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Influence of solar ultraviolet-B radiation in New Zealand on white clover (*Trifolium repens* L.), ryegrass (*Lolium perenne* L.) and pea (*Pisum sativum* L.)

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To my family

Abstract

New Zealand pasture plants have been exposed to increasing levels of ultraviolet-B radiation (UV-B) as a result of stratospheric ozone depletion during recent years. Thus far, there has been only limited published information on UV-B effects on pasture plants growing under field conditions. This study set out to investigate effects of natural solar UV-B radiation in the field for the pasture species white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) and compared those with pea (*Pisum sativum* L.), another economically important crop. Contrasting UV-B levels were created with two filter systems, using UV-B-absorbing glasshouse polythene film and UV-B-transmitting perspex glass. A further treatment included open plots that were not covered by filters. Ambient UV-B irradiance levels were recorded daily during the experimental period in summer from early December 1995 to late February 1996. The pasture species were investigated in monoculture as well as in their typical association in an established sward that was regularly grazed by sheep. Morphological measurements included leaf expansion, leaf initiation, leaf senescence, stem elongation, above-ground biomass and aspects of plant reproduction. To identify possible responses related to UV-B protection, measurements included specific leaf mass (SLM), accumulation of UV-absorbing compounds and of anthocyanins.

The highest UV-B levels occurred during the early- and midsummer period from mid December to late January. Clouding reduced UV-B irradiance by more than 70%. The UV-B-absorbing treatment reduced ambient UV-B levels by about 90%, and the transmitting filters by about 25%. Results from the monoculture trials revealed interspecific differences in UV-B sensitivity between the three plant species tested. Ryegrass and white clover appeared UV-B-sensitive in a number of vegetative morphological aspects, while pea generally displayed UV-B tolerance. Most features of UV-B sensitivity in the two pasture species were recorded during midsummer in January, with young plant parts particularly affected by the UV-B-transmitting treatments. The area of young white clover leaves was reduced by about 20%, and the length of young internodes by more than 25%. Solar UV-B increased the number of senescing ryegrass leaves in January and induced white clover inflorescence formation in February. Inflorescence numbers were also increased in pea under UV-B-transmitting filters.

While there was no clear relationship between SLM and UV-B susceptibility, the biochemical studies suggest that the interspecific differences in UV-B sensitivity may be due to differences in the accumulation of UV-absorbing compounds and of anthocyanins. Average levels of UV-absorbing compounds across treatments were about 50% higher in pea than in the two pasture species. Furthermore and in contrast to the pasture species, pea showed the highest levels of anthocyanins under solar UV-B in January.

The results from the sward trials were in general agreement with the findings from the monoculture studies, showing that morphological sensitivity under the UV-B-transmitting treatments could also be detected for white clover and ryegrass when grown in association. This was reflected in a reduction of white clover leaf area and of leaf elongation in mature ryegrass tillers by 13%, and by more than 20% in young ryegrass tillers. The effects on the two species under pasture conditions were also reflected in whole sward measurements, showing decreases in sward height of about 15% and in herbage accumulation of about 20% under UV-B-transmitting filters.

In conclusion, the findings from this study show that near-ambient solar UV-B levels can affect the morphology of the two most commonly sown pasture plant species in New Zealand. In contrast, pea showed tolerance to UV-B and this may at least be partly due to higher intrinsic levels of UV-absorbing compounds.

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1. General introduction

A recent New Zealand study shows that decreasing levels of stratospheric ozone result in increases of ultraviolet-B radiation (UV-B, 290 - 315 nm) reaching the Earth's surface (McKenzie et al., 1999). These increases are of particular importance in southern hemisphere regions such as New Zealand, where ambient UV -B levels already are higher than those at comparable northern latitudes. This is largely because the distance between the sun and Earth is shortest during the southern hemisphere summer and because of hemispheric differences in tropospheric and stratospheric ozone (McKenzie et al., 1999).

A number of studies have investigated effects of elevated UV-B radiation on plants, demonstrating numerous morphological and physiological responses. Detrimental UV-B effects include damage to DNA, to membranes, to phytohormones and to photosynthesis, ultimately resulting in reduced plant growth (Rozema et al., 1999c). However, plants have also developed a number of protective mechanisms against UV-B-induced damage. Morphological means of UV-B protection include reflection and scattering by the epidermis, or development of thicker leaves with higher specific leaf mass (Rozema et al., 1999c). Important physiological protective mechanisms comprise DNA repair, radical scavenging and production of UV-absorbing compounds. A predominant role in this regard has been attributed to phenolic compounds, particularly flavonoids such as flavonols and anthocyanins (Woodall and Stewart, 1998; Tsuda et al., 1996; Rozema et al., 1999c).

More than 1000 studies of UV-B effects on plants have been reported to date, and about 90% have been conducted in glasshouses, growth chambers or in the laboratory, using artificially elevated UV-B. Of the studies that have been carried out in the field, most were conducted under northern hemisphere conditions and used UV -B supplementation to study effects of elevated UV-B on plants (Searles et al., 2001). Less information is available on effects of ambient solar UV-B on plants. These effects can best be studied in the field by comparing plants growing under natural UV-B radiation with plants raised in conditions where UV-B is partially or entirely excluded, using filters (Sullivan and Rozema, 1999) or ozone-containing cuvettes (Tevini, 2000).

Recently there have been calls to intensify investigations on the effects of solar UV-B for plants (Paul, 2001). This is due to a number of reasons, e.g. provision of a more realistic spectral balance and the fact that plants are continuously exposed to natural fluctuations in solar UV-B, e.g. via changes in cloud formation. Numerous UV-B filtration studies have shown that plants can be affected by natural, present-day levels of UV-B (Deckmyn and Impens, 1995; Ballare et al., 1996; Mark and Tevini, 1996; Mark and Tevini, 1997; Saile-Mark and Tevini, 1997; Deckmyn and Impens, 1998a; Deckmyn and Impens, 1999; Hunt and McNeil, 1999). This is of importance for plants growing in New Zealand, where UV-B levels are already elevated in comparison with similar latitudes in the northern hemisphere (Madronich et al., 1998) and with pre-ozone depletion UV-B levels (McKenzie et al., 1999). Solar ultraviolet-B radiation in New Zealand has been shown to affect the morphology and physiology of native tree species (Hunt and McNeil, 1999). Potential effects of ambient UV-B could therefore also be expected for temperate species introduced from the northern hemisphere to New Zealand. This includes most plant species of agricultural importance to New Zealand. Conclusions from studies examining effects of ambient UV-B radiation on plants from the northern hemisphere may not necessarily hold true for New Zealand and other southern hemisphere regions.

The most commonly used pasture species introduced in New Zealand are ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), typically grown in combination as forage swards. New Zealand white clover cultivars have shown susceptibility to UV-B under laboratory conditions (Campbell and Hofmann, 1998). Glasshouse experiments have shown UV-B sensitivity of ryegrass to solar UV-B in the northern hemisphere (Deckmyn and Impens, 1999). In addition to having high nutritive value, the legume white clover provides a valuable source of nitrogen to the pasture association with ryegrass. Despite earlier recommendations (Laing, 1991; Laing, 1993), only limited information is available on the effects of UV-B on ecosystems *in situ*, particularly for agricultural ecosystems based on pasture plants.

UV-B effects that can be detected under controlled indoor conditions often disappear in field examinations. This has been related to the effects of other environmental factors that can alter (and in many cases reduce) sensitivity to UV-B. A general advantage of

field studies investigating UV-B responsiveness in plants is the inclusion of a number of other environmental conditions, including variation in the availability of moisture and of nutrients, changes in light intensity and temperature, insect herbivory and grazing by ruminants. Furthermore, indoor studies often include unrealistically high UV-B levels, frequently paired with simultaneously low levels of photosynthetically active radiation (PAR, 400-700 nm), resulting in lower capacity for repair (Allen et al., 1999). For example, indoor studies demonstrated detrimental UV-B responses for pea (*Pisum sativum* L.) (Jordan et al., 1992; He et al., 1993; Gonzalez et al., 1998; Nogues et al., 1998), while subsequent field UV-B supplementation trials found no such UV-B sensitivity (Allen et al., 1999; Stephen et al., 1999). Despite its agricultural importance, only limited information (Becwar et al., 1982) is available on the effects of ambient solar UV-B on pea.

Pea and white clover are members of the Fabaceae, and a number of species in this plant family have shown sensitivity to UV-B (Deckmyn and Impens, 1995; Singh, 1996; Adamse et al., 1997; Pal et al., 1997). There are also indications that UV-B might affect competitive interactions between plant species (Barnes et al., 1996). Of particular interest in this regard is the combination of a monocotyledonous and a dicotyledonous species in the New Zealand pasture association. Grasses have distinctly different morphological and physiological characteristics from dicotyledonous species, and this can be related to reduced UV-B sensitivity (Pal et al., 1997; Cybulski and Peterjohn, 1999; Musil and Wand, 1999).

This thesis reports in self-contained chapters the effects of solar UV-B in New Zealand on white clover, ryegrass and pea. The solar UV-B environment and treatment conditions for these studies are described in chapter 3 (Figure 1-1). Chapter 4 examines the morphological basis of UV-B responsiveness in the three plant species grown in monoculture. In chapter 5, the accumulation of UV-absorbing compounds and of anthocyanins is investigated in these plants to examine possible means of UV-B protection. Chapter 6 examines whether morphological changes to solar UV-B can also be observed in white clover and ryegrass plants growing *in situ* in their usual pasture association. Chapter 7 concludes the thesis with a general discussion on the results, written with a view towards identifying opportunities for future research resulting from the findings of this work.

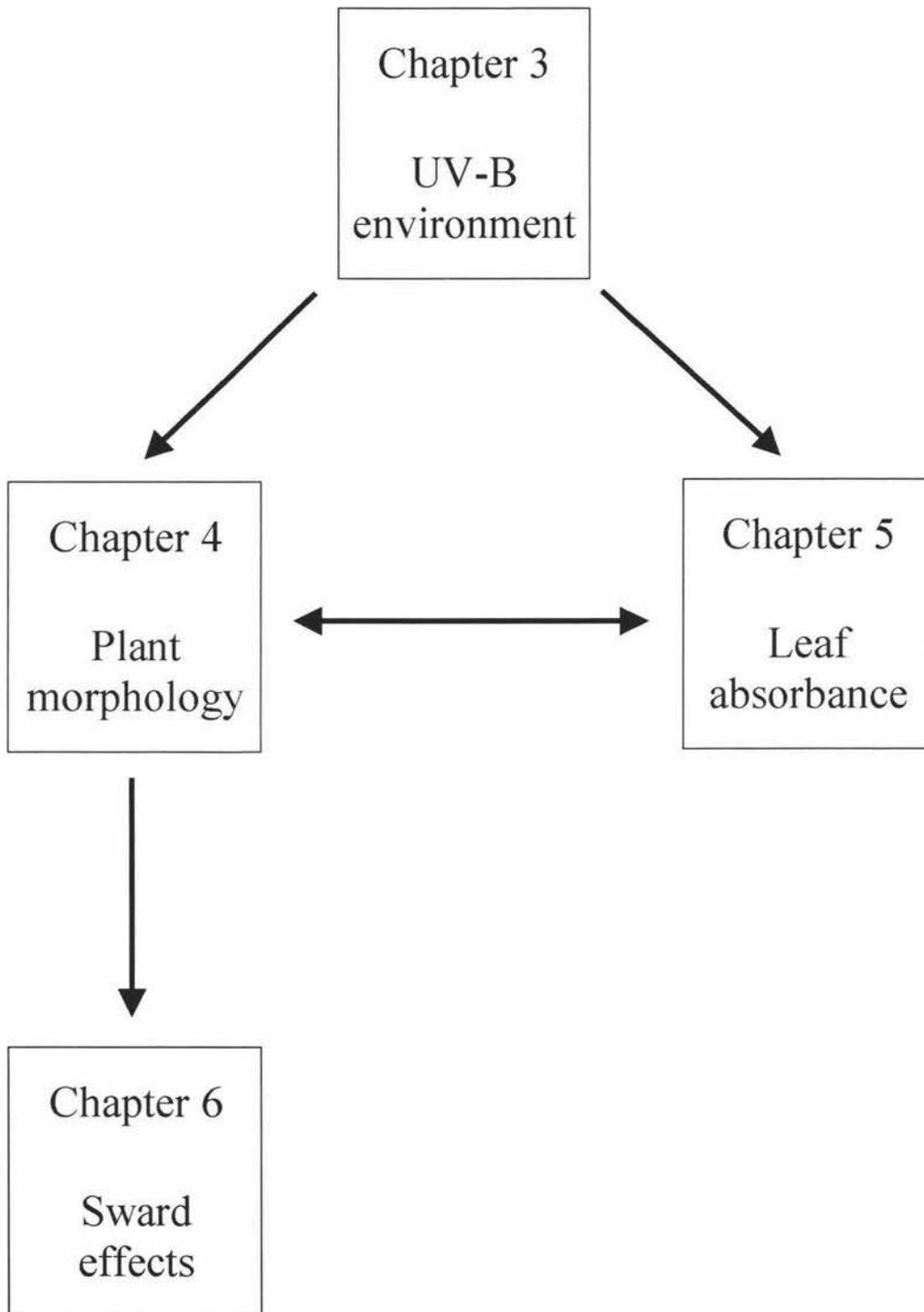


Figure 1-1. Flow diagram showing the main approaches used in this study.

The main hypotheses in this study were: (1) Natural UV-B levels in New Zealand affect growth or morphological development of three herbaceous crop plants. (2) UV-B sensitivity between white clover, ryegrass and pea show interspecific differences with lesser sensitivity in the monocotyledonous ryegrass. (3) Interspecific differences in sensitivity to solar UV-B are related to accumulation of UV-absorbing compounds or of related anthocyanin pigments. (4) Pasture species growing in their typical association in a grazed sward are sensitive to UV-B.

2. Literature review

2.1. Factors influencing UV-B

2.1.1. Ozone and ultraviolet radiation

Since its discovery two hundred years ago, the ultraviolet (UV) spectrum of solar radiation has stimulated a variety of research activities, ranging from investigations on UV absorption by gases in the stratosphere to UV effects on life on Earth. Together with oxygen, atmospheric ozone strongly absorbs the shorter wavelengths of the solar ultraviolet spectrum, UV-C (from 100 - 280 nm) and UV-B (280 - 315 nm), with decreasing efficiency towards longer wavelengths. Wavelengths below about 290 nm are practically entirely absorbed by atmospheric ozone, while UV-A (315 - 400 nm) is only weakly absorbed by ozone (Madronich et al., 1998). Solar UV-B in the lower atmosphere is present as a direct component (maximally 50% of total UV -B) as well as a diffuse component, the latter resulting from scattering and reflection.

2.1.2. Ozone depletion

The predominant effect of a depletion of the stratospheric ozone layer is the increase in irradiance at wavelengths from 290-315 nm (Caldwell et al., 1994; McKenzie et al., 1999). Thus, stratospheric ozone depletion largely affects the UV-B range of the solar spectrum. UV-C is not likely to be affected by the predicted levels of ozone depletion due to the highly efficient absorption by ozone and oxygen at these wavelengths.

