeter, and in controlled laboratory conditions using the combined oxygen electrode and fluorescence apparatus.

(b). UV effects

To monitor physiological effects of UV exclusion on selected plants by use of UV transparent (OXO) and UV opaque (VE) acrylic cloches.

Deployment of the above cloches for over-wintering over selected mosses growing under varying environmental conditions.

3. Short-term measurement of micro-climate conditions within selected cloches using Skye PAR, UV-A and UV-B sensors and Rotronic temperature/humidity sensor feeding to a Campbell CR-10 datalogger.

4. Collection of a variety of plant specimens for further work under controlled laboratory conditions in Cambridge.

Diary of activities

December 1995

5 Flight from Christchurch to Stazione Baia Terra Nova (Terra Nova Bay, TNB) by Italian C-130.

6 Helicopter to Edmonson Point (EP) field camp, brief inspection of local area, pm return to TNB.

7 To EP, setting up camp, tent fire, further inspection of site, pm return to TNB.

8 Set-up of Hansatech equipment at TNB, problem with thermocouple-oxygen electrode joint.

9 To EP, unpack IRGA and fluorimeter, preliminary testing hampered by lack of cuvette seal.

10 Return to TNB to collect more equipment for assistance with cuvette leakage.

11 Work with oxygen electrode, still joint problem.

12 Return to EP, continued testing of cuvette, set up CR-10 datalogger -faulty.

13 Continued testing on CIRAS cuvette, set up fluorimeter, problems with OSLOG program.

14 Leak testing on CIRAS cuvette.

15 Helo to Harrow Peaks, sample collection, some fluorescence measurements taken on selected lichens, return via EP North.

16 Work on IRGA, leak appears to improve (cuvette window not fully sealed).

17 Helo to TNB, send FAX concerning datalogger and CIRAS difficulties.

18 Work on oxygen electrode, try chlorophyll extractions on selected mosses.

19 Return EP, checked ambient CO_2 with CIRAS, functioning OK.

20 Arrival of 2nd party, CIRAS field test, CHECKSUM error occurs for first time, return to field camp to remedy fault.

21 Helo to Baker Rocks en route to Harrow Peaks sampling, return via Mt McGee.

22 CIRAS provisional field-work tests with *Ceratodon* cores by meltwater stream.

23 CIRAS field test on *Bryum argenteum* near meltwater stream (to be first cloche site, Site 1), close proximity to both penguin and skua activity.

24 Data sorting, reply to datalogger query had arrived, but information was incomplete, re-sent original request. Return to TNB for festive activity.

25 Return to EP.

26 Sampled *B. argenteum* at Site 1, CIRAS OK (return to field camp to collect more batteries).

27 Choosing and set up of UV+/- acrylic sheets over *Xanthoria elegans*.

28 Helo to Kay Island, set up of cloches over various lichen groups.

29 Helo to Gondwana station lake, sample collection, some fluorescence measurements taken. pm; CIRAS work on 1st cloche site, CHECKSUM error occurs twice, resulting in loss of all data for site.

30 Set up of *Umbilicaria* exposure expt at EP, samples collected from Kay Island.

31 Check existing sites for skua interference, data sorting, try lichen desiccation experiment.

January 1996

1 Sample sorting and data organising.

2 Work on Site 1, CIRAS crashes again with all data being lost, decide to stop work using CIRAS until can confirm crashes not harming CIRAS circuitry.

3 Return to TNB to fax PP Systems re-problem, and for BIOTEX lecture, leak testing on oxygen electrode and work on mosses.

4 am; Helo visits to Inexpressible Island and Cape Sastrugi, sample collection. pm; work with oxygen electrode and chlorophyll extraction trials.

5 Oxygen electrode work, joint problem finally resolved for electrode.

6 Oxygen electrode work with *Ceratodon*. Received complete wiring diagram for CR-10 datalogger.

7 am spectrophotometer work, then return to EP (still awaiting fax reply).

8 Try out IRGA again (Site 1), appears to function correctly, cores that were taken some have dried out. Tried out one core of *Bryum pseudotriquetrum*.

9 CIRAS work on new *B. pseudotriquetrum* patch (Site 2), close to Site 1, crashes again (4th time) reboot then continue, late pm; sampling trip to Harrow Peaks.

10 More sampling with CIRAS on Site 2, then placed two OXO cloches over the new site. Receive Fax (via e-mail) from PP Systems, return to TNB to carry out circuit test with suitable multimeter (loan HNZ), pm attend RILS lecture, oxygen electrode work with "Met" site samples.

11 At TNB, continued work on *C. purpureus* from Met. site.

12 Return to EP with new high power battery, trials of datalogger, now functioning satisfactorily, terminated Site 1 (too dry).

13 Sample collection, datalogger trial continuing, tried sample of *Buellia frigida* in CIRAS (with palmtop computer now connected to prevent loss of data) no substantive response.

14 Departure of 2nd party, re-organisation of camp. Collected samples for isolated core experiment (expt 3), carried out Fvm on samples. Leakage problems associated with extra equilibrator so was removed.

15 Expt 3; carried out gas exchange work using CIRAS.

16 Work on Site 2, sampling using CIRAS, noted flickering of palmtop screen, also battery life extended but not as long as expected, swapped one OXO cloche for VE.

17 Due to possible problems with excessive moisture, replaced extra equilibrator as safety measure. Tried taping ends on connection to solve leakage, appeared to work. Tried CIRAS P(blc) at sub-zero temps, minor problems.

18 Problems with erroneous recording with CIRAS at supra ambient CO_2 levels. Tested *X. elegans* in P(blc) too small for significant response (temperature was sub zero for recording).

19 Expt 3; performed Fvm. Return to TNB some work on *Tortula princeps*.

20 Return EP, yield for Site 2, glued beaker over crustose lichen for new trial.

21 Re-checked extra equilibrator, noticed loose connections with internal tubing, re-inserted and smeared with silicone grease. Trial of combined cuvette and fluorimeter stability and leakage problems.

22 Expt 3; work using CIRAS.

23 Helo from EP to Gondwana for CIRAS trial in two modes, but persistent zeroing problem with leakage around cuvette (warped beaker), sample collection. Late pm; electrode work with recently collected *T. princeps*.

24 Return to EP, watered Site 2 (dried out).
25 Work on Site 2, CIRAS crashes again, this time when reboot, the PAR sensor does not function. Return to field camp, sensor finally responds to another rebooting, data acquired, was saved on palm-top computer.
26 Helo to Kay Island, collection of samples from experiments set up at an earlier date, then to Harrow Peaks for further sample collection, return to TNB for oxygen electrode electrode work on fresh samples.
27 TNB work with oxygen electrode.
28 Oxygen electrode work (too windy to return to EP).
29 Oxygen electrode work moss core expt blown away, replaced by Ertz.
30 Moss core expt blown away again most samples lost, expt aborted. am try to fly to EP abandoned. Oxygen electrode work.
31 Oxygen electrode work.

February 1996

1 am electrode work, pm; return to EP, dismantle expt 3.
2 Helo to Cape King for sample collection, trial of CIRAS/fluorimeter combined system; stability problems.
3 Fluorimeter work on Site 2, pm return to TNB, oxygen electrode work.
4 Oxygen electrode work.
5 Return EP, final fluorimeter readings for Site 2. Set up over wintering cloche sites, sample collection, pack up camp.
6 Finish cloche set up, complete camp clearance, pm return TNB, oxygen electrode work.
7 Oxygen electrode work. Helo to Inexpressible island sample collection.
8 Oxygen electrode work. pm; pack up equipment, clean laboratory.
9 Finish cleaning lab, pm; assist in general base cleaning tasks.
10 Base cleaning tasks.
11 Finish base cleaning tasks, Helo around TNB, closing ceremony, to mv *Italica*.
12 mv *Italica* sailed.
13 Onboard mv *Italica*. Report writing for ENEA.
14 Onboard mv *Italica*. Report writing for ENEA.
15 Onboard mv *Italica*. Report writing for ENEA.
16 Onboard mv *Italica*.
17 Onboard mv *Italica*.
18 Onboard mv *Italica*.
19 Dock Lyttleton, NZ, am.

Experimental Work

1. Evaluation of Equipment

Unintentionally, this aspect of the work took up a significant amount of the two month field programme, delaying experimental work either directly by faults with equipment, or indirectly by waiting for corrective procedures to be sent.

1.1 CIRAS

The major approach was use of the IRGA with the custom cuvette. For full report see Appendix 3.

To summarise, whilst showing potential for both field and laboratory deployment, a number of difficulties were experienced, attributed to both the prototype design and instrumentation, and to the field environment. These need to be addressed, as does a more thorough evaluation of all four modes of operation, both in the laboratory and also in conditions which are likely to be experienced in the Antarctic Peninsula region. Tables 1 and 2 give a list of plant species used and a sample of data obtained for *Bryum argenteum*.

1.2 OS-100 modulated fluorimeter

See Appendix 3 for details.

As with the IRGA, the fluorimeter did demonstrate its value but again there were both instrument-based and environmental problems. The initial fluorescence ratio (Fvm) is obtained by the expression Fm-Fo/Fm; Fo is the minimal dark fluorescence where all Photosystem II (PS II) reaction centres are open in a non-energised state, obtained when switching on the modulated fluorimeter after a dark period. Fm is the maximal fluorescence level, when all PS II reaction centres have been closed by applying a saturating light pulse after Fo has been measured. The Fvm value gives an indication of photosynthetic competence of the plant, with a typical value for vascular plants being 0.7 - 0.8. The fluorescence yield F'm-Fo/F'm (where F'm is the light adapted maximum fluorescence level), is correlated to quantum yield and is thus an indicator of the photosynthetic rate. Fvm and yield values for lichens were generally lower than those for mosses. Mosses generally required about 40% of the maximum modulation intensity (MI, intensity of the modulated fluorescence measuring beam) whilst the lichens usually needed 80% or more of the MI to sustain a clear signal and to attain the minimum fluorescence intensity of 40 tics (arbitrary units of fluorescence signal recorded by the OS-100 detector) required to trigger the saturating pulse. A preliminary trace, showing the regain of chlorophyll *a* fluorescence on re-wetting after a period of desiccation in the lichen *Xanthoria elegans*, can be seen in Fig. 1. A "spike" can also be seen on the trace, the specific cause of these spikes is as yet unknown.

1.3 Combined oxygen electrode/fluorimeter

Although I have undertaken several studies using this technique on Antarctic lichens and algae at Cambridge, it is the first time that I have used it on Antarctic bryophytes.

The oxygen electrode is based on an aqueous electrochemical cell, when the oxygen is dissolved through the medium there is a proportional change in current over a certain range.

By using a sealed container, it is possible to measure rates of oxygen exchange of the plant. With addition of a modulated fluorimeter that is able to resolve quenching components of chlorophyll-a fluorescence, a powerful system is thus operable. When coupled with light response analysis, then even more parameters can evaluated: Blackman relationship of idealised photosynthesis; Genty plot expressing the relationship between F'm-Fo/F'm and apparent quantum yield; and quantum requirement (see Fig. 2 for example of data that can be produced).

After initial polarisation of the electrode (which can take up to two hours to provide a steady base-line), and checks for gas-tight seals, the system is then operable. With a 15-stage light response and a 30 min. dark period, a recording can take approximately one to one and a half hours. Temperature adjustment can take about one hour to change, depending upon the differential from the ambient temperature.

After an initial problem with the thermocouple joint, there were no other significant troubles, with data being obtained for several moss species.

1.4 Summary and Conclusion

The combining of fluorescence and gas-exchange measurements can create a powerful physiological tool, but systems need to have been fully evaluated before being deployed in the field if one wants to exploit full value from the data acquired, and also for it to be used most efficiently.