The first studies on the possible depletion of the stratospheric ozone layer by man-made gaseous substances were published thirty years ago (Johnston, 1971; Crutzen, 1972). Recent evidence links decreasing levels of stratospheric ozone to increases in UV-B radiation on the surface of the Earth (Madronich et al., 1998; McKenzie et al., 1999). Long-term studies of UV-B levels in New Zealand above Lauder (Central Otago) have provided the strongest evidence yet of a downward trend in stratospheric ozone, closely accompanied by increases in UV-B irradiance reaching ground levels at mid-latitudes (McKenzie et al., 1999). These studies showed that over the last decade, peak plant-

damaging summer UV-B levels increased at a rate of about 1.3% per year in New Zealand (McKenzie et al., 1999). The measured increase in UV-B levels was larger than that predicted from climatologic models and similarly the trend in ozone depletion also was larger than previously expected (McKenzie et al., 1999).

Catalytic destruction of stratospheric ozone during the last 25 years has been linked to anthropogenic emissions, especially halogenated compounds such as chlorofluorocarbons (CFCs) (Molina and Rowland, 1974). These substances diffuse from the troposphere, where they were created by human activities, to the stratosphere. Halogen radicals (mainly chlorine-based) can then be formed from these substances under cold temperatures, particularly in the very cold stratosphere in winter above Antarctica. When the sun appears in polar spring, these radicals catalytically destroy ozone, resulting in severe ozone depletion above Antarctica and spreading from there towards lower latitudes (Huiskes et al., 1999).

Measurements in Antarctica suggest that ozone depletion occurred since the late 1970s (Farman et al., 1985). The duration of the Antarctic ozone hole has also increased since 1990. Enhanced UV-B irradiance due to ozone depletion affects most strongly high latitudes (particularly in the southern hemisphere), followed by mid-latitude regions. Compared to (undepleted) 1970 levels, erythemally-weighted (sunburning) UV-B irradiance in spring has increased by 130% above Antarctica and by 22% in the Arctic, while the annual UV-B increase at mid-altitudes is around 6% (Madronich et al., 1998). The lowest UV-B increases can be found at the tropical lower latitudes, with little change between the equator and 25° latitude (Madronich et al., 1995; Bojkov and Fioletov, 1996). However, it is possible that even subtle increases at low latitudes could have biological consequences due to the already high natural UV-B irradiances present in these regions. Because UV-B levels are naturally highest in equatorial regions, the elevated levels of UV-B irradiance caused by ozone depletion - even the very strong increases in Antarctica - are usually below those found in the tropics.

2.1.3. Other factors influencing UV-B

UV-B levels on Earth are affected by a number of other factors in addition to stratospheric ozone, also resulting in differences in UV-B irradiation between latitudes

and hemispheres. These factors include the distance between sun and Earth, solar zenith angle (SZA), cloud cover and cloud geometry, surface albedo, altitude, volcanic impacts, tropospheric aerosols and gaseous pollutants (Table 2-1). The distance between sun and Earth is smallest in the Austral summer, contributing to the higher natural UV-B levels in the southern hemisphere. The solar zenith angle increases with latitude, and also depends on time of day and season (lowest at solar noon and in summer). The higher the SZA, the more solar UV is absorbed in the atmosphere. Decreases of UV-B irradiance with increasing latitude are also due to a natural latitudinal gradient as the ozone layer is thickest at the poles and thinnest above the equator. Because annual atmospheric decreases in ozone occur naturally after the summer solstice (21 December), the maximum levels of UV-B in the southern hemisphere are usually found in January, while annual minimum levels occur early in spring (Huiskes et al., 1999). The UV-B:PAR ratio is highest at low SZA, i.e. in summer and at solar noon, but can also be high when thick cloud cover affects absorption of PAR more than that of UV-B (Deckmyn et al., 1994). This ratio has important implications for the sensitivity of plants to UV-B because a number of protection- and repair mechanisms depend on adequate availability of PAR (Caldwell et al., 1994; Deckmyn et al., 1994).

Table 2-1. Factors influencing UV-B irradiance reaching ground levels (Madronich et al., 1995; Madronich et al., 1998; McKenzie et al., 1999).

Increase in	Influence on UV-B irradiance at ground level	
	Increase	Decrease
Stratospheric ozone depletion	✓	
Sun-Earth distance		✓
Solar zenith angle and latitude		✓
Cloud cover	✓	✓
Albedo	✓	
Altitude	✓	
Stratospheric aerosols	✓	✓
Tropospheric aerosols		✓
Tropospheric ozone and gaseous pollutants		✓

Furthermore, certain cloud conditions can increase ambient UV-B levels through scattering (e.g. sides of bright broken clouds, cumulus types), while full cloud cover can reduce UV-B irradiance by 80% or more (Madronich et al., 1995). On average, clouds in New Zealand reduce UV-B levels annually by ca. 30 % (McKenzie et al., 1996). Surface reflection (albedo) can increase UV-B irradiance, and values range from 10% for vegetation to up to 100% by snow (Madronich et al., 1995). Increases of UV-B with altitude are due to the thinner atmosphere at higher elevations and range from 4-10% per 1000m in unpolluted regions and up to 40% where lower elevations contain high amounts of pollutants and aerosols (Madronich et al., 1995).

Scattering by stratospheric aerosols (e.g. small particles such as ash and sulphur compounds from volcanic eruptions) can both enhance and reduce penetration of UV -B to ground levels, with the net effect of a slight reduction in UV-B (Madronich et al., 1995; Madronich et al., 1998). Stratospheric aerosols from volcanic eruptions also contribute towards the destruction of stratospheric ozone. Pollution of the troposphere with aerosols (e.g. sulphates, dust, soot), and gaseous UV-B-absorbing pollutants such as ozone and NO₂ can strongly reduce UV-B levels at ground levels, particularly in the northern hemisphere where levels of these pollutants can be high. Sulphate aerosols from fossil fuel combustion can result in marked reductions of UV-B by 5-18% in industrialised northern hemisphere regions (Liu et al., 1991). The higher tropospheric ozone levels in the northern hemisphere may have decreased UV-B levels in that hemisphere by 3-15% (Seckmeyer and McKenzie, 1992; Madronich et al., 1998).

In addition to this, ozone depletion in the southern hemisphere occurs throughout the year while in the northern hemisphere depletions are less pronounced in summer (McKenzie et al., 1999). Taken together, a number of factors, particularly the shorter distance between sun and Earth in summer, and hemispheric differences in tropospheric and stratospheric ozone result in UV-B levels in New Zealand that can be up to 40% higher than at comparable latitudes in the northern hemisphere (Madronich et al., 1998). Measurements in several other southern hemisphere locations (e.g. Argentina, Chile and Australia) have confirmed relatively high UV-B levels compared with similar northern latitudes (Madronich et al., 1995). Some of the most pronounced UV-B effects on plants can be observed using the solar irradiance of Antarctica where springtime UV-B levels can more than double as a result of ozone depletion (Xiong and Day, 2001).

2.1.4. Future trends

A number of very successful international political agreements initiated by the 1987 Montreal Protocol and its subsequent amendments have resulted in ozone-depleting substances in the stratosphere being close to their peak levels (Madronich et al., 1998). However, it is expected that UV-B irradiance at surface levels will continue to stay elevated for many years after the ozone minimum. There is considerable uncertainty about future emissions of ozone-depleting substances from regions in the developing world. Furthermore, there is growing concern about the interaction of global warming with ozone depletion, resulting in stratospheric cooling and possibly delaying the recovery of the ozone layer by decades (McKenzie et al., 1999). Notwithstanding these uncertainties, best estimates predict a return of UV-B irradiance to pre-ozone depletion values no earlier than during the middle of the 21st century (Madronich et al., 1998).

2.2. Effects of UV-B on terrestrial plants

2.2.1. UV-B, plants und action spectra

UV-B irradiance levels from the solar spectrum reaching Earth are much lower than those of longer wavelengths (UV-A and PAR), but the biological effectiveness of UV-B is usually much higher, triggering damage or protection responses in plants (Caldwell et al., 1986). Furthermore, the comparatively small increases in total solar irradiance caused by ozone depletion - less than 0.5% (Searles et al., 2001) - nevertheless also affect predominantly these biologically effective wavelengths.

Due to these reasons is necessary to use a weighting procedure to calculate the biologically effective UV-B irradiance. The wavelength dependence of a biological response or organism expressed as a function is called an 'action spectrum'. Biologically effective UV-B irradiance is simply obtained by multiplying the particular action spectrum (e.g. Caldwell, 1971; Quate et al., 1992a) with the spectral irradiance. The resulting area under the product function is the 'biologically-weighted' UV-B irradiance or dose rate (Madronich et al., 1998). By international agreement, most studies investigating UV-B responses in plants use the Generalised Plant Action Spectrum (GPAS) (Caldwell, 1971), standardised to action = 1 at 300 nm. This

spectrum was compiled 30 years ago from a number of plant responses and shows a strong increase in quantum effectiveness in the UV-B range from 313 nm downwards (Caldwell, 1971).

Despite their usefulness, action spectra have their limitations. Early action spectra have often been developed with monochromatic light. Time spans and irradiance levels of radiation emitted from monochromators are usually divergent from those received from the sun and the application of monochromatic wavelengths precludes detection of interaction effects with multiple wavelengths as in sunlight (Caldwell et al., 1994). The use of an inappropriate action spectrum can lead to over- or underestimation of effects of UV-B. Due to lamp constraints at the time, the GPAS does not include wavelengths longer than about 313 nm and has a very steep initial slope, which is seen to overestimate the UV-B response (Quaite et al., 1992b; Mark et al., 1996). Nevertheless, because it is widely applied in UV-B plant research it allows at least some comparability between experiments. From this, the radiation amplification factor (RAF) can be calculated, defined as the increase in biologically-weighted irradiance per 1% decrease in ozone (Madronich et al., 1995). Depending on ozone column thickness and SZA, a 1% decrease in ozone can result in an increase of up to 2% in plant-weighted UV-B (Madronich et al., 1995).

2.2.2. Two approaches for the study of UV-B effects on plants

More than 1000 papers have been published investigating numerous aspects of UV-B effects on terrestrial plants (e.g. reviewed by Caldwell, 1971; Stapleton, 1992; Bornman and Teramura, 1993; Tevini, 1993; Teramura and Sullivan, 1994; Caldwell et al., 1995; Rozema et al., 1997c; Caldwell et al., 1998; Rozema et al., 1999c; Searles et al., 2001). Approaches in UV-B plant research can broadly be divided in two categories: (1) studies supplementing UV-B using artificial lamp systems; and (2) studies excluding UV-B using absorbing materials (Table 2-2). In addition, some studies have used latitudinal (Robberecht et al., 1980) or altitudinal gradients (Rozema et al., 1997a) to examine plant responses to UV-B. However, such studies are confounded by concomitant changes in other environmental variables along the gradient, e.g. temperature (Rozema et al., 1999c).

Table 2-2. Experimental approaches in studies of UV-B effects on plants and some of their advantages (+) and disadvantages (-).

Approach	Features
I. UV-B supplementation	
1. Indoors	
+	<ul style="list-style-type: none"> → Controlled environmental conditions → Efficient screening of numerous plant species or cultivars → Facilitates study of mechanisms
-	<ul style="list-style-type: none"> → Dependence on weighting function → Lack of spectral balance → Discrepancies with results in the field
2. Outdoors	
a) Square wave	
+	<ul style="list-style-type: none"> → More realistic than indoor UV-B supplementation → Relatively inexpensive
-	<ul style="list-style-type: none"> → Dependence on weighting function → Lack of spectral balance
b) Modulated	
+	<ul style="list-style-type: none"> → Most realistic UV-B supplementation system → Simulation of future ozone depletion scenarios
-	<ul style="list-style-type: none"> → Expensive and complex design → Dependence on weighting function
II. UV-B filtration	
1. Filters	
+	<ul style="list-style-type: none"> → Wide range of field applications → Study of impacts of current UV-B levels → Realistic spectral balance → Inexpensive
-	<ul style="list-style-type: none"> → Interception of precipitation → Increased temperature and humidity → No simulation of future ozone depletion scenarios
2. Ozone cuvette	
+	<ul style="list-style-type: none"> → Study of impacts of current UV-B levels → Realistic spectral balance
-	<ul style="list-style-type: none"> → As for filters → Only suitable for small scale trials with pots → Exclusion of outdoor conditions → Expensive and complex design → Low true replication

2.2.2.1. UV-B supplementation

Most studies of UV-B plant research have been conducted indoors, in controlled environments such as laboratories, growth rooms or glasshouses. Using artificial UV-B supplementation from lamp systems, many of these studies have reported a variety of UV-B-induced effects on plants. However, subsequent UV-B supplementation investigations in the field have often failed to detect the strong responses observed indoors. One major factor that has been used to explain this discrepancy is the lower UV-B:PAR ratio in the field, resulting in increased potential for UV-B protection of plants growing outdoors.

Many UV-B supplementation studies in the field used a 'square wave' approach, i.e. supplementing set amounts of UV-B around solar noon. This can lead to very high UV-B:PAR ratios, particularly during cloudy periods. A more realistic spectral balance has been obtained with sophisticated (and expensive) modulation systems that continuously monitor changes in solar UV-B levels to allow realistic supplementation of UV-B (Rozema et al., 1999c). Even in these advanced modulation systems, however, application of supplemental UV-B is reliant on action spectra and biological weighting functions, which at best can only provide an approximation of biologically relevant spectral output. There is no perfect weighting spectrum and limitations in the exact simulation of the spectral distribution of solar radiation and inaccuracies in the calculation of ground-level solar irradiation by theoretical models (Fiscus and Booker, 1995) complicate modulation of UV-B from lamps.

A recent seminal review provides for the first time overall quantitative assessment of main plant responses to UV-B supplementation in the field (Searles et al., 2001). Using meta-analytic statistical methods, this survey examined findings in 450 reports from 62 papers, representing the majority of field studies from 1976 to 1999. A number of important plant features common to many UV-B studies were analysed, including aspects of plant morphology and growth, leaf pigmentation, as well as photosynthesis.

The main findings from this survey were the relative insensitivity of plant growth (measured as shoot biomass) to moderately enhanced UV-B, accompanied by an increase in UV-B-absorbing pigmentation (Searles et al., 2001). These findings could

therefore be interpreted as demonstrating that plants are generally protected against likely increases of UV-B radiation and that this protection may be due to increased amounts of compounds acting, amongst other functions, as sunscreens. Overall, plant growth was only inhibited when higher levels of UV-B supplementation were used (Searles et al., 2001). The survey also showed that specific leaf mass, reproductive yield, leaf photosynthetic processes and photosynthetic pigmentation were generally not affected by UV-B supplementation in the field. However, decreases could be found for some morphological measurements at all levels of UV-B supplementation, including leaf area per plant and plant height. Such morphological shifts may in turn lead to shifts in competitive relationships between plant species (Searles et al., 2001). Pasture species were not included in this analytical survey and inter- or intraspecific variation in the response of plants to supplemental UV-B was also not examined.

2.2.2.2. UV-B filtration

Given that UV-B levels are already elevated worldwide and are projected to stay elevated for years to come it is not surprising that research on effects of current solar UV-B effects has recently been intensified. Such studies utilise the sun as the source of UV-B irradiance, which is subsequently modified from near-ambient to near-exclusion levels by means of selective filtration (Table 2-2). The usual treatment set-up in these studies consists of (1) a UV-B-absorbing filter treatment providing near-exclusion of UV-B and (2) a UV-B-transmitting filter treatment with near-ambient UV-B levels (Table 2-3). In addition, some studies also include an ‘open’ treatment without plastic film covers, to separate UV-B effects from canopy effects (Table 2-3). The UV-B-absorbing treatment can be complemented by using filters that also absorb UV-A, or by using filters that absorb differential amounts of UV-B. UV-B filtration studies are characterised by a number of advantages and because the present thesis is based on this approach, the next paragraphs will discuss these in more detail.

Table 2-3. Treatments used in studies of UV-B filtration.

Treatments	Filter	UV-B exposure
Open frame		✓
UV-B-transmitting	✓	✓
UV-B-absorbing	✓	

Characteristics of UV-B filtration

Perhaps the biggest advantage of filtration systems is that the sun (rather than lamps) is used as the source of UV-B. Combined with selective filters this makes it possible to apply differential UV-B levels without having to use weighting functions. This set-up therefore allows better maintenance of the spectral balance between UV-B and longer wavelengths. Moreover, filtration of UV-B allows the examination of ambient UV-B effects on plants. This is important, because plants are continuously exposed to natural fluctuations in UV-B irradiance. The relative extent of natural variation in UV-B levels often strongly exceeds that caused by ozone depletion. This variation can even result in practically complete UV-B exclusion, depending on a number of factors including position within the plant canopy, cloud cover, solar zenith angle and levels of air pollution (see also Table 2-1 above). Ambient UV-B irradiance varies from continuous changes (e.g. due to clouding and SZA) to changes over seasons and even over decades (due to ozone depletion) (Huiskes et al., 1999). While certain cloud configurations and distributions can increase ambient UV-B, UV-B irradiance is generally reduced by clouds, reaching or exceeding 80% reduction of ambient solar UV-B (Madronich et al., 1995). Changes in the plant canopy can also result in marked effects of solar UV-B, e.g. exposing shade leaves to full sunlight can lead to marked photoinhibition (Krause et al., 1999). Dose-response studies suggest that even in the range of below-ambient UV-B levels there is no threshold level for detrimental UV-B effects to occur (de la Rosa et al., 2001).

Furthermore, filtration represents an inexpensive method that is relatively easy to construct and maintain to provide differential UV-B irradiation treatments. UV-B exclusion systems do not involve potentially hazardous lamps and the lack of electricity has provided numerous useful applications in a wide variety of environments, from remote regions in Antarctica (Xiong and Day, 2001) to the tropics (Searles et al., 1995). Because they are inexpensive, these systems allow higher replications with realistic plot sizes, which is of particular advantage for agricultural field trials. Although less complicated, filtration studies in general show the same direction of UV-B responsiveness to solar UV-B as is found under UV-B supplementation (Cybulski and Peterjohn, 1999).

Some of the limitations of the UV-B filtration approach include possible canopy effects and no possibility to examine future ozone depletion scenarios (Table 2-2). Canopy effects such as increased temperature or interception of precipitation under the filters can be reduced (e.g. by compensatory watering under the filters) and controlled by inclusion of an open frame control without filters to interpret the findings from near-ambient UV-B exposure. Furthermore, due to reductions in man-made ozone-depleting substances, it is possible that UV-B levels will not increase much further in the future and that for the next decades the question is more one of how plants will deal with UV-B irradiances similar to the currently elevated levels. UV-B filtration is a particularly suitable experimental approach in regions where UV-B levels are already elevated due to ozone depletion, such as in southern hemisphere mid to high latitudes (Searles et al., 1999). In contrast, UV-B field supplementation may be of more relevance in other regions where UV-B levels have not yet been substantially altered by ozone depletion (Searles et al., 1999).

The UV-B environment in New Zealand provides particular opportunities for the use of filtration studies to examine effects of stratospheric ozone depletion as well as of natural UV-B fluctuations. Compared to similar northern latitudes, temperate southern hemisphere regions are more affected by ozone depletion and receive up to 40% higher ambient UV-B levels (Madronich et al., 1995; Madronich et al., 1998). Furthermore, the geographical location of New Zealand favours relatively frequent environmental changes (particularly by clouds) in a relatively unpolluted troposphere.

A brief history of UV-B filtration studies

Filtration has already been used to study effects of solar UV-B on plants since early last century (e.g. Shibata and Kishida, 1915; Schanz, 1920; Popp and Brown, 1936; Foley, 1963; Caldwell, 1968; Bartholic et al., 1975; Becwar et al., 1982). Many of the earlier UV-B exclusion studies often were compromised by canopy effects, e.g. marked differences in PAR transmission and temperature between different filtration treatments (Caldwell, 1968; Caldwell, 1971). Other deficiencies included lack of appropriate controls, lack of true replication (i.e. plots rather than plants), and the use of pots rather than plots (Caldwell, 1971; Ballare et al., 1999).

Several research groups have formed worldwide to focus attention on solar UV-B effects on terrestrial plants. A Dutch group conducted several field experiments that investigated effects of solar UV-B in the Netherlands on dune grassland species and crops (Tosserams et al., 1996; Visser et al., 1997; Rozema et al., 1999b). Most of the recent field work was conducted by a US-Argentinean group using the elevated southern hemisphere UV-B levels in Argentina (Searles et al., 1995; Ballare et al., 1996; Rousseaux et al., 1998; Ballare et al., 1999; Mazza et al., 1999a; Mazza et al., 1999b; Rousseaux et al., 1999; Searles et al., 1999; Barnes et al., 2000; Mazza et al., 2000). Another research group recently utilised the even higher elevated UV-B levels in Antarctica to study solar UV-B effects on native vascular plants in the field (Day et al., 1999; Ruhland and Day, 2000; Ruhland and Day, 2001; Xiong and Day, 2001).

A German research group has developed the filtration approach further, using hollow UV-B-transparent plastic filters (cuvettes) that can be filled with various levels of ozone gas to screen UV-B (Mark and Tevini, 1996; Mark and Tevini, 1997). Ozone levels in these cuvettes can be accurately adjusted to expose plants to pre-depletion UV-B levels and thus are particularly suitable to study the effects of recent increases in UV-B. The German group used such ozone cuvettes (Table 2-2) and specialised glasshouses to study the effects of higher UV-B levels in Portugal on crops (Tevini et al., 1991; Mark et al., 1996; Mark and Tevini, 1996; Mark and Tevini, 1997; Saile-Mark and Tevini, 1997). However, due to a number of factors (Table 2-2), including size constraints and limited comparability to field situations, reliance on electricity, cost and low replicability, the cuvette technique is best kept for special investigations backed up by larger-scale filter studies in the field.

2.2.3. General plant responses

As UV-B filtration was the method chosen in the current project, the emphasis in further reviewing the relevant literature is on studies using similar approaches. There is an increasing database of more than 80 investigations that have utilised UV-B filtration to study UV-B responsiveness in terrestrial plants. Many of these experiments were conducted under field conditions and have revealed important UV-B-induced changes at molecular, morphological, biochemical, physiological and ecosystem levels.

Some of the main effects of solar UV-B on plant growth, morphology and physiology are summarised in Table 2-4. Several conclusions can be drawn from this table which is based on results from 24 UV-B filtration studies. (1) Most herbaceous dicotyledonous species are sensitive to solar UV-B. (2) In addition, several monocotyledonous species are affected, while trees (dicotyledons and gymnosperms) and a moss species are less sensitive to solar UV-B. (3) UV-B sensitivity expressed as biomass reduction is closely accompanied by leaf area decreases, particularly in dicotyledons. (4) Where change in shoot length occurs, this is towards a decrease, whereas SLM often increases. (5) Solar UV-B often enhances UV-absorbing compounds but this does not necessarily confer UV-B tolerance. (6) Solar UV-B effects on photosynthetic parameters show no general direction, nor do they correspond with changes in biomass.

A review of UV-B effects on plant functional types classified plant groups on the basis of 39 field studies published by early 1999 (Gwynn-Jones et al., 1999b). The ranking from lowest to highest UV-B sensitivity was: trees = grasses < shrubs < forbs < bryophytes. However, the majority of results used in that comparison were from UV-B supplementation studies (usually with a square wave function), which may help explain the partly differential picture emerging from the more recent data from UV-B filtration experiments (Table 2-4). While UV-B tolerance of trees is confirmed in filtration studies, grasses show a more variable biomass response (Table 2-4). The findings from filtration studies confirm general UV-B sensitivity of herbaceous dicotyledonous forbs, and comparisons for bryophytes are too early to tell. Preliminary results in *Sphagnum* show no responses to solar UV-B, but plant biomass was not measured (Searles et al., 1999). UV-B filtration studies have only recently become more common and it may well be possible that the UV-B sensitivity ranking of plant groups will change once an even larger sample size of studies on the effects of solar UV-B can be included.

Table 2-4. Results from recent studies investigating the morphological and physiological effects of solar UV-B. “+“ designates increase, “-“ decrease and “o“ no change in response to solar UV-B. D = herbaceous dicotyledon, M = monocotyledon, T = dicotyledonous tree, G = gymnosperm, B = bryophyte. Biomass = plant biomass, SLM = specific leaf mass, UV-abs = UV-absorbing compounds, PS = photosynthesis, which can include aspects of photophosphorylation, carbon fixation, stomatal control or photosynthetic pigment concentration. Where studies included other treatments (e.g. differential levels of CO₂ or temperature), the UV-B responses were reported at the treatment levels that were most similar to ambient conditions. If plants were examined in different seasons, results from the summer season are presented.

Species	Functional type	Bio-mass	Leaf area	Shoot length	SLM	UV-abs	PS
<i>Arabidopsis</i> ¹	D	o		o		+	
<i>Colobanthus</i> ²	D	-	-		+	+	o/-
Cowpea ³	D	-	-	-	+	+	-
Cucumber ^{4,5}	D	-	-	-	+	o	
<i>Datura</i> ⁶	D	-	-	-	+		
Dwarf bean ^{7,8}	D	-	-	-	-	+	+
Faba bean ⁹	D	o	-	-	o	o	o
<i>Gunnera</i> ¹⁰	D	-	-		o	o	
Lettuce ¹¹	D	-	-			+	+/o
Mung bean ¹²	D	-	-	-			-
New Zealand spinach ⁵	D	-	-	o	o	+	
Soybean ⁵	D	-	-			-	
Sunflower ¹³	D	-	-	-			-
Dune grassland spp. ¹⁴	M,D	o	o	o	o	+	o
Barley ¹⁵	M	-	o*		o	+	
<i>Bromus</i> ¹⁶	M	+	o	-	o	o	+
<i>Carex</i> ¹⁷	M	o	o*				
<i>Deschampsia</i> ²	M	-	-		+	+	o/-
Grassland spp. ¹⁸	M	+/-	+/-	-	-	+/o	+
Maize ¹²	M	o	o	o			-
Maize ^{13,19}	M	-	-	-		o	-
Wheat ²⁰	M	-	-	o		+	
Tropical plant spp. ²¹	T,D	o	-*	-	+/o	+	o
<i>Griselinia</i> ²²	T	o	o	o	o	+	o
<i>Nothofagus</i> ²²	T	o	-	-	o	+	o/-
<i>Populus</i> ²³	T	o	o	o	o	+	-
<i>Pinus</i> ²⁴	G	o	o			+	
<i>Sphagnum</i> ¹⁷	B			o		o	o

* Instead of leaf area, leaf elongation was measured.

¹ Fiscus et al., 1999; ² Xiong and Day, 2001; ³ Lingakumar et al., 1999; ⁴ Krizek et al., 1997; ⁵ Adamse et al., 1997; ⁶ Ballare et al., 1996; ⁷ Deckmyn and Impens, 1995; ⁸ Saile-Mark and Tevini, 1997; ⁹ Visser et al., 1997; ¹⁰ Rousseaux et al., 1998; ¹¹ Krizek et al., 1998; ¹² Pal et al., 1997; ¹³ Mark and Tevini, 1997; ¹⁴ Tosserams et al., 1996; ¹⁵ Mazza et al., 1999a; ¹⁶ Deckmyn and Impens, 1998a; ¹⁷ Searles et al., 1999; ¹⁸ Deckmyn and Impens, 1999; ¹⁹ Mark et al., 1996; ²⁰ Häder, 1996; ²¹ Searles et al., 1995; ²² Hunt and McNeil, 1999; ²³ Schumaker et al., 1997; ²⁴ Turunen et al., 1999

2.2.4. Damage

2.2.4.1. Vegetative growth and morphology

A number of filtration studies have revealed changes in plant morphology due to solar UV-B radiation. Ambient UV-B levels at high (Xiong and Day, 2001), middle (Krizek et al., 1997) and low latitudes (Searles et al., 1995) have all been shown to negatively affect plant growth or morphological characteristics. Typically, decreases in growth and morphological development (e.g. plant biomass or leaf area) due to solar UV-B range around 10-20% (Ballare et al., 1999).

Reduced plant biomass accumulation in response to solar UV-B can be found in most investigations of herbaceous dicotyledonous species (Table 2-4). The close relationship between reductions in biomass and leaf area support statements that leaf area reductions are the main cause of plant growth reduction under solar UV -B (Ballare et al., 1996). In cases where this relationship does not hold, it may be explained by no changes or even opposite changes in related morphological features. For example in mountain beech (*Nothofagus solandri* var. *cliffortioides*), solar UV-B reduced size per leaf but increased leaf number (Hunt and McNeil, 1999), with a net nil effect on total plant leaf area and biomass. The heterogeneous effects of solar UV -B on leaf appearance are also reflected by other studies, e.g. showing that leaf numbers are reduced in New Zealand spinach (Adamse et al., 1997) and increased in *Bromus* (Deckmyn and Impens, 1998a).

Compared to leaf area, decreases in shoot length appear less frequently and are less closely related to those in biomass accumulation (Table 2-4). Only a few studies have investigated UV-B effects on senescence. Some results suggest decreased senescence in response to solar UV-B (Tezuka et al., 1993; Tosserams et al., 1996). Other examinations, however, e.g. of a number of European heathland plants, reveal UV-B-elicited accelerated leaf senescence in several species (Björn et al., 1997). Effects on senescence have been related to presence of free radicals in response to changes in the radiation environment (Tezuka et al., 1993). Recent findings in *Arabidopsis* revealed UV-B-induced increases of senescence - in the form of loss of chlorophyll and increased lipid damage - were accompanied by up-regulation of a number of senescence-related genes (John et al., 2001).

Compared to studies on vegetative responses, effects of solar UV-B on regenerative growth have received lesser attention (Deckmyn and Impens, 1998a). Results suggest no distinct pattern in the direction of the UV-B-induced change for flowering, which can differ within a genus, and even a species (Musil and Wand, 1999). A number of studies report stimulatory effects of solar UV-B on timing of flowering, flower number, weight, seed production and other reproductive features (Visser et al., 1997; Deckmyn and Impens, 1998a; Cybulski and Peterjohn, 1999), but there are also reports of negative consequences, including delayed flowering and decreased flower production, lower weight of reproductive parts (Mark et al., 1996; Fiscus et al., 1999; Musil and Wand, 1999) or even no change in reproductive development (Searles et al., 1999). A more consistent response to UV-B seems to be the inhibition of pollen germination or tube growth, suggesting that pollination may be a particularly vulnerable developmental stage under UV-B (Musil and Wand, 1999). This has been related to increased UV-B transmission through pollen walls and subsequent damage to pollen DNA (Musil and Wand, 1999).