The test period before deployment of the CIRAS and OS-100 at Terra Nova Bay was brief and this subsequently lead to problems with the equipment. This inturn caused delays in the start of fieldwork.

Reasonable amounts of data were gained from the oxygen electrode over a relatively short period. There were no novel faults and it performed efficiently.

Poor communications between Terra Nova Bay and BAS did not help in the fault finding exercises, exasperating the situation when new faults occurred and rapid communication was essential.

2. Physiological data

Physiological research on Antarctic cryptogams has been restricted to a few species at a few sites and undertaken by very few researchers. The present work concentrated on the mosses and also made use of the UV cloches, used by other members of BIOTEX which were sited over several moss stands.

Samples have also been collected to continue and extend the work that was initiated during the field trip. See R. I. Lewis-Smith report Nº BIOTEX/1995/NT1 for an account of community composition and environmental conditions.

2.1 Mosses

Table 1 lists species that were sampled at Terra Nova Bay/Edmonson Point.

2.1.1 Experiments using CIRAS/fluorimeter

This was done *in situ,* with the work concentrating on the three dominant mosses; *Ceratodon purpureus, Bryum argenteum* and *B. pseudotriquetrum.* No supplementary light source was available, so the photosynthetic rates generally varied according to local photosynthetically active radiation (PAR) conditions. Rates per unit area did vary between species, generally *C. purpureus* < *B. pseudotriquetrum* < *B. argenteum,* as expressed per unit area; typically with rates of 2 to 3, 3 to 4, and 7 to 8.5 μmol m^{-2} s^{-1} respectively together with dark respiration of -5 to -8, -4 to -15, and -10 to -14 μmol m^{-2} s^{-1} respectively, based on PAR of c. 1800 μmol m^{-2} s^{-1}. Also to be noted is the rapidity of the induction of dark respiration in all species, being initiated typically after one minute of covering (Fig. 3). The CO_2 gush (that may be noted in C3 plants when undergoing a rapid change from the light to dark), was not immediately apparent in these mosses. The CIRAS recording function is only capable of one minute resolution and was not able to fully record the responses that were observed.

The Fvm ratio was again typically highest for *B. argenteum* then *B. pseudotriquetrum* and finally for *C. purpureus.* However, as can be seen in Fig. 4 the Fvm value for *C. purpureus* rose above that of *B. pseudotriquetrum* after samples had been placed under either OXO or VE cloches for five days.

This work will continue with more species in the controlled growth chambers in Cambridge. More attention will be paid to the physiological state of the specimen when sampling (hydration state, age etc). Also the photosynthetic rates need to be expressed by other ways than area to see if this differential between species is maintained.

2.1.2 Oxygen electrode/fluorimeter work

Note: pigment extractions have still to be completed so rates are only an indicative value and not absolute, unless otherwise stated.

Most work was carried out using this equipment, to produce light response curves. A dark period of 30 min was assumed to be sufficient to allow for complete relaxation of PS II reaction centres. The mosses sampled are listed in Table 1.

Experiments were carried out where possible using material that was as fresh as possible; this often entailed returning directly to Terra Nova Bay after a sampling session.

Experiments were carried out on a number of species at approximately 5, 10, and 20 °C, respectively. The data provisionally appear to show a substantial reduction in photosynthetic rates at lower temperatures, but Fvm data for *Sarconeurum glaciale* was slightly higher at 5°C than at 10°C, this also occurred with *C. purpureus* from the "Met." site cloche samples. These differences in Fvm were slight and would need further work for corroboration.

A short freezing test was carried out using small samples of *Tortula princeps* and *S. glaciale* which, after sampling were either frozen hydrated or in a dry state. Results showed that freezing whilst wet caused complete dysfunction of the photosynthetic capability, with Fvm values for *Tortula princeps* dropping from 0.7 (with a typical light response curve before freezing), to between 0.15 and 0.51 and with no significant net oxygen evolution for the samples that had been frozen whilst moist (fig. 5). Samples that had been slowly dried before

freezing exhibited no apparent differences in either oxygen evolution or Fvm. The hydration state of the sample may therefore be an important consideration when collecting samples to freeze for work in the UK.

Comparative studies on different moss species were also carried out. The Fvm for a number of species were usually in the range of 0.7 to 0.8, which is typical for a healthy vascular plant. *T. princeps, S. glaciale, B. pseudotriquetrum, B. argenteum* and *C. purpureus* appeared to behave reasonably well with the equipment, whilst *Pottia heimii, Grimmia lawiana* and *Pohlia nutans* all had less stable rates of photosynthesis as inferred by their erratic light response analysis and lower Fvm values. This may have been due to the state of the samples at the time of testing. Further work needs to be carried out to fully assess these early differences. The work on *P. nutans* is especially interesting for comparison as the moss was obtained from heated ground associated with fumaroles at c. 2,500 m altitude on Mt Rittmann.

2.2 Lichens

2.2.1 Work using CIRAS/fluorimeter

Little work was carried out on lichens using CIRAS. Wetting and drying studies attempted to correlate possible photosynthetic rate, with hydration status as expressed by sample mass, but were incomplete and will be continued at Cambridge with the samples that were collected. Fluorescence measurements were made *in situ* on a number of lichens which, whilst giving some information on the physiology of the re-instatement of photosynthesis after a dry period, was inconclusive. More work needs to be done to be able to compare different species, e.g. with hydration state and age. The regain of photosynthetic competence is apparent in lichens after only a short period (Fig. 6). Again this work will be extended at Cambridge, and will hopefully include more species.

2.2.2 Oxygen electrode/fluorimeter

Some work was carried but only on *X. elegans* and *Umbilicaria aprina* and/or *U. Antarctica*. Due to time constraints this work will be extended at Cambridge.

2.3 Summary and Conclusion

Physiological data has been collected from a wide range of moss species present in the Edmonson Point area. Work has ben carried out both on *in situ* rates of photosynthesis and in controlled conditions, varying both light and temperature. Limited work has been carried out on lichen species concentrating mainly on the effects of wetting and drying on photosynthesis.

The data collected for the three dominant moss groups present at Edmonson Point will provide a useful base-line for future work with the samples that will be taken to Cambridge. More work will have to be done to fully substantiate the differences tentatively highlighted between these mosses.

The Hansatech equipment performed well and produced reliable data. Again, more work will need to be carried out to evaluate the varying responses of the mosses at differing temperatures.

The work that has been done on the mosses will be expanded to include the lichens that were collected. This should help in the continued gross evaluation of the plants that have already been tested at Cambridge, and also to help assess possible differences between the

fresh and stored samples.

3. Experiments using UV cloches

(Note: all cloches had walls unless otherwise stated)

Cloches have been used for UV work by utilising the different transmission characteristics of various plastics. Whilst they alter various micro-climatic conditions to a greater or lesser extent depending upon the type of cloche used (open-sided or closed), they do however offer a simple and cheap way of altering UV transmission. Variations in micro-climate have been found to be generally the same for either type of cloche, UV opaque (VE) and UV transparent (OXO) (although the energetics of the UV stress may be shifted by changing the environment to a more favourable balance). Several groups of cloches were installed by Italian scientists for D. D. Wynn-Williams and R. I. Lewis-Smith in 1994-95, but a much larger scale series of experiments were set up by D. D. Wynn-Williams and R. I. Lewis-Smith in 1995-96. The locations of the cloches that were sited during the BIOTEX expedition are shown in Appendix 2.

3.1. 95/96 cloche work

3.1.1 Experiment 1

The first site (Site 1) that was chosen was a stand of *B. argenteum*, close to a melt water stream and near to the influence of both skuas and penguins. It was chosen for three main reasons: (a) size of patch allowing for up to four cloches to be sited; (b) uniformity of the site (the stand was almost pure *B. argenteum*), with little hummocking or obvious discolouration (this moss is liable to become bleached when dry); (c) the stand was generally moist due to its close proximity to an active melt-water stream.

The site was operative between 26 December and 8 January, and was sampled on a number of occasions, although most of the data were lost due to the IRGA crashing. It was because of this, and compounded by the drying out of the area that it was decided to terminate work at this site. Consequently no cloches were placed on the site.

3.1.2 Experiment 2

The second site (Site 2) was in close proximity to Site 1, but was a small stand of *B. pseudotriquetrum* amongst a community dominated by *B. argenteum* and *C. purpureus*. The site had been greening up for a number of weeks and was not yet in the stages of recession (i.e. drying out). Dates of operation: 9 January to 5 February.

20 cm^2 cores were taken using a steel borer approximately 5 cm deep, then inserted into perforated polystyrene weighing pots, these were then placed back into the moss carpet. Provisional measurements were made over a week to gain a background response of the moss prior to cloche emplacement. Then two OXO cloches were placed over two pure areas of *B. pseudotriquetrum*. Fluorescence yields were obtained for this experiment. One of the OXO cloches was then exchanged for a VE cloche on 16 January. There was no apparent visual difference between the moss under the cloches at the termination of the experiment, although

both were noticeably greener than that in the surrounding moss bank. Initial work using CO_2 assimilation and yield measurements indicated no differences between cloches. Further work needs to be carried out on the samples that were taken for follow-up studies in Cambridge.

3.1.3 Experiment 3

This was undertaken using 4 cores taken as described above, but in whole weighing pots, of each of the three dominant mosses present at Edmonson Point. They were then removed from the moss bank. Fvm measurements were taken, then placed under OXO cloches for 24 hrs. CO_2 assimilation rates were taken, and half of the samples placed under a VE and half under an OXO cloche. Sampling then continued on selected dates thereafter. Photosynthetic rates were found to be different within and between species, but not between cloches. Fvm data that were obtained for the experiment (Fig. 4), appears to show slight differences between OXO and VE cloches. Work with mosses is very problematical so the samples were always fully hydrated prior to recording (the samples were sprayed with water several hours before analysis). This experiment had to be abandoned due to adverse weather conditions twice damaging the experiment, the second time irretrievably. Dates of operation: 14 January to 30 January.

3.1.4 Experiment 4

This was undertaken at the primary micro-climate site (R. I. Lewis-Smith report N° BIOTEX/1995/NT1, "Met. site"). The site had both walled and un-walled cloches. Samples were taken for analysis from each set, but immediate analysis at Terra Nova Bay was done on those from walled cloches only.

This work was carried out using the Hansatech equipment at three different temperatures (see Fig. 7 for Fvm data). Unfortunately, no background data were obtained prior to the cloches being placed over the *C. purpureus* site. Data for the walled cloches and the control background material would appear at the moment to indicate that there were significant differences between the outside and the cloched material but were only slight between the OXO and VE cloches. There were colour changes found in the *Ceratodon* under the different cloches (see S. Ertz report N° BIOTEX/1995/NT3). Further work, using the equipment taken to Terra Nova Bay, will be carried out in Cambridge to assess any other possible effects not yet detected, and also to assess if there were any differences between the walled and un-walled cloches.

3.1.5 Experiment 5

Parts of cloches were placed over small stands of *Umbilicaria, Usnea, Buellia* and *Xanthoria*, primarily to check for any visual changes that may be induced and to obtain data using the fluorimeter. Samples of *Umbilicaria* and *Usnea* were also taken for further investigation. There were no immediate visual effects apparent in any of the species tested. It should be noted that the fluorescence signal for the *Buellia* sample was weak and unstable, so the fluorimeter study was not fully realised.

3.2 Over-wintering cloches

As a number of cloche experiments during BIOTEX were only for the duration of the summer, there was a large number of spare cloches in addition to those needed for possible replacement. As Edmonson Point is intended to be a long-term research site it was decided to place a number of these cloches on selected moss sites to over-winter and hopefully remain in place during the influence of the early Spring ozone depletion.