2.2.4.2. DNA damage

Several mechanisms of UV-B-induced growth reductions in plants have been proposed, but clear evidence is still outstanding (Ruhland and Day, 2000). DNA is probably the most important target for UV-B damage (Björn, 1997). Inhibition of transcription and replication under solar irradiation is often caused by UV-B-induced formation of pyrimidine (6,4) pyrimidinone dimers (6,4 photoproducts) and cyclobutane pyrimidine dimers (CPDs) (Stapleton et al., 1997; Ballare et al., 1999). Investigations with mutants show that plants lacking DNA repair mechanisms are strongly damaged by UV-B (Britt et al., 1993). A number of UV-B filtration experiments have clearly shown that the UV-B portion of the solar spectrum induces CPDs in several plant species (Ballare et al., 1996; Stapleton et al., 1997; Mazza et al., 1999a; Rousseaux et al., 1999; Mazza et al., 2000). Above-ground tissue is more affected than roots and the large majority of damage occurs in the epidermal cells (Stapleton et al., 1997). Compared to UV-B filtration, ambient UV-B levels can double DNA damage (Ballare et al., 1996). Studies in the southern hemisphere have shown close relationships of DNA damage to fluctuations in day-to-day UV-B irradiance under springtime ozone depletion in southern South America (Rousseaux et al., 1999). Furthermore, DNA damage increases

with seasonal increases in solar UV-B (Ballare et al., 1999). However, at higher summer UV-B levels, CPDs in *Datura* increase to a lesser degree than at subambient UV-B levels (Ballare et al., 1996). This has been attributed to several mechanisms of protection, which are all elicited by higher levels of PAR and UV radiation, including higher UV-absorbing compound formation, increased activity of photolyase and higher SLM (see below). UV-B exclusion work has also shown that growth reductions due to ambient UV-B are usually accompanied by increased DNA lesions in leaves, e.g. in barley (Mazza et al., 1999a) and in soybean (Ballare et al., 1999).

2.2.4.3. Reductions in photosynthesis

A number of studies in controlled environments have shown that all three major areas of photosynthetic function can be affected by UV-B, i.e. photochemical reactions in the thylakoids (He et al., 1993), carbon fixation (Vu et al., 1982) and stomatal control of CO₂ supply (Negash and Björn, 1986). While UV-B treatment often reduces leaf area, decreases in photosynthetic rate per unit area are rare, especially under realistic field conditions (Xiong et al., 1997). For a number of years, photosystem II was seen as a particularly sensitive target for UV-B. However, the overall outcome from field investigations using UV-B supplementation shows no detrimental effect of UV-B on photosystem II (Searles et al., 2001). Other photosynthetic functions such as carbon fixation or stomatal control could still be affected by UV-B, but this is only likely at very high, unrealistic levels of UV-B supplementation (Allen et al., 1998). Similar conclusions can be drawn from the existing knowledge of solar UV-B effects on photosynthesis in plants. Recent studies show a heterogeneous picture with no clear direction of photosynthetic changes under solar UV-B (Table 2-4). Some studies show no effects of ambient UV-B levels on net photosynthesis, chlorophyll levels and stomatal control, e.g. in faba bean (Visser et al., 1997). Other results point at positive effects by solar UV-B on photosynthetic parameters, e.g. on maximal photosynthesis and on chlorophyll levels in *Phaseolus* (Deckmyn and Impens, 1995), lettuce (Krizek et al., 1998) and *Bromus* (Deckmyn and Impens, 1998a). This is balanced by studies demonstrating damaging effects of solar UV-B on photosynthesis, e.g. on net photosynthesis and stomatal conductance in *Populus* (Schumaker et al., 1997), net photosynthesis in mung bean (Pal et al., 1997) and photoinhibition in young mountain beech leaves (Hunt and McNeil, 1999). Exposure of shade leaves of tropical plants to

direct sunlight resulted in reduced photosystem II efficiency, probably due to protein damage in photosystem II (Krause et al., 1999). This was only slowly reversible (requiring at least 10 days), when leaves were placed back in shade. In Antarctic vascular plants, efficiency of photosystem II was not affected and it was suggested that light-independent enzymatic, rather than photosystem II limitations were responsible for reduction of photosynthesis in the upper mesophyll of leaves, represented by reduced quantum yield of photosystem II (Xiong and Day, 2001). While rates of photosynthetic O₂ evolution were reduced by solar UV-B on a chlorophyll and dry-mass basis, they were not sensitive on a leaf area basis (Xiong and Day, 2001). Plant growth reductions can nevertheless be observed in the absence of changes in photosynthetic rates (Xiong et al., 1997). Overall, however, there is general agreement that neither realistic supplementation nor ambient levels of UV-B have a marked detrimental effect on photosynthesis in plants (Ballare et al., 1999; Searles et al., 2001).

2.2.4.4. Other possible mechanisms

Another mechanism responsible for UV-B-generated damage is stimulation of a photoreceptor, e.g. flavin, that reduces stem elongation (Ballare et al., 1995), or cell division in leaves (Nogues et al., 1998). Other photoreceptors that have been proposed in this regard include phytochrome (Stapleton, 1992) and auxins (Ros and Tevini, 1995). Inhibition of production (e.g. via photooxidation by UV-B or oxidation by UV-B-induced peroxidases) or of transport of plant hormones such as auxins and gibberellins is seen as a major cause of UV-B-induced growth reduction (Kulandaivelu et al., 1989; Tevini, 1994; Ros and Tevini, 1995). In addition, concentrations of the hormone ethylene can be increased by UV-B, resulting in reduced cell elongation (Predieri et al., 1993).

UV-B-induced increases in secondary compounds can also limit growth. For example, wall-bound phenolic acids can constrain cell expansion by forming cross-links between cellulose and hemicellulose microfibrils in the cell wall (Liu et al., 1995; Ruhland and Day, 2000). A study in the native Antarctic grass *Deschampsia* indicated a trend towards such increases for wall-bound ferulic acid in response to solar UV-B (Ruhland and Day, 2000). Furthermore, certain flavonoids (mainly quercetin compounds) that are enhanced by UV-B also act as inhibitors of auxin function (Meijkamp et al., 1999).

Finally, there could be protein damage, e.g. of enzymes with important functions in cell expansion (Schumaker et al., 1997), or general changes in membrane activity due to peroxidation of membrane lipids by free radicals (Kramer et al., 1991).

2.2.5. Protection

Important biochemical and molecular protective mechanisms against UV-B damage include the accumulation of UV-absorbing “sunscreen” compounds and of antioxidants as well as photoenzymatic DNA repair. Important morphological means of protection against damage include scattering and reflection of UV-B, thickening of the epidermis or of entire leaves and growth delay. These mechanisms of protection can be separated into processes that either prevent or repair UV-B damage.

2.2.5.1. Prevention (avoidance)

UV-absorbing compounds

Accumulation of UV-absorbing compounds is one of the most important mechanisms of plant defence against UV-B. Increased levels of UV-absorbing compounds under UV-B were often observed in indoor experiments, but this is now also considered a general response to UV-B supplementation in the field (Searles et al., 2001). Among the UV-absorbing compounds, a large part of prevention of UV-B damage in plants can be attributed to secondary phenolic compounds. A number of colourless pigments (mainly phenylpropanoids) allow transmission of PAR, while acting as “sunscreens” in the UV region. Key enzymes in the phenylpropanoid pathway are induced by solar UV-B, including phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) (Liu and McClure, 1995; Krizek et al., 1998). This in turn can lead to increased levels of numerous phenolic UV-absorbing compounds, including flavonoids and hydroxycinnamic acids. Mutants lacking in these compounds show reduction in plant growth in response to UV-B (Landry et al., 1995; Bieza and Lois, 2001). In many herbaceous plants, flavonoids are the main UV-absorbing compounds in the vacuoles of epidermal cells and their concentration can be increased by UV-B, while hydroxycinnamic acids form a more intrinsic shield in cell vacuoles of internal leaf layers (Bornman et al., 1997). The regulation of flavonoid biosynthesis is seen to

involve phytochrome, blue light and UV absorbing photoreceptors (Beggs and Wellmann, 1994).

UV-B filtration studies also show a predominant trend towards increased levels of UV-absorbing compounds under solar UV-B (Table 2-4). This has been shown for numerous plant species, e.g. *Phaseolus* (Deckmyn and Impens, 1995), wheat (Häder, 1996), conifers (Turunen et al., 1999) and dicotyledon tree species (Searles et al., 1995). Filtration experiments with soybean have demonstrated that absorption of UV-B is strongly increased in the epidermis of leaves exposed to solar radiation (Ballare et al., 1999). This response was wavelength-dependent, largely triggered by the UV-B component of the solar spectrum (Mazza et al., 2000) and strongly resembled the general plant action spectrum (Caldwell, 1971). Thus, accumulation of protective UV-absorbing compounds is particularly induced by the more damaging portion of the solar spectrum. The studies in soybean also demonstrated that the functional significance of increased levels of UV-absorbing compounds under solar UV-B was the protection of leaf tissue against DNA damage (Mazza et al., 2000).

In addition to UV-absorbing compounds in the vacuole, the accumulation of such compounds in anticlinal walls, particularly phenolics such as ferulic and coumaric acids, can strongly reduce transmission of UV-B through the epidermis (Ruhland and Day, 2000). This is of particular importance for herbaceous angiosperms, which usually show relatively high UV-B transmission through anticlinal walls (Day et al., 1993).

Morphology and growth delay

In addition to biochemical changes, plants can also develop morphological mechanisms of UV-B avoidance. An important trait in this regard is the ratio of leaf dry weight over leaf area (specific leaf mass, SLM), which often increases in indoor UV-B supplementation studies. While also related to higher leaf density (e.g. increased concentration of starch and other molecules) (Britz and Adamse, 1994), this response has generally been interpreted as a thickening of plant leaves. This can increase scattering, optical path length and absorption of UV-B (Ballare et al., 1996). Where changes in SLM occur in solar exclusion studies (mainly in herbaceous dicotyledons), the direction is predominantly towards increased SLM (Table 2-4). A study of pot-

grown grasses in the glasshouse showed that tolerance to northern hemisphere solar UV-B levels was partially related to increased SLM (Deckmyn and Impens, 1999).

Because SLM can also be affected by leaf density, direct measurements of thickness provide further structural information. Increases in epidermal thickness have been observed in cucumber in response to a 25% enhancement of ambient New Zealand UV - B levels (Hunt and McNeil, 1998). Solar UV-B has been shown to increase epidermal and whole leaf thickness in southern hemisphere native plants (Hunt and McNeil, 1999; Ruhland and Day, 2000). Interspecific comparisons in New Zealand temperate trees suggest that species with intrinsically thicker leaves are less responsive to UV-B (Hunt and McNeil, 1999). The species that was more responsive to solar UV-B (mountain beech) had thinner leaves with a thinner epidermis (Hunt and McNeil, 1999). These filtration studies also showed for mountain beech that ambient New Zealand UV-B levels can decrease transmittance of wavelengths > 380 nm, concomitant with an increase in reflectance between 280 nm and 450 nm (Hunt and McNeil, 1999). In mountain beech and broadleaf plants, transmittance of PAR was decreased and reflectance increased by solar UV-B. Absorptance of PAR in leaves of broadleaf plants also decreased under solar New Zealand UV-B levels (Hunt and McNeil, 1999). Thus, while largely opaque to UV-B and UV-A radiation, ambient New Zealand UV-B levels affect optical leaf properties in leaves of native plants in the PAR range, with increased reflectance and decreased transmittance. It was suggested that this was related to increased leaf thickness and could also be related to alterations in surface waxes (Hunt and McNeil, 1999). Three epidermal coverings can protect plants from solar UV-B, namely thick cuticles, leaf surface waxes and dense trichomes (Musil and Wand, 1999).

UV-B-induced thickening of the epidermis could increase the pathway of UV-B and absorption through UV-absorbing compounds such as flavonoids, which are usually present in epidermal vacuoles (Bornman et al., 1997). For example in pine needles, reductions in epidermal transmittance under solar UV-B were accompanied by increases in UV-absorbing compounds and in epidermal thickness (Turunen et al., 1999). Another morphological mechanism of UV -B avoidance is heliotropism. In soybean for example, UV-B-tolerant cultivars have been observed to move their leaves to intercept light at more oblique angles, thus ameliorating UV-B damage (Young et al., 1993). UV-B-sensitive cultivars did not show such heliotropic movements. However, while alteration

of leaf angle may change exposure to direct UV-B irradiance, it does not affect exposure to diffuse UV-B.

UV-B has more recently been regarded as a regulatory, rather than merely a plant stress factor (Rozema et al., 1997c; Rozema, 1999). In fact, detrimental plant responses elicited by UV-B can also serve as morphological avoidance strategies against UV-B. This mechanism is termed “growth delay” (Beggs et al., 1986). For instance, reduction of leaf area or of plant height can reduce the extent to which cellular targets are exposed to incoming UV-B radiation (Ballare et al., 1996). Furthermore, it has been suggested that other protection mechanisms can take effect due to growth delay, e.g. the accumulation of UV-absorbing compounds (Ballare et al., 1995). Similarly, UV-B-induced reductions in cell division rates can increase time available for repair of DNA damage. However, the classification of such responses as mechanisms of protection or expression of damage is to some degree open to interpretation.

2.2.5.2. Repair (tolerance)

DNA repair

In addition to these mechanisms of avoidance, plants possess a variety of means to deal with UV-B radiation that does enter plants (e.g. via anticlinal cell walls). The main processes of UV-B tolerance are DNA repair and radical scavenging. Repair of DNA damage by UV-B is predominantly effected by two mechanisms (Stapleton et al., 1997). ‘Photoreactivation’ happens quickly (within the first few hours of DNA damage) by specific photolyase enzymes which reverse CPDs or 6,4 photoproducts. ‘Dark repair’ or excision repair occurs more slowly and involves excision of damaged bases and renewed DNA synthesis to fill the gaps (Stapleton et al., 1997). DNA damage in plants due to ambient solar UV-B is 60-fold less than what would theoretically be possible and it has been shown that this is largely due to effective photoreactivation (Stapleton et al., 1997). Photorepair can also undo damage of photosystem II proteins (Christopher and Mullet, 1994).

Radical scavenging

As briefly mentioned above, UV-B-induced plant damage can be caused by the formation of free radicals in plant tissues. However, this mechanism can also trigger the expression of radical-scavenging antioxidants (Balakumar et al., 1997). Results from recent indoor studies in sorghum, maize, potato and wheat suggest that plant sensitivity to UV-B may be related to a lack in the capacity to increase antioxidant activity (Santos et al., 1999). Findings from filtration studies in South America (34°S) show that solar UV-B leads to increased activity of the hydrogen peroxide-scavenging antioxidant enzymes catalase and ascorbate peroxidase in barley (Mazza et al., 1999a). Mutant barley plants lacking antioxidants also showed increased sensitivity to ambient UV-B exposure with reductions in biomass accumulation (Mazza et al., 1999a). Both intrinsic and UV-B-induced antioxidant capacity appear important in UV-B tolerance (Mazza et al., 1999a). UV-B filtration studies in soybean show that the more UV-B penetrates through the epidermis, the higher the antioxidant activity becomes (Ballare et al., 1999).

Anthocyanins are specific flavonoids that can act as antioxidants (Tsuda et al., 1996). Furthermore, they can conjugate with phenolic compounds and thus also contribute to UV-absorption (Woodall and Stewart, 1998). A number of photoreceptors regulate anthocyanin biosynthesis, including phytochrome, blue light and UV-B receptors (Krizek et al., 1998).

2.2.6. Inter- and intraspecific effects

UV-B effects differ between plant species, cultivars and populations, and this affects the balance between co-existing plant groups, ultimately changing the structure of plant communities. For example, while no changes in biomass accumulation could be found for wheat or a weedy competitor (wild oat) under enhanced UV-B treatment, the competitive balance was altered due to differential morphogenetic shifts between the species (Barnes et al., 1988; Ryel et al., 1990). In wild oat, leaf insertion heights and leaf blade lengths were reduced by UV-B and this resulted in wheat overtopping its competitor under UV-B, with more light interception and larger rates of canopy photosynthesis in wheat (Barnes et al., 1988).

There are indications that plant species and populations from higher natural UV-B backgrounds are less sensitive to solar UV-B. For example, southern hemisphere wheat cultivars from lower latitudes were less sensitive to the solar UV-B levels than northern hemisphere cultivars from higher latitudes (Häder, 1996). This was related to higher intrinsic amounts of UV-absorbing compounds in the tolerant cultivars. Intraspecific differences in sensitivity to solar UV-B can also be observed, e.g. for maize, where a cultivar used in Europe (Mark and Tevini, 1996) was more sensitive to UV-B than one used in India (Pal et al., 1997). In European maize cultivars, Central European cultivars were more UV-B-sensitive than those from southern Europe (Mark et al., 1996).

For a number of years, the Fabaceae have been considered a plant family particularly sensitive to UV-B. Sensitivity to UV-B was found in many leguminous species, even in tropical legumes (Singh, 1996). However, overall examination from plant responses to UV-B supplementation in the field did not suggest particular UV-B sensitivity for the Fabaceae (Searles et al., 2001). This may nevertheless be different for effects of ambient solar UV-B. Findings from UV-B filtration studies suggest that natural UV-B levels decrease plant biomass in a number of legumes, including soybean (Adamse et al., 1997), *Phaseolus* (Deckmyn and Impens, 1995) and mung bean (Pal et al., 1997).

There are also indications that herbaceous dicotyledons may be more sensitive to UV-B than monocotyledonous species (Pal et al., 1997; Cybulski and Peterjohn, 1999; Musil and Wand, 1999). Such differential sensitivity has been related to a number of factors, including molecular features, high foliar alkaloid levels, phenology and leaf morphology (Stapleton et al., 1997; Musil and Wand, 1999). Some grasses - including ryegrass - contain alkaloids that can have UV screening properties (Musil and Wand, 1999). It has been suggested that monocotyledons may have a more diverse distribution in photorepair enzymes which could be of advantage for UV-B-induced DNA repair (Stapleton et al., 1997). The phenological growth patterns of many monocotyledons also favour avoidance of UV-B, with often lower above-ground growth rates and growth predominantly occurring in late winter and spring, when solar UV-B levels are relatively low. Furthermore, it has been suggested that grasses are better protected against UV-B by the well-shielded meristematic region, the protective basal sheath, and their more vertical leaf orientation, compared to the typically more horizontal dicotyledon leaves (Van and Garrard, 1976; He et al., 1993; Pal et al., 1997).

2.2.7. Interactions and ecosystem effects

2.2.7.1. Natural and agro-ecosystems

Only a limited number of studies have measured effects of UV-B radiation on whole ecosystems *in situ*. The UV-B-induced changes on plant morphology, growth, physiology and biochemistry of individual plant species ultimately have ramifications on ecosystem levels. Rather than directly affecting photosynthesis or the genome, under realistic field conditions UV-B is more likely to affect ecosystem structure and composition, e.g. through species-specific changes in morphology which in turn alter canopy structure and light interception (Barnes et al., 1996; Rozema et al., 1997c; Rozema, 1999). This would be of particular relevance for plants growing in close association, such as ryegrass and white clover in New Zealand pastures.

Preliminary results from a New Zealand study using enhancement of UV-B suggest that cyanogenesis is induced by higher UV-B levels in white clover plants growing in association with ryegrass (Campbell et al., 1999). The stimulation of this secondary metabolic process was seen as a signal of heightened stress occurring in the white clover plants. Several studies have investigated the effects of elevated UV-B on European heathland species *in situ*, using sites in High Arctic Greenland, northern Sweden and Greece (Björn et al., 1997). Findings point at species-specific responses with premature senescence, reduced shoot growth, increases in flowering and in some photosynthetic processes (Björn et al., 1997). In the Greek ecosystem, enhanced UV-B improved drought tolerance of Mediterranean pines via increased cuticle thickness (Petropoulou et al., 1995).

Studies in subarctic ecosystems revealed a number of effects of enhanced UV-B on heath and moss species, including stunting, decreased dry mass, lower respiration, changes in tannin levels and in litter decomposition (Gehrke et al., 1995; Gehrke, 1998; Gehrke, 1999). Sensitivity to enhanced UV-B was also observed in studies of subarctic grass species (Gwynn-Jones and Johanson, 1996). In contrast, filtration studies in a dune grasslands ecosystem in The Netherlands showed no effect of solar UV-B on biomass production, morphology and photosynthesis in a number of dicotyledonous and

monocotyledonous species (Tosserams et al., 1996; Rozema et al., 1999b). Findings from examinations of Mediterranean-climate ecosystems in South Africa suggest particular sensitivity of herbaceous dicotyledons to increased UV-B (Musil and Wand, 1999).

Recent filtration studies have investigated in detail effects of solar UV-B radiation on terrestrial ecosystems in southern South America (Ballare et al., 1999). The findings from several plant species showed UV-B-induced inhibition of biomass accumulation, leaf area expansion and yield, while UV-absorbing compounds and antioxidant activities were enhanced. Growth reductions were usually accompanied by DNA damage. These effects were even exceeded by much stronger effects of solar UV-B on plant herbivore interactions, by decreasing leaf damage and the number of phytophagous insects. A number of studies have also investigated effects of solar UV-B on terrestrial ecosystems in Antarctica (Day et al., 1999; Huiskes et al., 1999; Ruhland and Day, 2000; Ruhland and Day, 2001). Several solar UV-B effects were found for a vascular plant community consisting of the grass *Deschampsia antarctica* and the dicotyledon *Colobanthus quitensis*. In *D. antarctica* this included smaller and shorter leaves, associated with shorter epidermal cells at the leaf base and tip, reductions in leaf number and in branching as well as increases in UV-B-absorbing compounds (Ruhland and Day, 2000; Ruhland and Day, 2001). Similar responses were found in *C. quitensis*, with reduced biomass, and smaller and shorter leaves due to shorter epidermal cells at the leaf base as well as increased levels of UV-B-absorbing compounds (Ruhland and Day, 2000; Xiong and Day, 2001).

2.2.7.2. Trophic levels

Effects of UV-B on plants need to be seen in conjunction with simultaneously occurring impacts on herbivores and pathogens. For example while natural levels of UV-B represent a limiting factor for plant growth, this may be counterbalanced by inhibitory effects of UV-B on leaf herbivory by insects (Ballare et al., 1999).

Several studies have investigated whether morphological (e.g. increases in SLM or leaf thickness) or biochemical changes (e.g. higher levels of general or specific UV-absorbing compounds) due to UV-B are likely to affect herbivory on these plants. For

example, increases in SLM and in leaf thickness can affect tissue toughness and attractiveness to herbivorous insects (Bernays and Chapman, 1994). Bioassays in the laboratory have shown changes in herbivory patterns when insects were fed plant material exposed to enhanced levels of UV-B (Hatcher and Paul, 1994; McCloud and Berenbaum, 1994; Lindroth et al., 2000). Reports from earlier UV-B filtration studies indicated a beneficial role of solar UV-B for plants by reducing herbivory (Bartholic et al., 1975). This was recently substantiated in a series of filtration studies using solar UV-B (Ballare et al., 1996; Rousseaux et al., 1998; Mazza et al., 1999b). In these experiments, herbivory was strongly reduced by ambient southern hemisphere UV-B. Furthermore, this effect was dependent on the UV-B dose applied. For example, leaf damage in *Datura* (Ballare et al., 1996) and soybean (Mazza et al., 1999b) declined steadily with increasing levels of solar UV-B. Concomitantly, the density of insect herbivores declined strongly with reduced filtration of solar UV-B (Ballare et al., 1996). Furthermore, some of these results showed that changes in herbivore response were not necessarily related to plant tissue quality such as SLM, relative water content, hemicellulose, phenolic compounds or nitrogen levels (Rousseaux et al., 1998).

This could be at least partly due to direct effects of UV-B on plant consumers. For example, direct UV-B effects have been observed for juvenile amphibians (Blaustein et al., 1994) and chironomid larvae (Bothwell et al., 1994). Recent filtration studies in southern South America also showed that microfauna (testate amoebae and rotifers) in a *Sphagnum* bog ecosystem can be favoured by solar UV-B radiation (Searles et al., 1999). Solar UV radiation has also been shown important for normal dispersal activity, flight orientation, host recognition or landing behaviour in whiteflies, thrips and aphids (Antignus et al., 1996; Antignus et al., 2001). In other UV-B exclusion studies, thrips appeared to directly sense and avoid exposure to solar UV-B (Mazza et al., 1999b). Comparing the magnitude of UV-B-induced responses, it is now thought that changes at trophic levels may be more affected by solar UV-B radiation than direct effects on plant growth and physiology (Ballare et al., 1999; Searles et al., 1999).

2.2.7.3. Decomposition

UV-B-induced changes in leaf morphology, senescence and chemistry can ultimately affect litter decomposition and biogeochemical cycling (Zepp et al., 1995; Rozema et

al., 1997b; Rozema et al., 1999a). Changes in decomposition can affect production of trace gases by microbes, and the availability of nutrients that are important for plant growth (Zepp et al., 1995). Decomposition can be accelerated when surface litter is photo-degraded by UV-B, and delayed when bacterial activity is suppressed or the chemical composition in living plant tissues is changed towards one that later reduces its biodegradability as litter (Zepp et al., 1995). Higher amounts of secondary compounds in leaves can inhibit litter decomposition, e.g. in the dune grassland species *Calamagrostis epigeios*, where lignin content increased while decomposition rate decreased under enhanced UV-B (Rozema et al., 1997b).

2.2.7.4. Stress interactions

Incidence of high solar UV-B radiation can - particularly in summer - be accompanied by a number of other plant stress forms, including low water availability, high temperature and high levels of UV -A and of PAR. The majority of studies investigating the interaction of UV-B and water stress suggest that drought can lessen plant sensitivity to UV-B. For example in soybean, droughted plants were not affected by enhanced UV-B while well-watered plants were UV-B-sensitive (Caldwell et al., 1995). This may be related to the fact that the droughted plants had higher levels of flavonoids compared to the well-watered plants. In turn, UV-B has also been shown to alleviate the adverse effects of drought under field conditions (Petropoulou et al., 1995).

Higher PAR and UV-A levels generally also result in the amelioration of UV-B damage (Cen and Bornman, 1990; Caldwell et al., 1994). This is due to a number of factors, e.g. stimulation of photorepair and of UV-B-protective compound formation (Cen and Bornman, 1990). Levels of UV-A and PAR are low in many indoor studies, strongly increasing UV-B sensitivity. Field studies providing a realistic spectral balance of UV-B, UV-A and PAR are therefore essential to allow meaningful ecological examinations of possible UV-B effects for plants.

Interactions with temperature and CO₂ are also important as higher temperatures often accompany higher UV-B levels and because of the rise in temperature and CO₂ due to global climate change. Furthermore, the functioning of the UVB-protective photolyase enzyme is sensitive to changes in ambient temperature (Young et al., 1993). Using the

ozone cuvette technique under solar UV-B levels in Portugal, a study with sunflower and maize seedlings showed that increased temperatures and higher CO₂ ameliorated several aspects of UV-B sensitivity (Mark and Tevini, 1996; Mark and Tevini, 1997). Alleviating effects for UV-B sensitivity may include higher metabolic and photosynthetic rates under elevated temperature and enhanced CO₂ (Mark and Tevini, 1996; Mark and Tevini, 1997). Interaction effects of UV-B and CO₂ are species-specific and beneficial CO₂ effects are particularly important for C₃ plants, because of reduced photorespiration and higher photosynthetic rates under higher CO₂ levels (Mark and Tevini, 1997; Tevini, 1999). Beneficial effects of enhanced CO₂ are often not found in studies using unrealistic enhancement of UV-B (Tevini, 1999).

Nutrient stress can reduce plant sensitivity to UV-B, although in some cases additive stress effects are also possible (Krupa et al., 1998; Tevini, 1999). Using solar New Zealand UV-B levels as a background, cucumber plants grown under high nitrogen availability were sensitive to supplemental UV-B, with decreases in leaf area, plant height and biomass as well as increased midday photoinhibition (Hunt and McNeil, 1998). Such sensitivity was not observed when nitrogen stress (lower nitrogen availability) was applied. In addition, increases of protective UV-absorbing compounds under UV-B supplementation were particularly observed in nitrogen-depleted leaves (Hunt and McNeil, 1998). From this it was concluded that changes in the plant nitrogen status (e.g. via fertilisers or pollution) could also affect UV-B sensitivity (Hunt and McNeil, 1998).

Anthropogenic pollution e.g. by tropospheric ozone in the air (Feder and Shrier, 1990) or heavy metals in the soil can aggravate UV-B effects (Dube and Bornman, 1992). UV-B can also interact with stress caused by pathogens. Several findings point at additive effects, e.g. in sugar beet infected with the fungus *Cercospora beticola* (Panagopoulos et al., 1992). There may however also be beneficial effects via the stimulation of secondary pathways by either stress that also have mutual protective functions (Tevini, 1999).

3. Solar UV-B environment

3.1. Introduction

Most studies of UV-B effectiveness on plants have been conducted indoors under controlled environmental conditions or in the glasshouse. As outlined in the previous chapter, extrapolation from these studies to the field situation is complicated by a number of factors, e.g. unrealistically high UV-B doses and unnatural spectral distribution. Examinations under more realistic field conditions are thus needed to obtain meaningful environmental information about possible effects of UV-B on plants. Studies filtering solar UV-B radiation are of particular importance in this regard because they provide more realistic spectral balance and allow testing the effects of present-day levels of UV-B on plants. This is especially important for plants growing in New Zealand, where UV-B levels can be 40% higher than at comparable northern latitudes (Madronich et al., 1998).

For the experiments of this thesis, filters with differential UV-B absorbance were used to expose plants growing under New Zealand field conditions to contrasting levels of UV-B. This chapter presents results from testing of the equipment used, and reports levels of solar UV-B irradiance and related parameters that were present during the plant studies. UV-B irradiance levels per day were recorded with a UV-B broadband sensor during the experimental period in summer from early December 1995 to late February 1996. To relate these measurements to the generalised plant action spectrum (Caldwell, 1971), the unweighted output of the broadband sensor was calibrated with a spectroradiometer. The transmittance of the UV-B-absorbing and transmitting filters was determined in the laboratory with a spectrophotometer. Filter comparisons in the field are also presented, together with tests of spatial variation of UV-B incidence.