It is intended that the cloches can be sampled 1996-97, for further analysis in Cambridge, or at least photographed again to assess possible UV effects.
The following sites had cloches placed on them (see Appendix 2):
(i). Full set of cloches (UV open/walled & VE open/walled) over a *C. purpureus* dominant stand, which had been fed by meltwater briefly during the early spring.

(ii). Full set of cloches five metres east of site (i) over a colonising patch of *B. argenteum.*

(iii). Two walled cloches, again in the same basin as i and ii over *C. purpureus,* but apparently more moist than (i).

(iv). Two walled cloches on area near to experiments at Sites 1 and 2, over *B. argenteum* patch.

(v). Two walled cloches over a *large B. argenteum* stand close to BIOTEX campsite.

(vi). Two walled cloches, 30 m down stream from the "Met. site" covering *B. argenteum.*

3.3 Summary and Conclusion

The full value of the cloches could not be realised during the BIOTEX expedition as the work was started after the UV-B levels had reached their maximum (October). The cloche study was itself brief, with a lack of background data prior to cloche establishment. This means that any differences between cloches may be speculative as any possible pre-treatment differences will not have been revealed. Again, this work was influenced by the knock-on effects of the equipment troubles.

Trying to find an homogeneous site was difficult. The "Met. site" was quite heterogeneous and, even with a uniform-looking site, there were actually significant differences in photosynthesis between sample cores of the same species within close proximity. The physiological state of the sample at time of recording needs to be assessed to ascertain whether it may play a role in these differences.

It would have been preferable to set up this type of experiment over one and half summers, with the first summer spent obtaining the necessary background data and setting up overwintering experiments. Sampling could then have been carried out as soon as possible in the second spring.

Temperature differences may also ameliorate any negative effects of the UV exposure. Also, as there is some UV penetration in the open cloche (see S.Ertz report N° BIOTEX/1995/NT3), it needs to be checked how much this may affect the response of the plant. A 5°C regime above ambient may be considered to be favourable, but as studies at higher temperatures have shown, such an increase is not always beneficial (Fig. 7). Care has

therefore to be taken in interpreting data from the cloche experiments.

4. Micro-climate data

These were obtained using Skye UVB & UVA sensors, a PAR sensor, and a Rotronic temperature/humidity sensor, feeding back to the Campbell CR-10 datalogger.

Due to an omitted wire in the wiring diagram, delays occurred with the use of the data logger and also due to problems with Terra Nova Bay to UK communications. Data were collected both with and without the different types of cloches (see also S. Ertz report N° BIOTEX/1995/NT3). The data are still to be fully processed and the UV values corrected using the radiation transfer model of T. Martin. They have also to be correlated with the data acquired by the primary micro-climate station. These will be held on a TFLSD Data & Resources Centre database.

There was generally a temperature differential between the cloches and ambient air e.g. on one occasion when ambient was 6°C, the temperature in the walled cloches was 18.7 and 16.4°C, for OXO and VE respectively. When the temperature goes below 0 ℃, the relative humidity readings rise dramatically (this will be checked to ensure the sensor was functioning correctly). UV-B levels were different as expected but, there was little difference in PAR between the walled OXO and VE cloches; most climatic variables apart from UV that have been altered, are coincident with both OXO and VE cloches. A greater appreciation of cloche variables should be found from examination of the data from the primary micro-climate station.

5. Sample Collection

As well as use of other samples collected by fellow BIOTEX members, specimens were also collected for continued use with the ongoing physiological research in the growth cabinets at Cambridge. Table 1 lists samples that were collected. Samples were also taken from "Met. site"; Site 2; *Umbilicaria* and *Usnea* cloche site; and all sites chosen for over-wintering. The samples from remote sites were needed to complement and increase the range of plants that were available at Edmonson Point.

General Summary & Conclusion

The work undertaken at Edmonson Point and Terra Nova Bay has been mainly involved in the development of the use of a combined IRGA system and fluorimeter. This work was frustrated by novel problems, and difficulties with cuvette design were also encountered. In addition, some problems arose due to lack of time for adequate preparation.

Of the work that was carried out successfully, it was shown that the equipment does have a tremendous potential in Antarctic fieldwork and in laboratory studies on the experimental plants.

Work was carried out on as many moss species as possible, primarily using the Hansatech equipment. The value of this equipment is that there can be replication of the conditions under which the samples were tested, allowing comparison of samples that were tested at Terra Nova Bay, then frozen for return to Cambridge.

Cloches or acrylic sheets (as used to make the cloches) were placed over a variety of mosses and lichens for a limited period to study their response to UV radiation. Because only a small amount of work could be carried out, research was concentrated on the three dominant mosses present at Edmonson Point. Over-wintering cloches were also placed over selected mosses.

Samples of a range of plants present in the area were taken for further analysis and continued studies using the UV conditions provided by the growth cabinets at Cambridge.

The data that have been obtained are still being processed and re-analysed. Chlorophyll extractions still need to be carried out on a number of samples used with the Hansatech equipment and all of the work undertaken with the CIRAS. The soil analysis carried out R. Bargagli and the pigment analysis undertaken by S. Ertz will aid in the analysis of the data, and in further work to be carried out. The community analysis by R. I. Lewis-Smith should help in the possible interpretations of the data gathered for the different mosses. Identifications by R. I. Lewis-Smith and F. Bersan will ensure the correct nomenclature and labelling of samples.

Mosses appeared to exhibit high net rates of photosynthesis under conditions of extremely high PAR. This will be investigated further, especially with regard to the possible relationship between photoinhibition and the effects of increased UV-B radiation. Also the work on inter-specific differences, especially with respect to extremes of temperature and light, will help to assess possible responses to UV changes.

Unfortunately, the UV work was minimal. In such a rapidly changing and unpredictable environment, any experimentation needs to be carried out as efficiently as possible with utmost expedience. When this is delayed, as was the case with the new equipment, then time is lost that will inevitably result in reduction of potential work. UV cloches should ideally be placed over the experimental plants as soon as possible in the season (even before our arrival the maximum ozone depletion had already occurred). As this was not possible, all work on the samples had to be short-term, and at a time when UV-B levels were becoming increasingly normal.

To try to compare the effects of UV within and between species, it is necessary to be sure about the conditions experienced for growth in the field, and under the respective experimental regimes, as well as during the period when measurements are being made. If a sample is being measured at sub-optimal rates such as an inhibitory hydration state, then apparent differences that could be highlighted with UV treatment may be an artefact of the data and not actually related to a UV effect. This, therefore, makes studies using cloches, and comparison between cloches, difficult and results must be interpreted with caution.

Cloches can only deplete existing UV-B levels. A way to complement cloche studies (and also laboratory-based investigations) by increasing ambient UV-B radiation, would be to use a UV-B supplementary system, using an array of UV-B emitting fluorescence tubes. This would enable fieldwork to be carried out after the decline in the ozone hole. Long-term studies of increased UV-B exposure could also be carried out, thus assisting in predictive studies of future affects of increased UV-B radiation. However, design and modulation of the UV-B dose can be troublesome, and would have to be thoroughly evaluated.

In hindsight, for the most reliable results it would probably have been better to have

worked only with the oxygen electrode/fluorimeter system that was already proven, and in the end provided the majority of the data. Other problems, could have been overcome if the planning and preparation period had been longer. However, it must be borne in mind that the objectives included the assessment of new or previously untested equipment in an Antarctic environment. This was broadly achieved, together with recommendations for possible improvements. Some of the difficulties could have been identified with a longer trial period, but not all. This should make the IRGA/fluorimeter a much more powerful tool when it is next deployed.

The overall brevity of the Antarctic summer, and the swift transience of the environment once ice-free during the season, had not been fully appreciated, and this hindered finding suitable sites. Moss stands that had been covered by snow one week were uncovered and moist the next, and dry again by the subsequent third or fourth week. With such a quick turnover time and short growing season, it is essential to have experimental sites established with the equipment fully operational as soon as possible.

To conclude, this was a very enriching expedition for me in many aspects. Not all objectives were satisfactorily achieved and, as with most experimental fieldwork, there are still many questions to be answered.

7. Acknowledgements

I would like to thank the team leaders, Dr D. D. Wynn-Williams and Dr R. Bargagli and all members of the BIOTEX team for making this experience so worthwhile. Also to Dr R. Bargagli for all his hard work and support with communications between TNB and EP. I am also very grateful to Ing. M. Zucchelli for allowing my extended stay at Edmonson Point and all of ENEA's TNB personnel for their help and logistical support. My thanks also to the Helicopters New Zealand pilots for logistical support (and especially to Dave Lewis for loan of multimeter).

I am especially grateful to the British Antarctic Survey for providing equipment and the opportunity to work as a member of the BIOTEX "team", to Anglia Polytechnic University (APU) for financial assistance and for permission to work in the Antarctic and to Dr D. Keiller (APU) and Dr P. O. Montiel for invaluable advice and correspondence.

I would also like to thank Dr. R. I. Lewis-Smith for his help with editing the script.

Table 1 Plants collected at Edmonson Point and surrounding areas for experimental studies using equipment as detailed in text.

SPECIES	CIRAS	OS-100	Hansatech #	NOTES
MOSSES				
Bryum argenteum	✓	✓	✓	**
B. pseudotriquetrum	✓	✓	✓	**
Ceratodon purpureus	✓	✓	✓	**
Grimmia lawiana			✓	**
Pohlia nutans			✓	1 trial **
Pottia heimii			✓	**
Sarconeurum glaciale			✓	**
Tortula princeps			✓	**
LICHENS				
Acarospora sp.		✓		Brief trial
Buellia frigida	✓	✓		Brief trial **
Rhizocarpon melanophthalma		✓		Brief trial
Rhizoplaca sp.		✓		**
Umbilicaria aprina/antarctica		✓	✓	**
U. decussata		✓		**
Usnea antarctica		✓		**
U. sphacelata	✓	✓		**
Xanthoria elegans	✓	✓		**

** sample(s) also collected for work in UK.

Hansatech, combined oxygen electrode and modulated fluorimeter.

Table 2. Sample of data produced using CIRAS from the moss *Bryum argenteum*.

Measurements made without extra equilibrator.

Ref.....Reference measurement.
Diff.....Difference between analysis tubes.
CO2.....CO_2 (ppm).
Wvap....Water vapour (mBar).
Cuv.....Cuvette.
Temp....Temperature (°C).
Transp...Calculated Transpiration rate.
Stom C..Apparent stomatal conductance.
Ebal.....Method of temperature determination.
PSN.....Photosynthetic rate (umol m^{-2} s^{-1}).
Cint.....Calculated intercellular CO_2 concentration (ppm).
Code.....CIRAS error code.

sample1	rec No	ddmm	hhmm	CO2 Ref	CO2 Diff	PAR	Wvap Ref	Wvap diff	Cuv temp	area"
1	1	1501	1455	222.3	-1.9	1330	2.3	6.47	9.9	10
1	2	1501	1456	320.2	42.7	410	1.5	6.15	8.3	10
1	3	1501	1457	347.1	9.5	1499	2.9	1.94	9.3	10
1	4	1501	1458	347.6	-60.5	1466	2.9	4.47	9.5	10
1	5	1501	1459	355.8	-65.9	1444	2.9	4.8	9.6	10
1	6	1501	1500	358.6	-66.7	1433	2.9	4.2	9.5	10
1	7	1501	1501	359	-69.1	1491	2.9	4.29	9.6	10
1	8	1501	1502	359.1	-71.3	1535	2.9	4.28	9.6	10
1	9	1501	1503	359	-73.6	1557	2.9	4.85	9.7	10
1	10	1501	1504	359.1	-73.3	1539	2.9	4.07	9.7	10
1	11	1501	1505	359.1	-73.8	1513	2.9	4.15	9.8	10
1	12	1501	1506	359.2	-73.1	1473	2.9	4.59	9.8	10
1	13	1501	1507	359.2	-72.6	1411	2.9	4.55	9.8	10

Flow rate	Transp	Stom C	Ebal	leaf temp"	PSN	Cint	AtmP	code	sample1	rec No
301	1.36	283	0	11.9	0	218	987	10	1	1
301	1.29	409	0	8.5	-9.2	400	986	10	1	2
298	0.4	43	0	12	-2.1	428	986	10	1	3
301	0.94	146	0	11.9	12.2	147	986	10	1	4
301	1.01	165	0	12	13.3	155	987	10	1	5
301	0.88	132	0	11.9	13.5	122	987	10	1	6
301	0.9	133	0	12.1	13.9	115	987	10	1	7
301	0.9	130	0	12.2	14.4	104	987	10	1	8
301	1.02	160	0	12.3	14.8	129	987	10	1	9
301	0.85	118	0	12.3	14.8	78	987	10	1	10
301	0.87	121	0	12.4	14.9	81	987	10	1	11
301	0.96	146	0	12.2	14.8	117	987	10	1	12
301	0.95	146	0	12.1	14.6	119	987	10	1	13

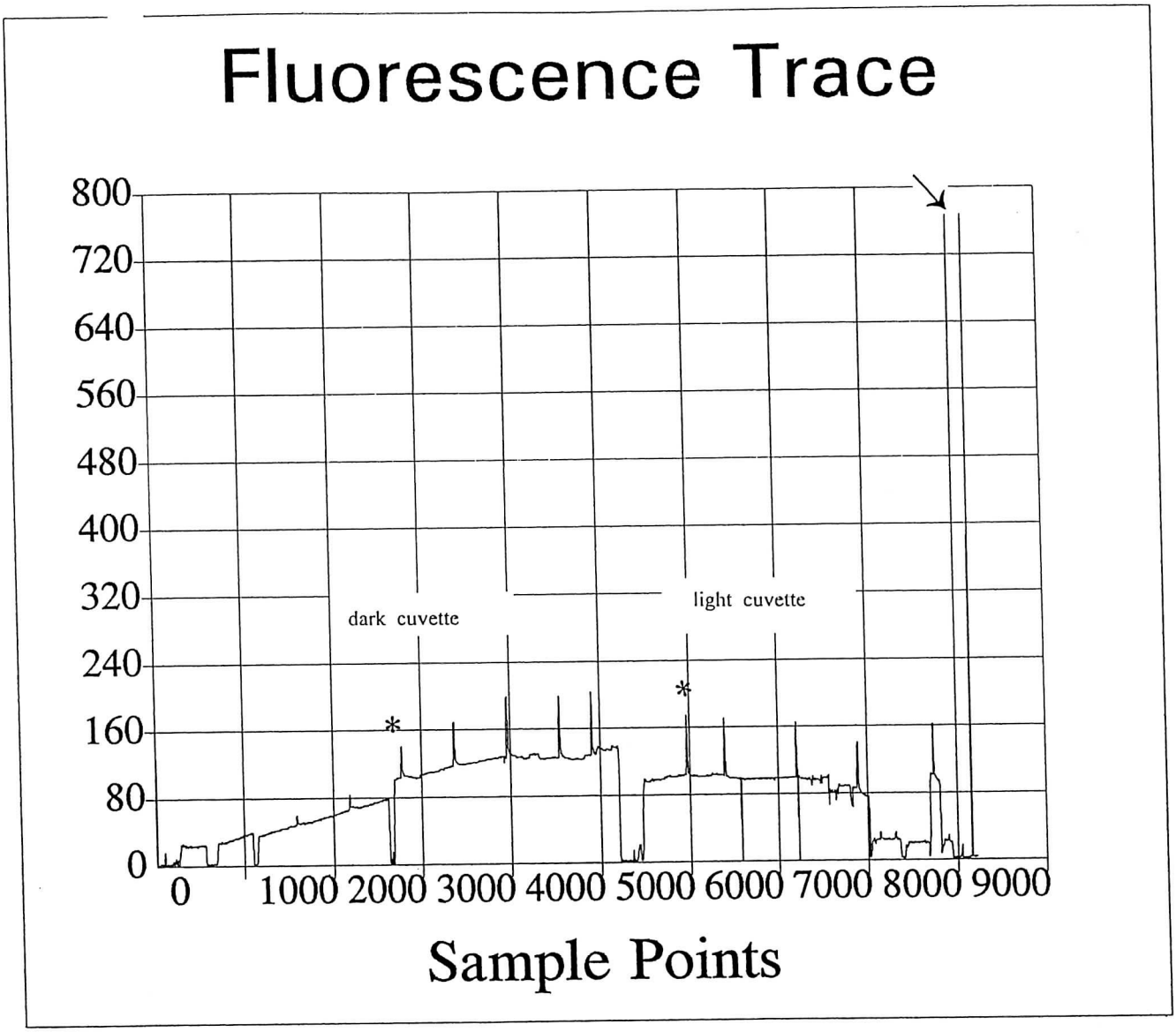

Fig. 1. Fluoresence trace from a test thallus
of *Xanthoria elegans* re-hydrated after a dry period.
Vertical scale, fluorescence signal (relative units).
(*) Saturating pulses applied, (←) spike generated by PC mouse.

SA2 RUN 2 SHELTER (EP130196SA2SHELTER) 29/1/96
LEAF DISC AREA WAS 2.50 cm2
fo= 494.00 fm= 1743.00 fvm= 0.72

INCIDENT LIGHT	ABSORBED LIGHT	RATE PSIS	F'o	F	F'm	qP	qN	qQ	1-qP	Q.YIELD*10	Q.REQ	F'm-F/F'm	TEMP
0.00	0.00	-2.09	442.32	431.21	1039.09	1.02	0.52	0.10	-0.02	0.00	0.00	0.59	11.39
6.87	6.32	-1.54	434.03	446.72	991.09	0.98	0.55	0.12	0.02	0.22	45.89	0.55	10.78
14.76	13.58	-0.18	486.03	512.74	1087.09	0.96	0.52	0.02	0.04	0.35	28.32	0.53	10.68
23.00	21.16	0.78	493.94	511.21	1055.09	0.97	0.53	0.00	0.03	0.34	29.48	0.52	10.80
40.40	37.17	1.94	423.15	428.67	815.09	0.99	0.69	0.14	0.01	0.27	36.85	0.47	10.81
58.10	53.45	3.72	375.31	378.58	655.09	0.99	0.78	0.24	0.01	0.27	36.78	0.42	11.55
76.00	69.92	5.63	329.84	338.35	559.09	0.96	0.82	0.33	0.04	0.28	36.20	0.39	11.70
103.50	95.22	5.76	299.82	320.42	479.09	0.89	0.86	0.39	0.11	0.21	48.53	0.33	11.93
153.50	141.22	7.05	286.96	288.79	399.09	0.98	0.91	0.42	0.02	0.16	61.79	0.28	11.83
268.00	246.56	9.06	282.32	276.37	335.09	1.11	0.96	0.43	-0.11	0.11	88.40	0.18	11.94
448.00	412.16	10.80	264.46	268.51	335.09	0.94	0.94	0.46	0.06	0.08	127.89	0.20	10.74
668.00	614.56	11.41	245.20	262.64	335.09	0.81	0.93	0.50	0.19	0.05	182.05	0.22	12.32
974.00	896.08	11.42	238.03	253.93	303.09	0.76	0.95	0.52	0.24	0.04	265.30	0.16	12.12
1060.00	975.20	12.46	216.98	237.81	335.09	0.82	0.91	0.56	0.18	0.04	268.10	0.29	11.53

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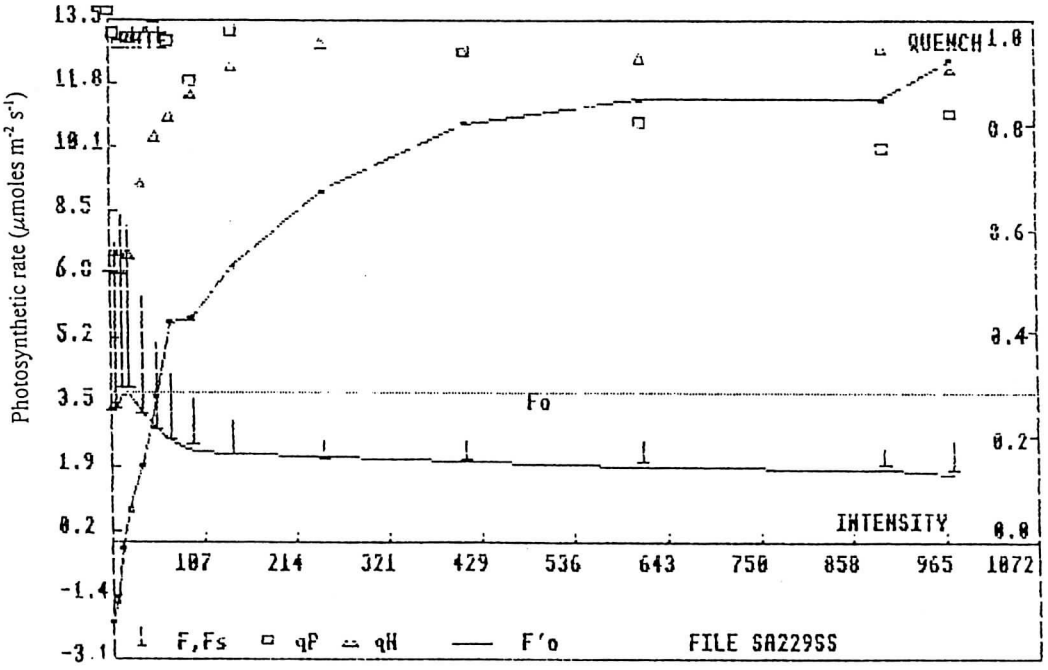


OXYGEN RATE DATA FROM FILE SA229SS

Fig. 2. Sample of a combined oxygen evolution and fluorescence trace and the data acquired from the moss *Sarconeurum glaciale* using the Hansatech equipment.