Aims for the investigations in this chapter were: (1) to obtain a detailed picture of UV-B irradiance conditions experienced by the plants during the trials; (2) to test how the broadband measurements are related to those conducted with a spectroradiometer in the UV-B range; and (3) to obtain radiation comparisons under the UV-B-absorbing and transmitting filters.

3.2. Materials and methods

Contrasting UV-B environments were created using UV-B-transmitting perspex glass (Casocryl, Chemiplas, Auckland, New Zealand) and UV-B-absorbing glasshouse polythene film (Polycrop, Cosio Industries, Auckland, New Zealand). The filters were fastened over the top of wooden frames, oriented with a diagonal axis aligned in a north-south direction. The filters also covered the two north-facing sides, while the two south-facing sides were left open to reduce temperature build-up under the filters. Experimental plots were thus protected from direct sunlight except for early morning and late afternoon. Spectral transmittance of the two filter types was examined with a Hitachi U-2000 scanning spectrophotometer. A third set of plots was maintained with frames but without filters to act as an 'open control' for the covered plots. Thus three treatments were applied in this set-up: '-UV-B' for UV-B-absorbing filters, '+UV-B' for UV-B-transmitting filters and 'Open' for unfiltered frames.

Ambient UV-B irradiance was measured continuously using a SKYE SKU-430 broadband UV-B sensor (SKYE Instruments Ltd., Powys, UK) linked to a SKYE Instruments data logger, except when recording was interrupted for the downloading of data and for maintenance. The data logger was also used to connect two SKP201/I PAR sensors and two SKTS200/I air temperature sensors. To examine the extent of penetration of diffuse UV-B from the open southern sides under the filters, a spatial test of UV-B incidence was conducted using the SKYE SKU-430 sensor. Sequential readings were taken 150 mm from the closed end, in the middle of the filter and 150 mm from the open ends above and below the filters under clear sky conditions. To check performance of the SKYE SKU-430 sensor, its output was calibrated during a 10 day period in mid February 1996 against a UV spectroradiometer at Lauder, Central Otago by the National Institute of Water and Atmospheric Research (NIWA) over the UV-B spectral range. Comparisons of readings from the two UV-B-monitoring instruments were analysed with the Linear Regression Analysis procedure in GENSTAT (Genstat, 1993).

3.3. Results

3.3.1. Sensor comparisons

Figure 3-1 compares the output from the SKYE SKU-430 sensor and the NIWA spectroradiometer at 10° intervals of solar zenith angle in February 1996. There was a linear relationship between the outputs of the two instruments and a fitted quadratic term was not significant (Figure 3-1). A similarly significant linear relationship was observed when the SKYE sensor output was plotted against plant-weighted UV-B values (Caldwell, 1971) from the NIWA instrument (Figure 3-2).

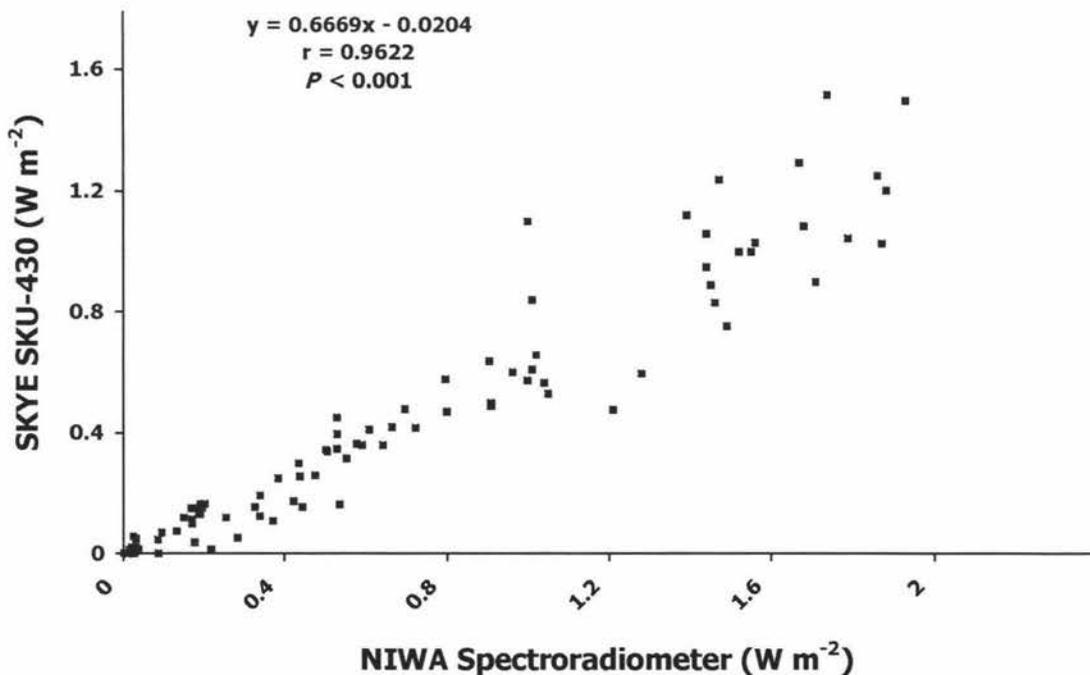


Figure 3-1. Comparison between the NIWA spectroradiometer and the SKYE SKU-430 broadband sensor output over the UV-B wavelength range.

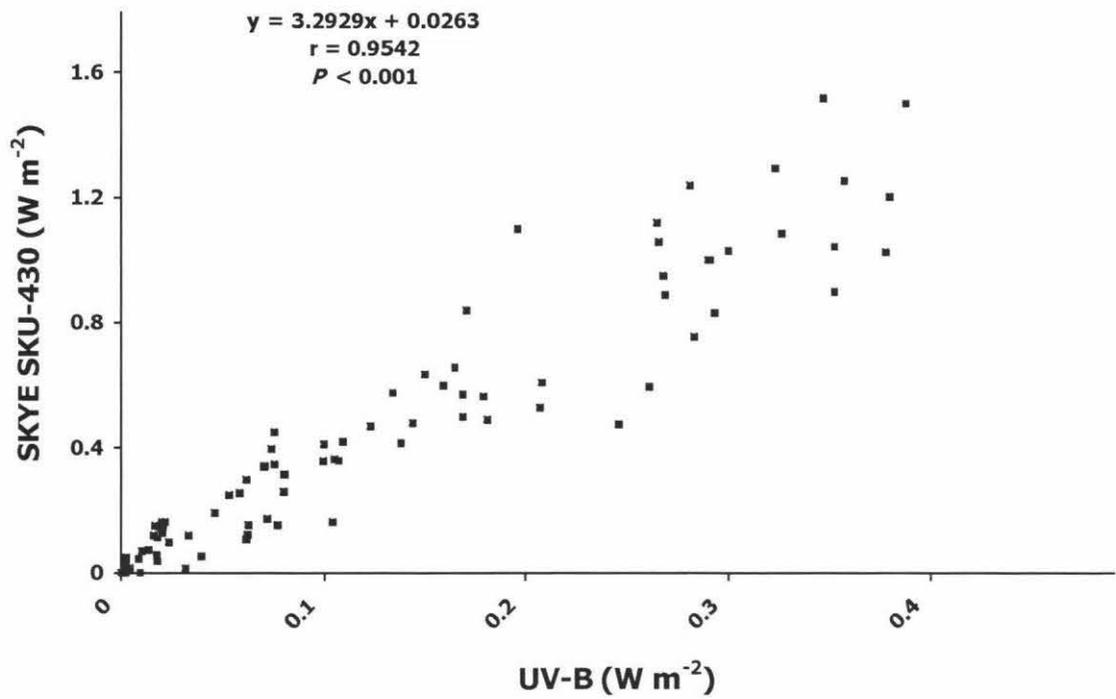


Figure 3-2. Comparison between plant-weighted UV-B irradiance (calculated from the NIWA spectroradiometer readings) and the SKYE SKU-430 broadband sensor output.

3.3.2. UV-B radiation levels

UV-B levels (Figure 3-3 to Figure 3-7) are shown as plant-weighted irradiances, calculated from the linear relationship in Figure 3-2. Daily average UV-B irradiance in December was $6.2 \text{ kJ m}^{-2} \text{ d}^{-1}$, with the maximum of $8.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ recorded on 16 December, followed by the minimum of $2.4 \text{ kJ m}^{-2} \text{ d}^{-1}$ one day later (Figure 3-3). The highest UV-B irradiances were measured in January, exceeding $8 \text{ kJ m}^{-2} \text{ d}^{-1}$ four times (3, 6, 16 and 20 January). The maximum (20 January), minimum (26 January) and mean values for January were 8.5 , 2.6 and $6.5 \text{ kJ m}^{-2} \text{ d}^{-1}$ UV-B, respectively (Figure 3-4). In February, the maximum irradiance of $7.9 \text{ kJ m}^{-2} \text{ d}^{-1}$ UV-B was recorded at the beginning of the month, while the minimum of $2.0 \text{ kJ m}^{-2} \text{ d}^{-1}$ occurred towards the end of the measurement period on 21 February (Figure 3-5). The mean for the measurement period in February was $5.6 \text{ kJ m}^{-2} \text{ d}^{-1}$. The average maximum UV-B irradiance (mean of the ten highest values) across the summer 1995/1996 measurement period was $8.1 \text{ kJ m}^{-2} \text{ d}^{-1}$. This compares well with calculated recent average maxima for Palmerston North of $8.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ plant-weighted UV-B in 1997, $8.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ in 1998, $8.3 \text{ kJ m}^{-2} \text{ d}^{-1}$ in 1999, and $8.1 \text{ kJ m}^{-2} \text{ d}^{-1}$ in 2000 (C.L. Hunt, pers. comm.).

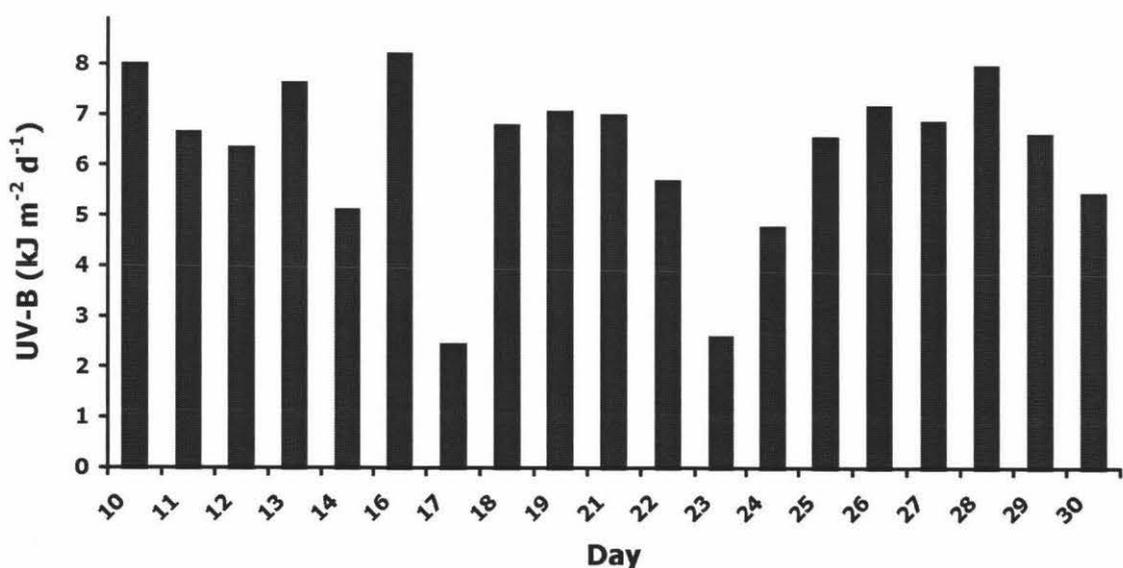


Figure 3-3. Plant-weighted UV-B irradiance in December.

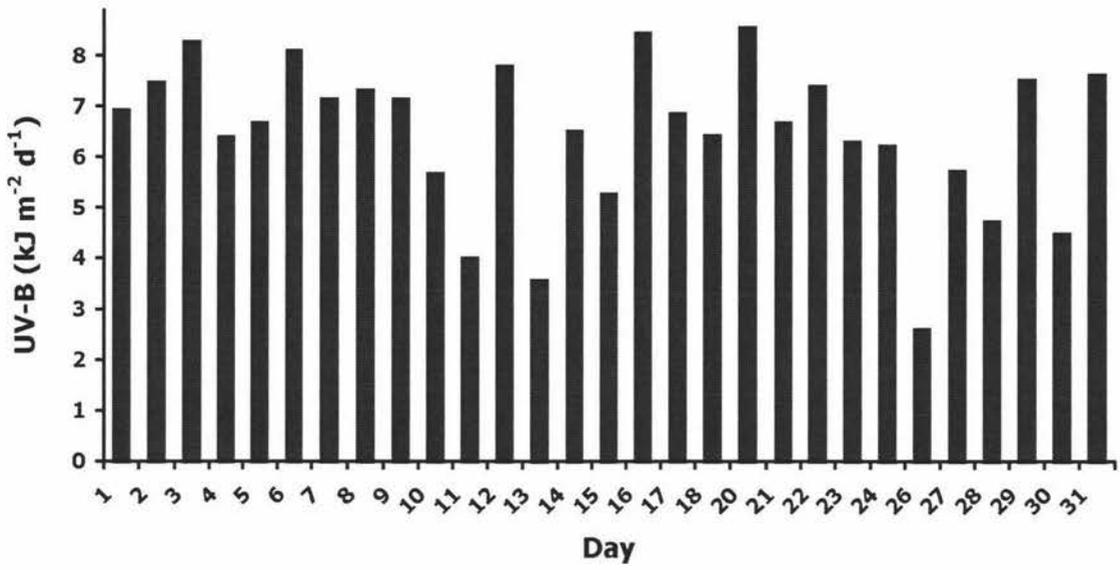


Figure 3-4. Plant-weighted UV-B irradiance in January.

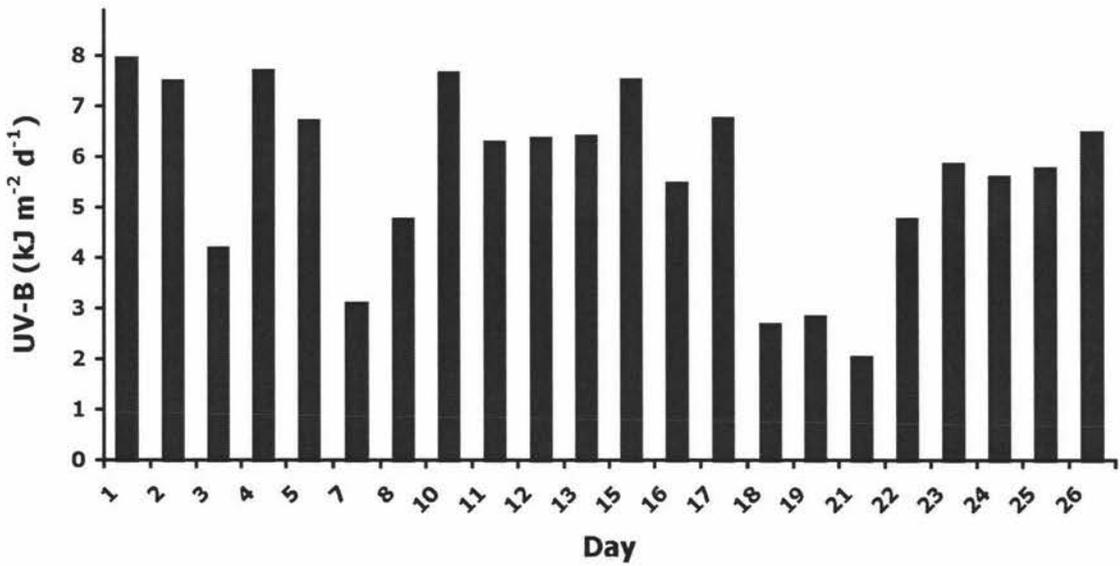


Figure 3-5. Plant-weighted UV-B irradiance in February.