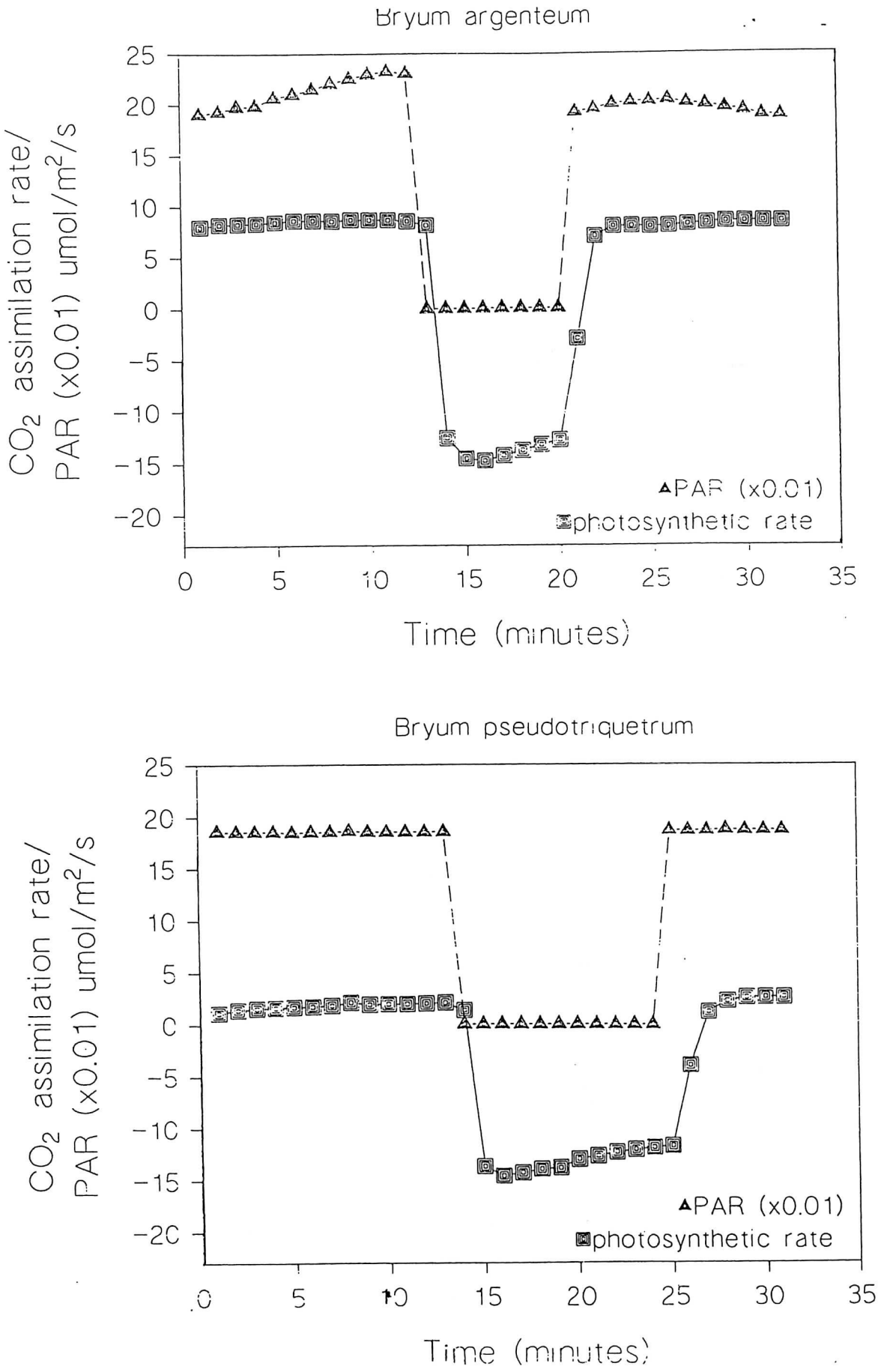


Fig. 3. The effect of applying a dark period on two mosses taken *in situ*. Recordings made at one minute intervals.

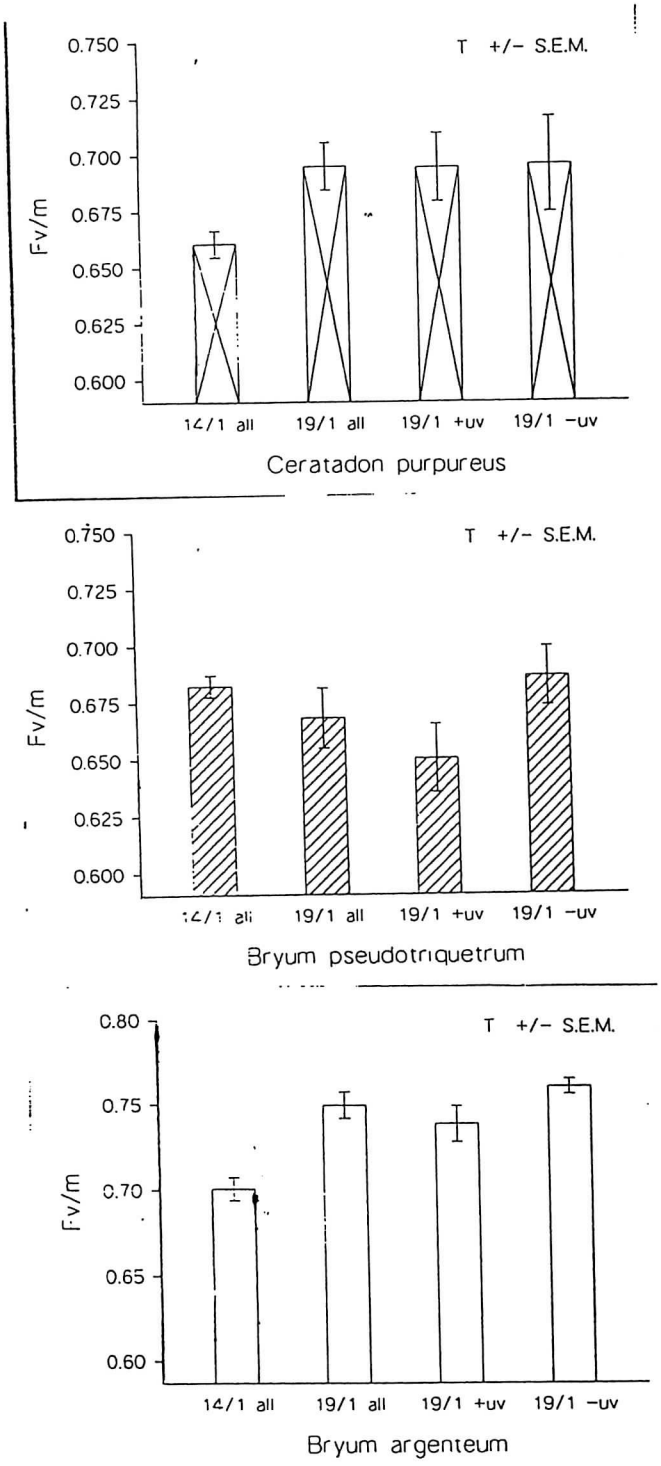


Fig. 4. Initial fluorescence ratio (Fvm) measured for three mosses after a 30 min. dark period using OS-100 modulated fluorimeter. Measurements taken prior to emplacement of cloches over mosses (14/1) and five days later (19/1). All, mean of all samples; +uv, mean of samples pkaced under UV transparent cloche; -uv, mean of samples under UV opaque cloche.

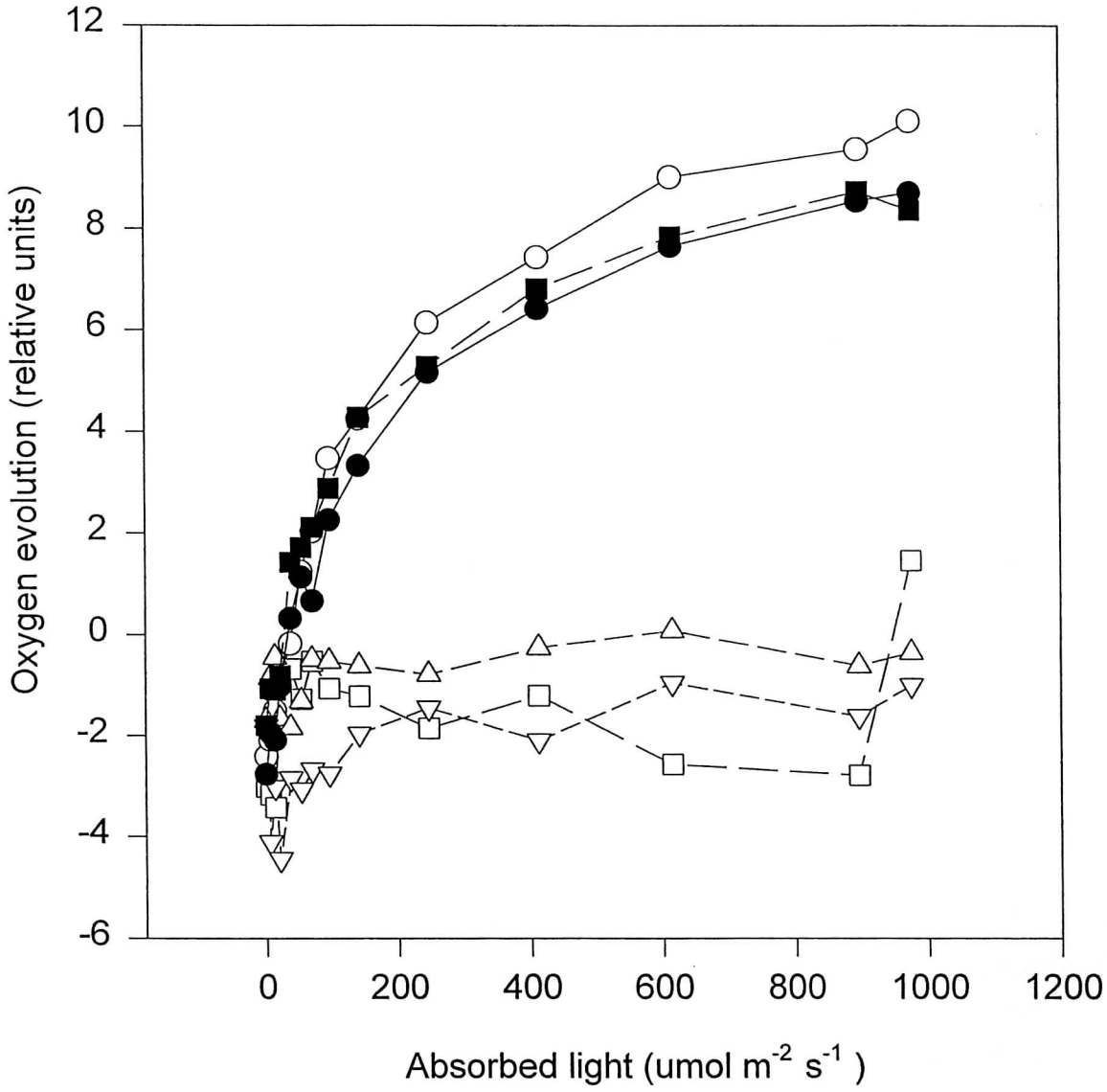


Fig. 5. Effect of freezing treatment on the moss *Tortula princeps.*
Open symbols, frozen whilst moist; closed symbols, frozen after drying.
Circle, before freezing; square, one day after freezing;
upward triangle, 2 days after freezing; downward triangle, 3 days after freezing.

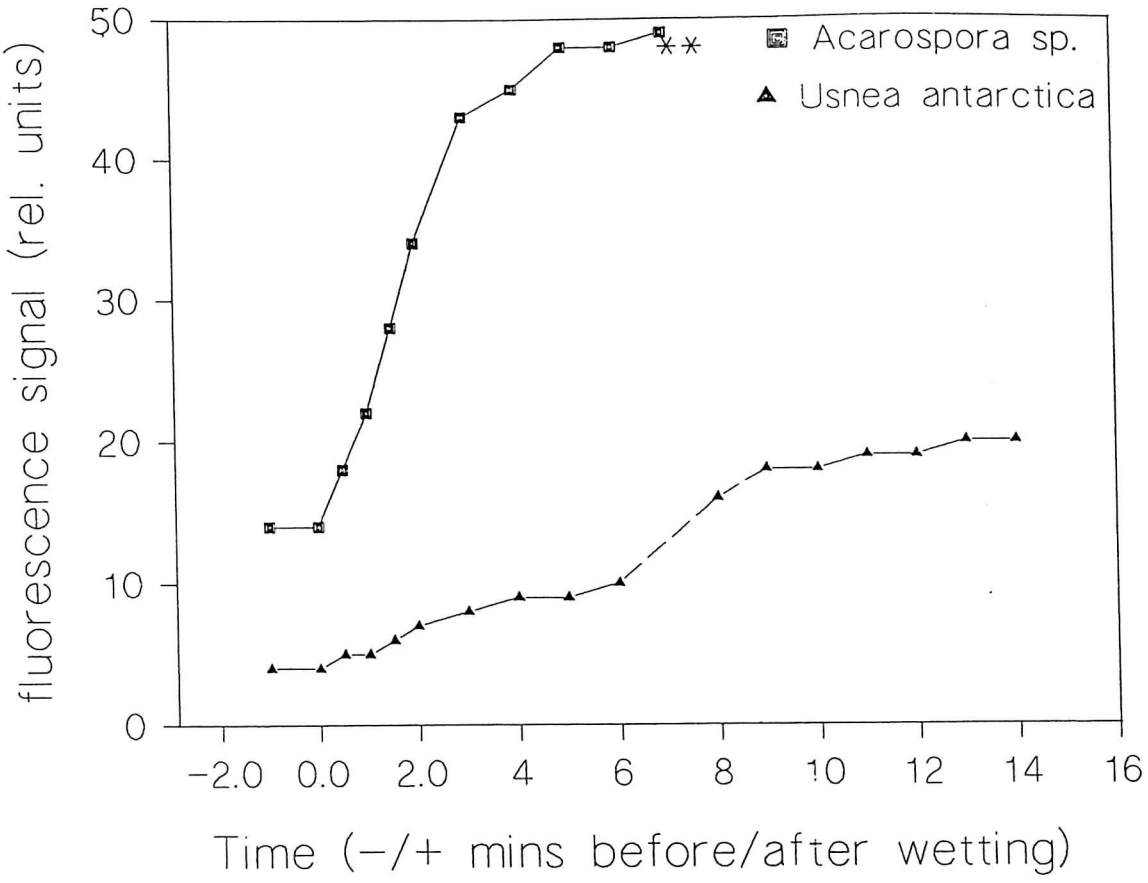


Fig. 6. Effect of hydrating two lichens after a dry period on dark level fluorescence (Fo). Both samples measured with same modulation intensity (175 *tics*). Fvm for *Acarospora* 0.414.
** saturating pulse applied.
Note: Fo was too low in *U. Antarctica* for a saturating pulse to be applied.

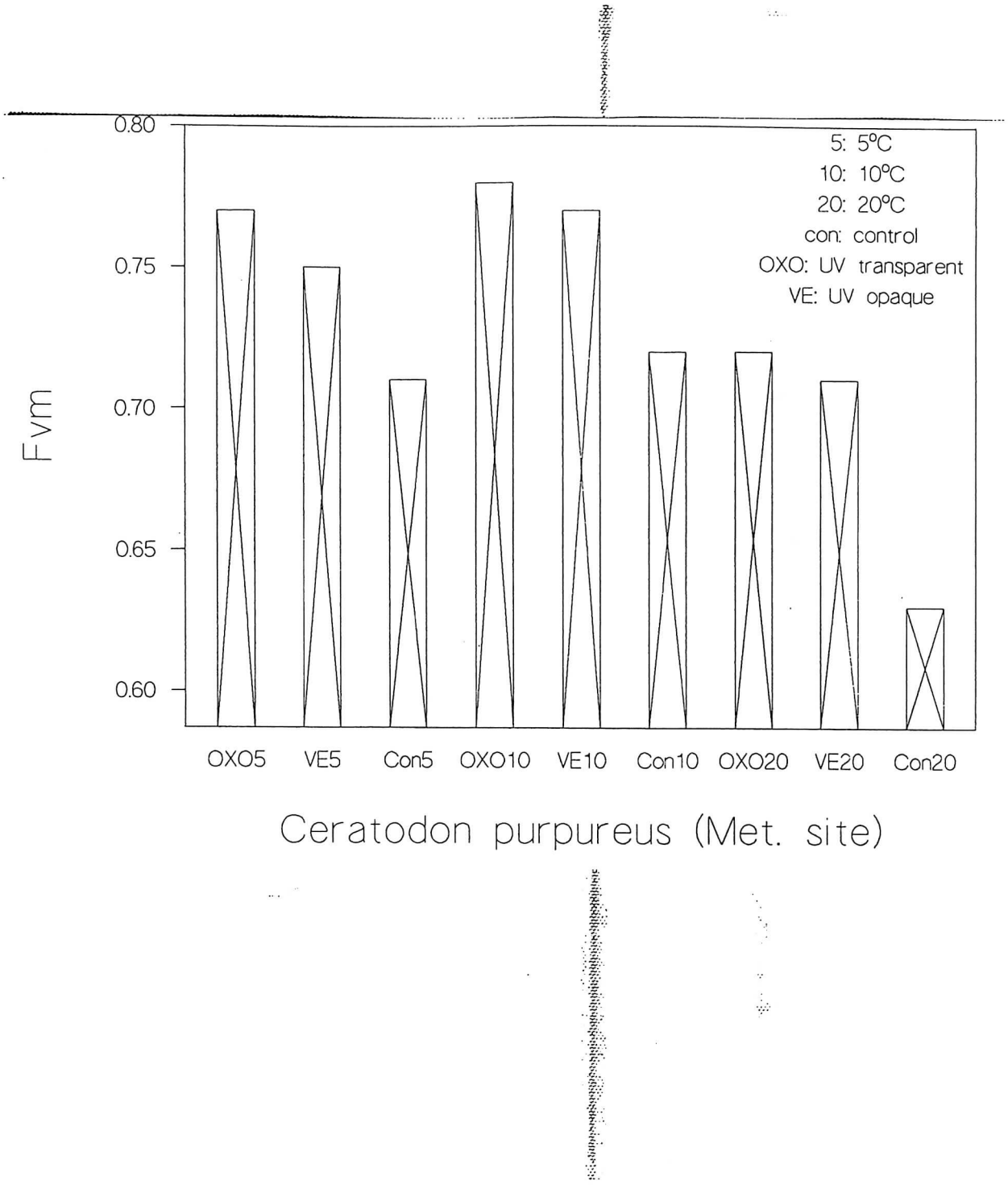


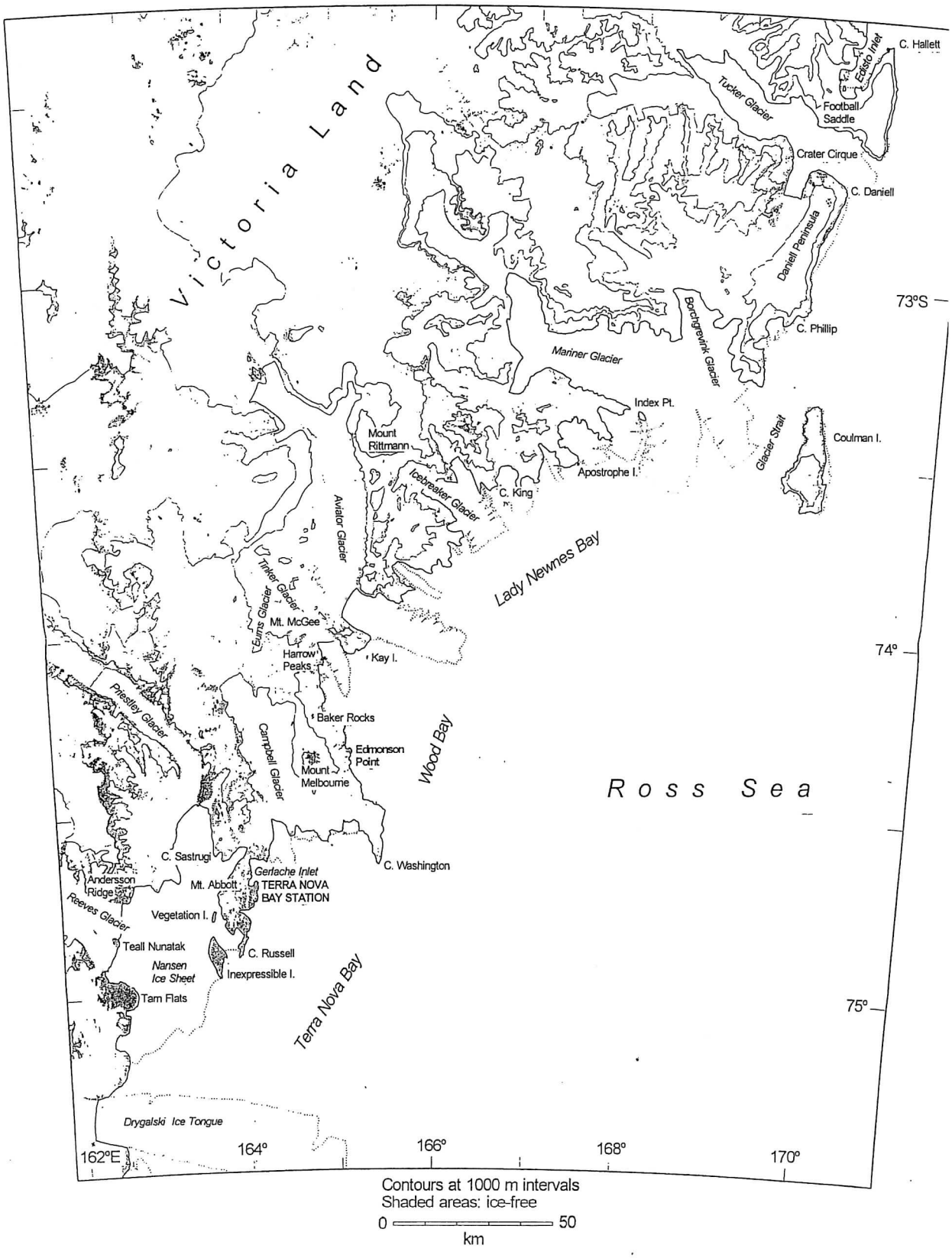
Fig. 7. Variation of the initial fluorescence ratio Fvm (taken after 30 min. dark period), either with cloches (OXO or VE) or without cloches (control), and at different temperatures in the moss *Ceratodon purpureus*.

Appendix

1.Map of study area & locations of remote sites visited (from R I. Lewis-Smith report N° BIOTEX/1995/NT1).