Figure 3-6 depicts the daily course of UV-B irradiance during a largely cloudless day in midsummer (16 January), with maximum irradiance at 2pm. The effect of intermittent clouding is highlighted in Figure 3-7, resulting in distinct changes in the UV-B flux.

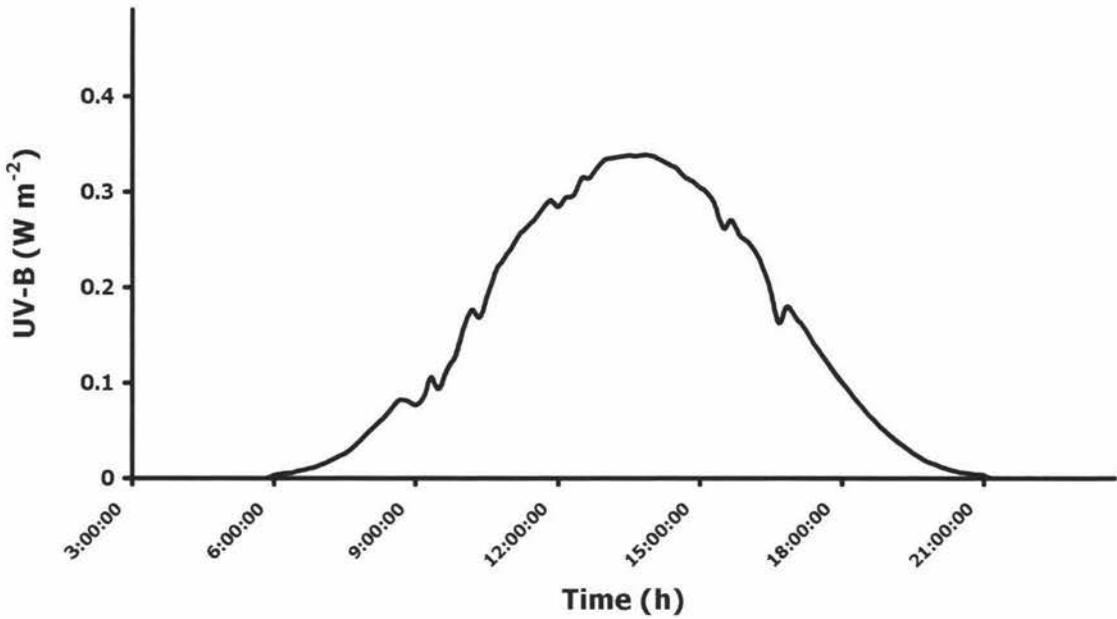


Figure 3-6. Plant-weighted UV-B irradiance on a largely cloudless day in midsummer (16 January).

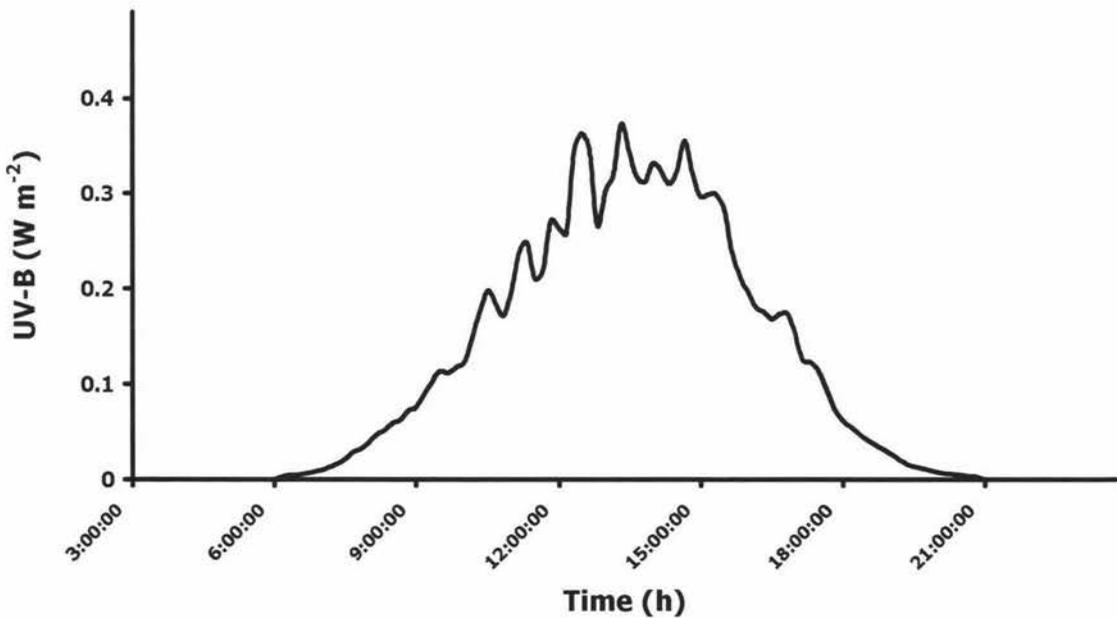


Figure 3-7. Plant-weighted UV-B irradiance on a partly cloudy day in midsummer (12 January).

3.3.3. Comparisons of UV-B filters

Spectrophotometric examination of the two filters revealed similar transmission of PAR, but contrasting transmission in the UV region, with about 80% transmittance of UV-B for the perspex glass and negligible transmittance for the polythene film (Figure 3-8). The cut-off point for the UV-B-transmitting perspex filter was at 291 nm and that of the UV-B-absorbing polythene filter at 330 nm (Figure 3-8). Transmittance of UV-A in the latter filter was about 40% of the perspex glass.

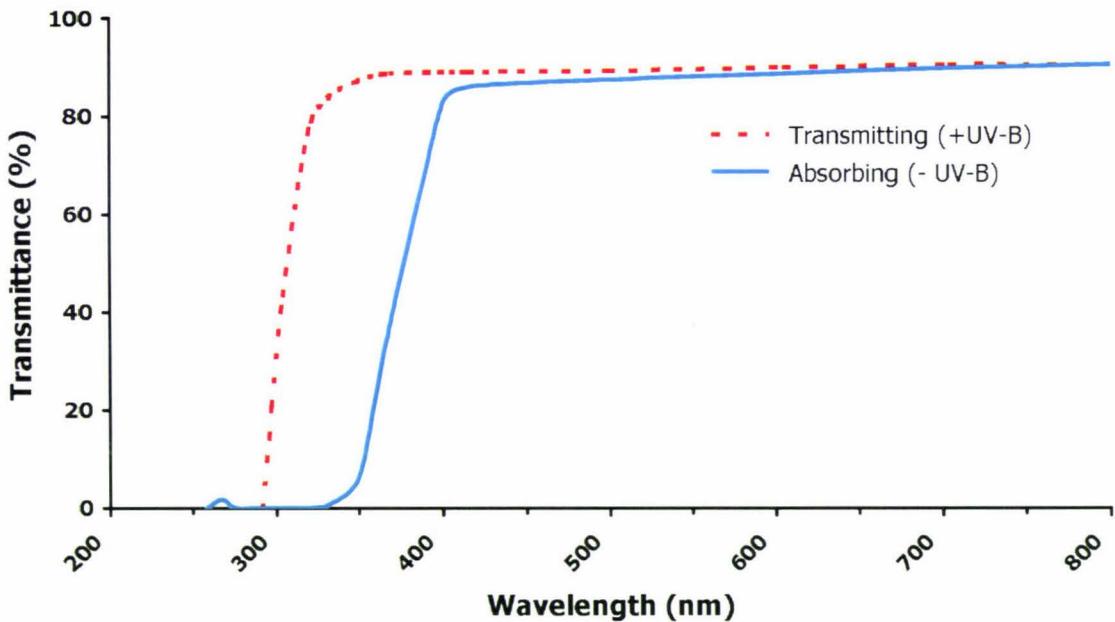


Figure 3-8. Spectral transmittance of UV-B-transmitting (Casocryl perspex glass) and UV-B-absorbing filters (Cosio Polycrop glasshouse film).

To examine the extent of penetration of diffuse UV-B in the field from the open southern sides of the filters, a test of spatial variation in UV-B incidence was made. Under UV-B-absorbing filters, values ranged from 9% of ambient UV-B near the closed ends to 11% near the open ends (Figure 3-9). Similarly, an approximately linear increase from 72% to 78% of ambient UV-B towards the open end was found under the UV-B-transmitting filters.

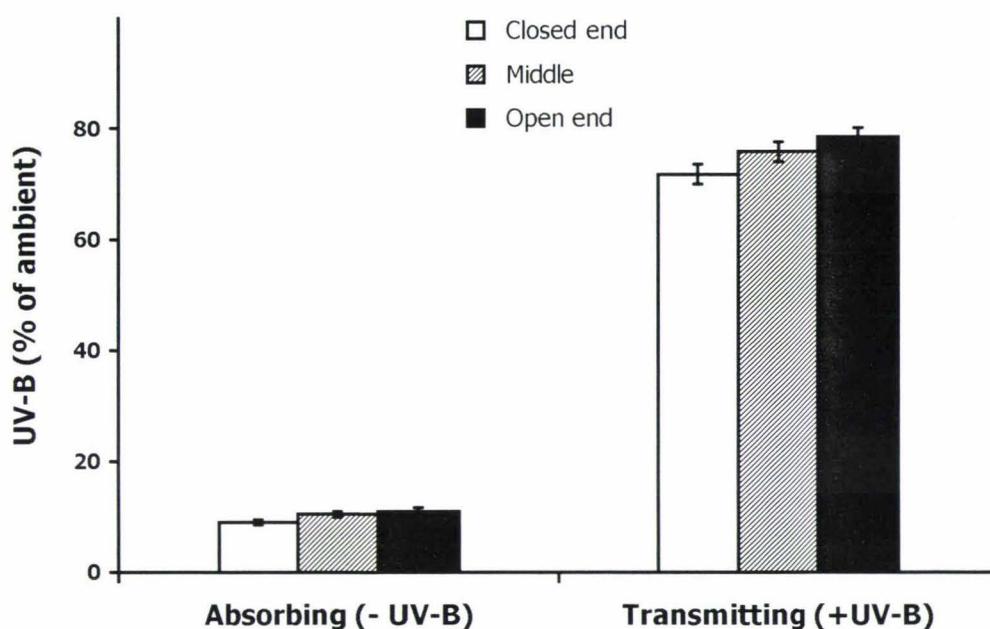


Figure 3-9. Spatial variation of UV-B incidence under UV-B-absorbing and UV-B-transmitting filters. Sequential readings were taken above and below the filter 150 mm from the closed northern ends, in the middle section of the filter and 150 mm from the open southern ends.

Comparisons of PAR between the two filters in the field showed about 4% lower levels under the UV-B-absorbing filters (Figure 3-10). Temperatures in the latter were on average 0.5°C lower compared to the UV-B-transmitting filters (Figure 3-11). Temperatures under both filters were about 0.5 – 1 °C higher at night and up to 4 °C higher in strong sunlight when compared to open plots.

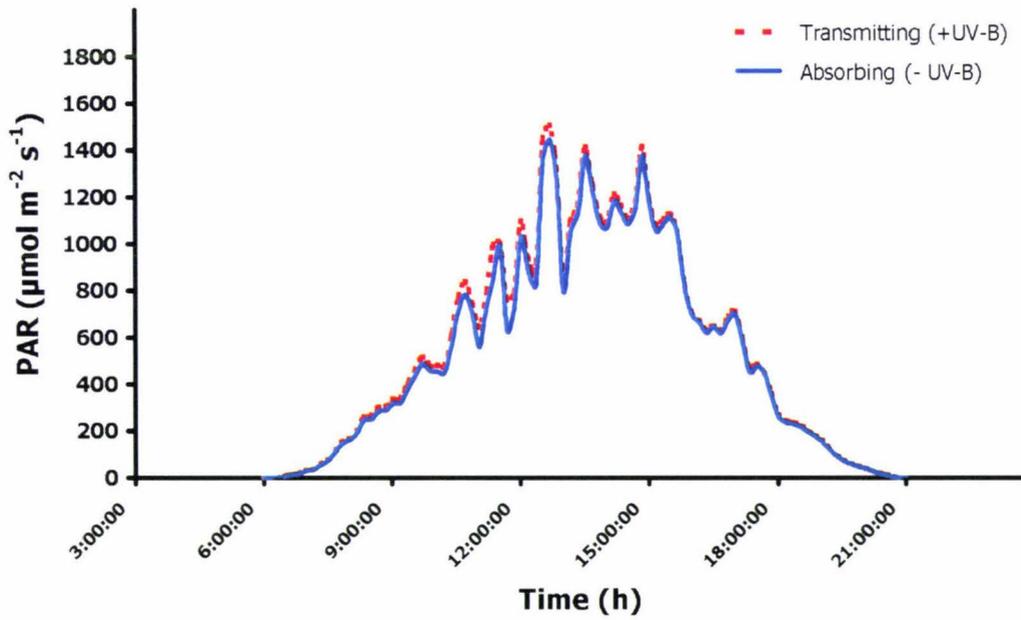


Figure 3-10. Comparison of PAR levels under UV-B-transmitting and UV-B-absorbing filters (12 January).

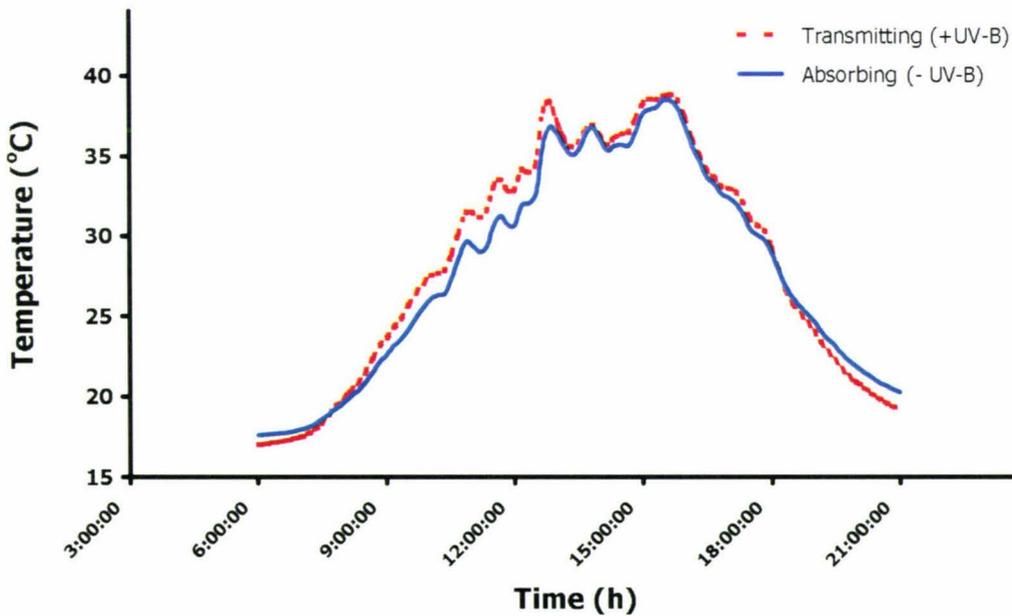


Figure 3-11. Comparison of temperatures under UV-B-transmitting and UV-B-absorbing filters (12 January).