2. Map of local study area (adapted from ENEA map provided by R. Bargagli).

3. Report on CIRAS and OS-100 modulated fluorimeter.



Appendix 1 Map showing area around study site and remote locations

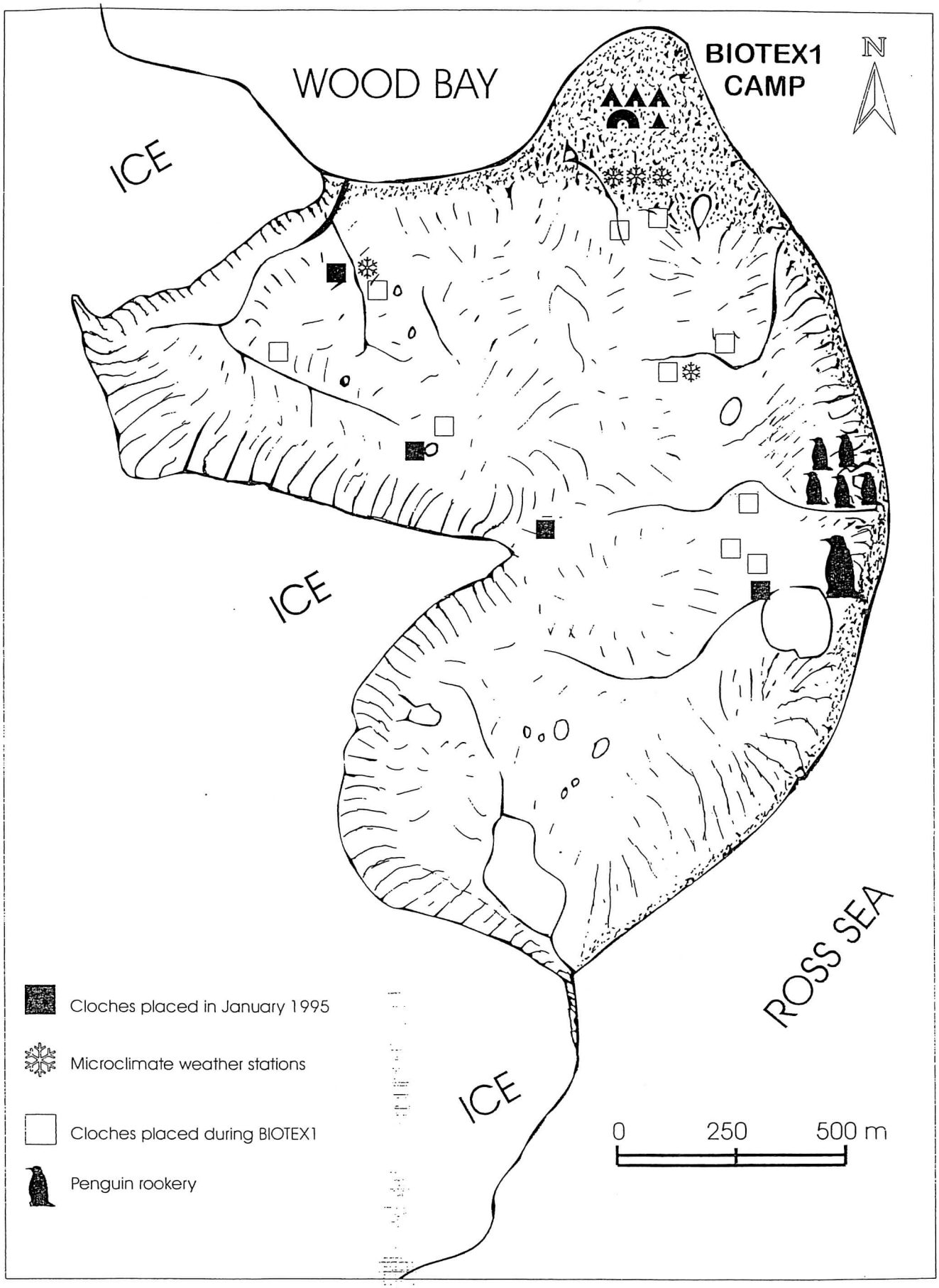


Fig. 1 - A sketch map of the Edmonson Point area

Appendix 3

Report on field performance of PP Systems "CIRAS" Infra-red gas analyser and "moss-cuvette" and the Opti-Science "OS-100" modulated fluorimeter.

A. E. Smith

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1. CIRAS infra-red gas analyser and custom "moss" cuvette

Introduction
The Equipment
Modes of Use

2. OS-100 Modulated Fluorimeter
Introduction
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Modes of Use

3. Fieldwork Problems

4. Summary & Conclusion

Report on field performance of PP Systems "CIRAS" Infra-red gas analyser and "moss-cuvette" and the Opti-Science "OS-100" modulated fluorimeter.

1. CIRAS infra-red gas analyser and custom "moss" cuvette.

1.1 Introduction

Measurement of primary production in plants can be carried out by using two approaches, (i) a long-term method generally using dry weights and analysing biomass production; (ii) a generally short-term approach utilising biochemical and physiological techniques. To assess shorter term performance of plants and to help identify how the plant adapts to a changing environment and any additional stresses, other methods than harvesting can be of more use. Radio-labelled carbon dioxide (CO_2) is useful for determining products and intermediates, but will result in the destruction of the sample and is also not generally suitable for continuous measurements. Measurement of gas exchange can be most useful in short term assessment, and combined with fluorescence studies, can help to identify sites within the photosynthetic processes that may be affected.

Oxygen (O_2) flux can be measured primarily by two techniques, manometry and polarography, but neither type of equipment is suited for field use. Measurement of net CO_2 exchange can also be carried out using the basis of infra red absorption of hetero-atomic molecules such as CO2, each of which have a characteristic emission. Water vapour also has a similar peak emission.

Infra red gas analysers (IRGAs) have been used for a number of years in the study of photosynthesis. There are three main approaches to IRGA design: a closed system which is the simplest, where the sample is kept in a sealed container and the air is continually re-circulated thereby resulting in a gradual decline in CO2 partial pressure thus causing CO2 measurement not to be in steady state. Also water vapour may gradually increase thus affecting the pressure of the system; an open system where the sample is kept in a steady state. After recording CO2 concentration the gas is exhausted, care needs to be taken with accurate measurement of flow rates; a semi closed system where the CO2 concentration is maintained by constant analysis of the

air flow. An open, differential system is employed by CIRAS; which also has the advantages of controlling both CO_2 and water vapour concentration. To assist in the measurement of gas exchange, specially designed enclosures (cuvettes) have been devised. Cuvettes used for enclosure with CIRAS have in the past been designed for economically important species, cereals, legumes and conifers. None of these designs are adapted for cryptogam measurement, where larger samples are generally required to perform a more reliable analysis, and must be able to cope with the immense amounts of water loss associated with these plants.

1.2 The Equipment

1.2.1 Infra-red gas analyser

Standard CIRAS model with automatic CO_2 and H_2O control.

1.2.2 The custom cuvette

The prototype custom cuvette handle (Fig. 1) differs from the normal Parkinson (broad leaf cuvette, P(blc), Fig. 2) in several ways, it has only one stirring fan located on the upper arm, to cope with the additional water vapour release, an extra "water vapour equilibrator" is attached to the return pipe of the cuvette arm before entry into the IRGA. This creates problems with water vapour measurements and other water vapour related parameters. A UV transparent window (OXO-acrylic)is glued on instead of the usual quartz window, covering a chamber with a surface area of approximately 20 cm^2. To cope with the different situations that arise with studying lower plants, specially interchangeable cuvettes were constructed (see below).

1.3 Modes of Use

1.3.1 The IRGA

As per manual, as either stand-alone or combined with various attachments; either in differential or absolute mode; either closed or an open system, with or without CO_2/H_2O conditioning.

1.3.2 The Custom Cuvette

The cuvette handle has been designed to be used with four different attachments.

1. As an enlarged P(blc) (Fig. 2).
2. For adhesion on to an external surface eg covering crustose lichens.
3. Plant core cuvette accommodating up to c. 20 cm^2 surface area, with three different length beakers (short, medium, and long) to allow for varying sample depths (Fig. 1).
4. As (3) but also employing the OS-100 fluorimeter (see later for description), with the fibre-optic cable attached to an adapted beaker.

CIRAS & custom cuvette, mode (1)

The lower aluminium support plate is attached by three small screws with the addition of silicone grease to help ensure an airtight seal. A silicone o-ring is put in place then the top plate of the cuvette is tightened using the adjustable nut. The seal is checked by blowing around the gasket. The extra equilibrator is an optional attachment (see later note). Central positioning of the sample is essential to prevent shading by the vertical aluminium cuvette sides. The rate of air flow may be adjusted depending upon the rate of CO_2 uptake/evolution according to other applications.

This set up is generally used with foliose and fruticose lichens. A small stage for the sample may be used in low temperatures to prevent freezing to the aluminium base-plate.

Cuvette mode(2)

The base-plate is removed and the custom aluminium disc with outer silicone o-ring is fixed into position with three screws, again with silicone grease applied to the metal contacts. The upper and lower plates are closed. The cuvette can then be placed on the exposed surface. Associated problems: the tightening screw thread is too long for the cuvette to be placed horizontally on a rock surface. As the whole screw-thread length is required for mode (4), it was decided not to shorten the thread; moreover a flat, smooth slate-like non-porous rock is really required to ensure an adequate rock-cuvette seal. There was not enough time to evaluate this with CIRAS properly. Modifications would be recommended with this mode: a deeper rubber skirt may assist, or a mounting ring that has previously been attached to the

substrate (a method was tried by fixing a cuvette on to the rock with sealing compound).

Plant core cuvette mode (3)

This employs an adapted nalgene beaker (approx. 6.5 cm dia.) which has had the base removed and is upturned. The screw-lid acts as a removable stage for the sample (usually a moss core or higher plant sample). The lower removable aluminium base-plate is taken out, the rim is smeared in silicone grease and an o-ring is positioned on top of the lower base plate. The rim of the beaker base is smeared in grease, then inserted through the lower base plate and the o-ring placed around the beaker. The upper plate is then secured, ensuring that the beaker rim is not thrust too far up into the cuvette chamber thus preventing an adequate seal. The sample is then placed on the stage (having had the screw thread previously greased), and the stage is tightened until airtight; this may be checked by blowing around the seals. Care must be taken not to over-tighten the stage in case of warping the beaker. Ideally the sample should be as high up in the chamber as possible to alleviate any shading effects of the chamber's vertical sides. It is usually necessary to tilt the chamber arm. At present there is no dedicated artificial light source available for the system; this creates difficulties when trying to perform A/Ci curves in the presence of variable natural daylight conditions. A supplementary light source similar to the combined P(blc) OS-100 system for both field and laboratory work would be useful for the custom cuvette. It is preferable that the sample does not touch the window surface as any moisture on the surface could lead to problems with gas exchange. Flow rates may then be adjusted to suit the sample involved to improve the resolution of the IRGA (as stated in the manual). Care must be taken when removing the sample (by unscrewing the stage), not to dislodge the beaker from its position in the plate, as this may disrupt the seal. Furthermore, care must be taken not to contaminate the greased lid with grit and moss fragments.

Combined cuvette mode (4)

This uses the specially adapted beaker (15 cm long) which has a small aperture drilled two-thirds up to fit the field type fibre-optic cable of the fluorimeter. First the beaker is set up as per (3) except that as a longer beaker is used, it has an increased taper at its top. To ensure the beaker retains its

position, an extra o-ring has to be placed over the beaker rim. The fibre-optic cable is inserted through a padded collar and the holding screw tightened. Problems with the angle of insertion and depth of chamber penetration may arise. As there is no supporting structure for the fibre-optic cable and fluorimeter box, great care has to be taken in keeping both cable and box stable, so as not to tug on the beaker which, can lead to displacement. Compounded with the difficulties mentioned earlier, this system was not adequately tested.

1.3.3 Water vapour equilibrator note

Initial studies were carried out with the equilibrator attached, but leakage problems occurred which, at certain times were associated with the equilibrator. Taping of connections did not always remedy the situation, so the equilibrator was removed. Temporarily this solved leakage problems, but due to the large amount of moisture contained in the air flow this could not be continued due to the potential contamination of the analysis tubes with water. The capillary pipe connections were then checked for firm positioning, a number were found to be loose, these were reinserted and silicone grease was smeared over the joints to help in sealing the pipes. I was still not completely happy with the sealing of the extra equilibrator.

1.3.4 Note on calculated parameters

With the normal P(blc), a detailed history documenting its development is available while, for the custom cuvette, no such description is available. Apart from the CO_2 assimilation rate, which is compensated with a water vapour dilution factor, this value is not physiologically correct for the plant but is physically correct as it is a measure of the amount CO_2 dilution. As the extra equilibrator is attached water may be lost prior to measuring and thus any water vapour-based parameters will probably be erroneous, i.e. transpiration rate, evaporation rate, conductance/resistance and internal CO_2 concentration. Obviously not all these parameters can be applied to lower plants, but water loss is an important factor and any possible help in determining this would be of use. Further work in clarifying the water vapour-based data would therefore be helpful.

2. OS-100 Modulated Fluorimeter

2.1 Introduction

Measurement of photosynthesis by way of fluorescence is a well-established technique, although the characteristics of fluorescence have not yet been fully resolved, and there is still much debate about the source of certain fluorescent parameters.

A modulated fluorimeter has a number of advantages over a non-modulated fluorimeter: it can be used in the light; also dark adapted initial fluorescence (Fo) when all the reaction centres are assumed to be open, can be measured more accurately, which is important when determining both yields and the initial fluorescence ratio Fm-Fo/Fm (Fvm). Some work has been carried out with lower plants but is still minimal when compared to higher plant species.

2.2 The Equipment

The OS-100 fluorimeter is a portable battery operated modulated fluorimeter (see manual for technical specification), one fibre-optic cable carries the saturating light source, weak modulated measuring light source and the fluorescence detector. Flash intensity and duration can be altered and the intensity of the measuring light can be changed.

2.3 Modes of Use

The fluorimeter can be operated as a complete unit or by a dedicated "Windows" program, thereby allowing the storage of multiple traces. When in operation with the Toshiba 1910 PC, movement of the mouse (that is essential for operation), can lead to spiking and possible distortion of the trace. Also whenever the program menu is not run correctly, such as when the wrong drive is selected or was not in the directory that was last selected (even after close down of the system), then the program crashes and resorts back to "program manager"; this not only means that the fluorimeter screen needs reloading and resetting, but data obtained before the crash is invariably lost.

3.3 Fieldwork Problems

Note: Precipitation was not generally present during deployment at Edmonson Point, so that performance under these conditions could not be assessed.

3.1 CIRAS/cuvette

There were three main difficulties associated with the CIRAS/cuvette in field use:

3.1.1 Plant core cuvette leakage (see earlier notes)

This was apparently from variable sources and was never satisfactorily resolved (corrective methods were used as described in the manual, but were not always successful). Adequate gas mixing needs evaluation at varying flow rates to check whether stirring of the air in the chamber is fully effective at the desired flow rates.

3.1.2 Battery longevity

A fully charged battery rarely lasted more than 1 hr of continuous operation (without the palmtop computer attached). This generally meant that carriage of five spare batteries did not even ensure a days work (eight hours). A greater capacity trickle feed battery helped alleviate the situation and provided for up to three hours of use with one battery inclusive with the palmtop. The changeover of batteries may have also contributed to the next problem.

3.1.3 Memory chip crash

This appeared to have no directly attributable cause although might be associated with this battery changeover. I have never come across this problem before, nor does the manual express that it should occur (especially more than four times in matter of three weeks). The memory chips are involved with the operation of CIRAS including the calibration state of the analyser tubes, storage and processing of raw data. Initially this caused a considerable predicament, as well as the need to reset the IRGA all data that had been previously accrued were irretrievably lost. As this problem was an unusual one, inevitably delays had to be anticipated in communication between the Antarctic and the manufacturers, during which no work could be carried out with CIRAS for the fear in irreversibly corrupting

the memory chips and rendering the IRGA inoperable. After one crash following subsequent rebooting the PAR sensor did not operate adequately. Data corruption was solved by using the palmtop but this contributed to the increased depletion of the battery. The problem of data corruption was never finally resolved although the circuitry appeared to be operating properly after each crash. Both the battery changeover and the dry environment that the equipment was being operated in, together with the artificial fibres in the clothing contributed to an unusually high level of static electricity being produced (the engineer responsible for the Adelie penguin monitoring system avoided wearing artificial fabrics when testing the electronic equipment. When I tried to "earth" myself the crash frequency appeared to decline).

3.2 Fluorimeter

The fluorimeter in "field" mode requires a minimum fluorescent response from the sample before allowing the saturating light source to be triggered, this therefore means that because of the low fluorescence intensity of some specimens, such as black lichens, e.g. *Buellia frigida*, they cannot be fully investigated. This "buffer" can be overridden when using the "OSLOG" program.

4. Summary & Conclusion

The Custom cuvette for operation with the CIRAS did have good potential, but a number of problems, concerning design and field operation, need to be overcome.

4.1 CIRAS

Summary of faults, problems and possible remedies

4.1.1 Cuvette window

The glue used to fix the OXO sheet to the cuvette frame did not appear to be fully sealed. Movement on one edge of the window

was noticed, extra glue was thus added. The window was not exchangeable, this would have allowed for interchange with UV opaque material when carrying out UV studies.

4.1.2 Cuvette chamber

The screw-lid (stage) was not always airtight, sometimes due to warping and plant fragments or grit that clogged up the greased lid. Also it was time consuming when changing samples. A more user-friendly approach would be to employ catches with o-rings. A flanged chamber top would help in the handle-cuvette seal instead of the double o-rings presently employed.

4.1.3 Cuvette chamber modified for fluorimeter

This beaker tapered too greatly to fit acceptably (three o-rings being required). The fibre-optic cable being positioned halfway down the chamber did not help in light penetration with the sample having to be in a similar position. I believe that if it were possible to secure the cable on to the chamber handle by making a 60° bore through the handle into the chamber, then this could help to improve the application, either the cable could penetrate the chamber directly or there could be a window to seal the borehole. Moreover the combined system similar to that employing the P(blc) and OS-100, could be used if a suitable actinic light source is available to cope with the larger surface area of the cuvette.

4.1.4 Cuvette chamber handle

This had three problems: a, stability of the chamber with the greased o-ring seal. Again this could be overcome by using a flange type chamber and would still allow for the flexibility of chamber type and function; b, the sides of the handle were vertical and thus caused shading on the sample when upright, this could be solved by angled walls and a slightly curved window, which may improve light distribution within the chamber; the hinged handle on the cuvette was too close to the handle base-plate, this led to an angle being created between base-plate and handle. A larger gap between plates would remedy this.

4.1.5 Enlarged P(blc) chamber

This appeared to be fine except secondary problems such as shading.

4.1.6 Rock application

Not yet fully evaluated due to problems with the tightening screw, etc.

4.1.7 Extra water vapour equilibrator

This had problems with leakage, most of which should be solved with adequate protection of connections. The leak could have stemmed from the capillary piping, although unlikely, in which case possible ways of removing the equilibrator may have to be sought or other water vapour traps tried such as enclosure of the equilibrator in a large sealed container. Water vapour measurements are useful; ways of avoiding the extra equilibrator may therefore be more fruitful in assessing water exchange with the sample.

4.2 Fluorimeter

4.2.1 Fluorimeter minimum pulse requirement

This can hopefully be overridden on the stand alone system, or the "Windows" based system can be made more portable.

4.2.2 Fluorimeter PC difficulties

Both the ease of program crashing and the spike generation of the mouse cause annoyance and both can probably be eliminated. As the stand alone system can only keep one trace at a time, it would be advantageous to use the pc operated system. An alternative would be to find a way of downloading the trace to a palm-top computer.

4.3 Field problems

4.3.1 Cuvette leakage

See earlier notes, generally most of the primary problems were solved. Secondary problems such as the equilibrator caused additional trouble, as it was initially an unexpected source of difficulty.

4.3.2 Battery longevity

This could be solved by using a larger capacity battery to trickle charge. Modifications would need to be made and confirmation needed (by PP Systems) that trickle charging was actually appropriate.

4.3.3 Memory-chip crashing

This a problem that is still to be resolved in both source, remedy and prevention.

4.4 Conclusion

The system is suitable for field analysis of Antarctic cryptogams, but as it stands now does need some revision and further assessment. Ideally before the next Antarctic field deployment, there should be a field test matching similar conditions that are experienced in this country so as to be able to assist, not only in the procuring of helpful comparative data, but of possible highlighting of other potentially difficult areas such as precipitation and further work at low temperatures could also highlight any other future problems.

Figures attached:-
Plant core cuvette (Fig. 1).
P(blc)(Fig. 2).

Copies to:
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PP Systems
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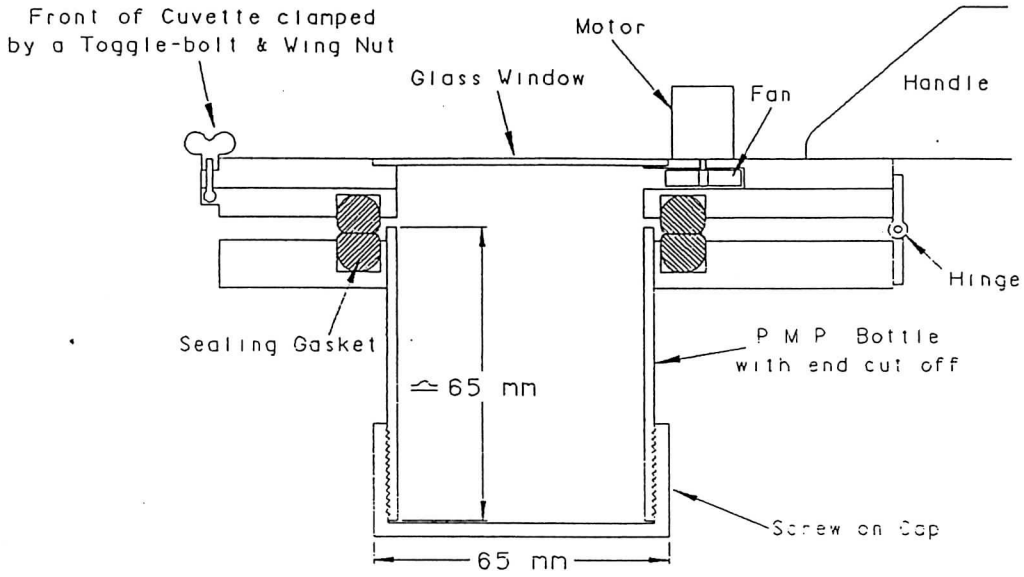
MOSS CHAMBER



Transparent cuvette for Ciras to allow open system measurements of photosynthesis and respiration in mosses and lichens. It uses a modified TPX bottle as the base which is supplied in a range of sizes. A sample of the moss can be placed in the bottle which is then fixed into the lower plate of the cuvette. Alternatively, by leaving off the bottle completely, the lower plate can be pressed directly onto the ground for measurement of plants such as lichens.

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PP SYSTEMS

Fig 1; custom cuvette in mode (3), with adapted beaker.

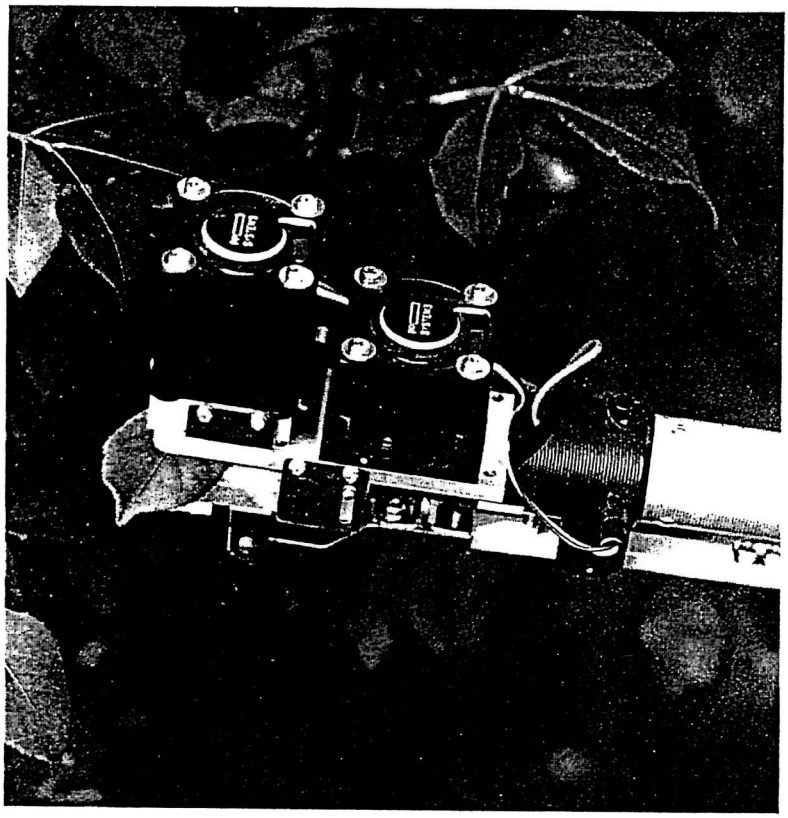

Fig. 2 Photograph of Parkinson broad leaf cuvette, P (blc).