3.4. Discussion

The SKYE SKU-430 broadband sensor and the NIWA spectroradiometer readings showed good comparability in the UV-B range (Figure 3-1 and Figure 3-2). The spectroradiometer at NIWA in Lauder is designed to measure the spectral distribution of solar UV irradiation. Based on a Bentham DM300 double monochromator, it has a spectral resolution of approximately 0.9 nm and is programmed to automatically log data from 290 – 450 nm at 5 degree steps of solar zenith angle throughout the day. In addition, it and makes several scans around noon. Such systems have been in use since 1989 at Lauder, Otago (McKenzie et al., 1992), and have been compared with similar systems in Australia, Germany, USA and Antarctica (McKenzie et al., 1993).

Broadband UV-B sensors have been used in a number of experiments to measure levels UV-B irradiance (e.g. Krizek et al., 1997; Krizek et al., 1998; Mazza et al., 1999a). More recently, the SKYE SKU-430 broadband sensor used here has also been utilised in other studies of effects of solar UV-B on plants (Cybulski and Peterjohn, 1999; Day et al., 1999; Ruhland and Day, 2000; Ruhland and Day, 2001; Xiong and Day, 2001). Disadvantages of broadband sensors such as the SKYE SKU-430 include, for example, inability to distinguish which wavelengths within the sensor band contribute to a particular reading, and also inclusion of some short UV-A wavelengths (Day et al., 1999). While accurate calculation of biologically-weighted UV-B is not possible from broadband sensor readings, calibration against spectroradiometer readings has been used frequently to address some of the deficiencies of broadband sensors and to estimate plant-weighted UV-B doses (Cybulski and Peterjohn, 1999; Day et al., 1999; Ruhland and Day, 2000; Ruhland and Day, 2001; Xiong and Day, 2001). In these studies, the correlations between readings of the two instruments gave equally strong linear relationships as in the present work (Figure 3-1 and Figure 3-2).

While changes in ozone and other factors such as clouds can affect UV-B and longer wavelengths differently, day-to-day variation in UV-B can be reasonably accurately estimated with broadband sensors. Although there are some limitations in the direct conversion between the two instrument readings (Figure 3-1), many of the outliers in this comparison occurred during periods of intermittent cloud cover and can be explained by differential integration intervals of the two instruments. The SKYE sensor

integrated data over a 10 minute interval, while the NIWA spectroradiometer had a scanning cycle of about 3 minutes. During a scanning cycle, variation in cloud cover could therefore affect the instruments differently. There were few clear sky values during the observation period and thus it was not possible to compare this relationship under clear sky conditions.

Under cloudless conditions, the highest UV-B levels at temperate southern latitudes would be expected early in January (Huiskes et al., 1999). However, this is strongly influenced by the presence of clouds and accordingly the data from this study show high UV-B levels ($> 8 \text{ kJ m}^{-2} \text{ d}^{-1}$) from mid December to mid-late January (Figure 3-3 and Figure 3-4). The influence of clouds was apparent in the comparison of irradiance levels between consecutive days. Irradiance levels of $2.4 \text{ kJ m}^{-2} \text{ d}^{-1}$ on 17 December were more than 70% lower than the $8.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ measured on the previous day (Figure 3-3). Very thick cloud cover can reduce UV-B levels even further, by 80% or more (Madronich et al., 1995). Strong reduction of irradiance levels by clouds could also be observed during the course of a day with intermittent clouding for levels of UV-B (Figure 3-7) and PAR (Figure 3-10). Close examination of these levels suggests that clouding reduced PAR levels to a greater extent than UV-B (thus increasing the UV-B:PAR ratio), e.g. when comparing the cloud-induced drop in irradiance levels around 13:00 h between UV-B (Figure 3-7) and PAR (Figure 3-10). UV-B is generally less reduced by clouding than wavelengths in the PAR spectral range, due to the presence of a higher amount of diffuse radiation in the UV-B range (compared to PAR).

The plant-weighted levels of summer UV-B irradiance measured in this experiment (Figure 3-3 to Figure 3-7) were similar to values reported at similar latitudes in other studies. A study conducted in the northern hemisphere summer of 1996 at a similar latitude (39°N) in West Virginia (USA) also calculated plant-weighted UV-B values from a SKYE UV-B sensor calibrated against a spectroradiometer (Cybulski and Peterjohn, 1999). Minimum and maximum daily UV-B irradiance in that study were 1.8 and $8.1 \text{ kJ m}^{-2} \text{ d}^{-1}$, compared to the 2.0 (21 February, Figure 3-5) and $8.5 \text{ kJ m}^{-2} \text{ d}^{-1}$ (20 January, Figure 3-4) measured during the course of the present study. At a latitude of 34°S in South Africa, daily minima of 2.6 kJ m^{-2} , daily maxima of 8.9 kJ m^{-2} and maximum cloudless solar noon levels of 0.38 W m^{-2} were reported (Musil et al., 1999). The validity of plant-weighted irradiance levels calculated from the relationship

between SKYE sensor and spectroradiometer readings is further substantiated by the fact that the average maximum summer UV-B level of $8.1 \text{ kJ m}^{-2} \text{ d}^{-1}$ measured in this study is very similar to values calculated for Palmerston North during subsequent years (C.L. Hunt, pers. comm.).

UV-B-transmitting Casocryl perspex glass and commercially available UV-B-absorbing glasshouse polythene proved stable in the windy climate of the experimental site. The slope of the curve under the Casocryl filter and transmission of about 80% irradiance in the UV-B region is similar to that found with cellulose acetate (Ballare et al., 1996). Transmission of UV-B by about 10% in plots under the polythene filter (Figure 3-9) is similar to values in PE filters (Adamse et al., 1997; Day et al., 1999; Ruhland and Day, 2001). The reduction of UV-B under these UV-B-absorbing filters was therefore similar to that on a day with thick cloud cover (Madronich et al., 1995).

In addition to UV-B exclusion, the polythene cover also filtered some of the short -wave UV-A portion of the spectrum (Figure 3-8). Plant responses to UV-A have been demonstrated and it could be argued that the partial UV-A filtration may also influence plant responsiveness in the present study. Alternatively it could be argued that UV-A is a factor that often ameliorates UV-B damage (Caldwell et al., 1994) and that the methodology used here represents a more conservative approach as in addition to the removal of damaging UV-B, some of the more beneficial UV-A wavelengths were also partially removed by the absorbing filters of the control treatment. Experiments on spectral balance showed no differences in responses of plants exposed to the same amounts of UV-B, but with higher or lower amounts of UV-A (Caldwell et al., 1994). Rather, the level of PAR was the deciding factor in altering UV-B sensitivity.

In numerous other examinations of solar UV-B filtration, the use of UV-B-excluding filters also reduced portions of UV-A to various degrees (Tezuka et al., 1993; Ballare et al., 1996; Pal et al., 1997; Krizek et al., 1998; Rousseaux et al., 1998; Huiskes et al., 1999; Krause et al., 1999; Turunen et al., 1999; Cockell et al., 2000; Mazza et al., 2000). To be exact, the use of these filters investigates the effect of reduction in solar UV-B and various portions of UV-A on plants. However, the common denominator in the present work and in these other studies is the sharp contrast in UV-B between filter treatments. For reasons of clarity this contrast is commonly used as the prime

distinction in spectral distribution between filters and accordingly, the treatments are described as relative amounts of UV-B received, namely +UV-B for the transmitting and -UV-B for the absorbing filter (Deckmyn and Impens, 1995; Searles et al., 1995; Mark et al., 1996; Adamse et al., 1997; Mark and Tevini, 1997; Saile -Mark and Tevini, 1997; Krause et al., 1999).

Investigation of spatial variation of UV-B incidence showed that the contrast in UV-B transmission between the two filter types remained intact across much of the plot area. As expected, the results show a gradient of increasing UV-B irradiance towards the open ends in both filters (Figure 3-9). However, this gradient was small, with minimal variance in UV-B across the sampling area. The average 10% transmittance of UV-B in the absorbing treatment (Figure 3-9) can largely be attributed to UV-B from the diffuse component of solar radiation entering the frames from the open ends. Figure 3-9 shows that this did not cancel the effect of UV-B-absorbing filters near the open sides. It can also be assumed that some diffuse short-wave UV-A reached the plants from the open sides.

The majority of field studies examining UV-B effects on plants compare *enhanced* UV-B levels to ambient irradiation. While this allows projections of UV-B effects under possible future ozone depletion scenarios, most of the supplemental UV-B irradiation protocols use levels that significantly exceed current predictions. Such methodology is thus likely to also exaggerate possible UV-B effects on plants. Furthermore, many of these studies show remarkable differences in plant responses between treatments. Between all field examinations, there is some divergence in ambient UV-B levels reaching plants due to differential study location. However, this is further compounded in studies of effects of enhanced UV-B by considerable differences in the application and degree of UV-B supplementation. It can be suggested that results from UV-B filtration experiments are more comparable to each other, given the more realistic spectral balance and that in most cases near-ambient levels of UV-B are compared against near-exclusion UV-B controls.

Most UV-B exclusion studies compare near-ambient to near-exclusion levels of UV-B, while only a subset include an open frame treatment. Some studies have even used no near-ambient treatment and compare UV-B-absorbing treatments directly with

unfiltered plots (Sharma et al., 1991; Häder, 1996; Dong et al., 1998; Steel and Keller, 2000). This however needs to be regarded with caution, as a number of other environmental variables are also affected by filtration, including temperature, humidity and herbivory. An open unfiltered treatment - in addition to near-ambient and reduced UV-B treatments - can be used to test for effects on plants of a closed canopy *per se*, exemplified by the slightly higher temperatures under filters measured here, and a presumed increase in relative humidity. Inclusion of an open frame treatment is therefore important when interpreting plant responses due to differential filtration of UV-B. Small differences between filters were exemplified by the slightly higher temperatures measured under UV-B-transmitting perspex (Figure 3-11), as has also been observed in other studies using UV-B filtration (Xiong and Day, 2001).

Taken together, the information in chapter shows that the highest UV -B levels occurred during early and midsummer from mid December to mid-late January. The UV-B measurements from the broadband sensor were similar to spectroradiometer readings and to values reported in other studies. There were marked differences in UV-B transmittance between the two filters, with minimal variance across the sampling area.

4. Effects of solar UV-B radiation in New Zealand on the morphology of pasture and crop species

4.1. Introduction

Decreasing levels of stratospheric ozone result in increases of wavelengths in the UV -B band range of the solar spectrum (290 - 315 nm) during summer in New Zealand (McKenzie et al., 1999). These increases and the fact that solar UV-B levels in the southern hemisphere are naturally higher compared to similar northern hemisphere latitudes (Madronich et al., 1998) indicate a need for studies on the effects of ambient New Zealand UV-B levels for plants. This is further warranted as most of the agronomically important plant species in New Zealand originate from temperate northern hemisphere regions. The vast majority of studies examining UV-B responses in plants have been conducted on vegetable and grain crops, while relatively little information exists on the effects of UV-B on species of natural or agricultural grasslands (Tosserams et al., 1996; Deckmyn and Impens, 1998a). The most important pasture species in New Zealand are white clover and ryegrass, which are grown in combination as forage swards.

Solar UV-B can affect various morphological and growth-related plant attributes, including decreases in biomass, leaf area, shoot length, as well as increases in specific leaf mass or leaf thickness (e.g. Ballare et al., 1996; Lingakumar et al., 1999; Xiong and Day, 2001). Furthermore, there could be differences in the extent of solar UV -B effects between morphological functions. For example, effects on leaf area can be independent on those on shoot length (Adamse et al., 1997; Deckmyn and Impens, 1998a). Few studies have examined effects of UV-B on the area of individual leaves and leaf number, both contributing to total leaf area (Nogues et al., 1998; Hunt and McNeil, 1999). There is a need to relate UV-B responsiveness to the stage of plant or organ development. Other findings suggest that younger plant tissue may be more affected by UV-B than more mature plant parts (Mark et al., 1996; Rozema et al., 1997c; Hunt and McNeil, 1999). Moreover, there is a need to conduct investigations on the influence of

ambient UV-B on reproductive development, as such effects can differ depending on the species investigated. Solar UV-B can have stimulatory (Visser et al., 1997; Deckmyn and Impens, 1998a; Cybulski and Peterjohn, 1999), decreasing (Mark et al., 1996; Fiscus et al., 1999; Musil and Wand, 1999) or no effects on reproductive development (Searles et al., 1999). The few investigations of UV-B effects on senescence give a similarly diverse picture with decreases (Tezuka et al., 1993; Tosserams et al., 1996) as well as increases in leaf senescence (Björn et al., 1997).

The New Zealand white clover cultivar 'Huia' has shown sensitivity to enhanced UV-B under controlled environmental conditions (Hofmann et al., 2000; Lindroth et al., 2000). Studies on white clover plants in the tropics showed increased leaf thickness along an elevational gradient (Rozema et al., 1997a). The prostrate growth habit of white clover can also be conveniently used for investigations of ontogenetic effects of UV-B by comparing measurements along its stolon.

Some studies suggest that monocotyledons such as grasses may be less sensitive to UV-B than dicotyledonous species due to differential morphological and physiological characteristics (Pal et al., 1997; Cybulski and Peterjohn, 1999; Musil and Wand, 1999). A field study using ryegrass showed that transmission of UV-B into the canopy is significantly higher than of PAR (Deckmyn and Impens, 1998b). However, that study did not specifically investigate UV-B sensitivity of ryegrass. Studies conducted in the northern hemisphere have shown susceptibility of glasshouse-grown ryegrass to solar UV-B (Deckmyn and Impens, 1999), while a study using elevated UV-B found no effects on ryegrass biomass production in the field (Newsham et al., 1998). Other examinations with elevated UV-B showed reductions in plant height, leaf number and leaf area in pot-grown ryegrass plants (Nakayama et al., 1996).

UV-B effects under controlled indoor conditions are often not observed in field examinations. A prime example in this regard is pea, a leguminous vegetable crop species of agronomical importance to New Zealand. A number of indoor experiments showed UV-B susceptibility for this species (Jordan et al., 1992; He et al., 1993; Gonzalez et al., 1998; Nogues et al., 1998), while findings from UV-B enhancement studies in the field found no such sensitivity (Allen et al., 1999; Stephen et al., 1999). Little information is available on the influence of ambient UV-B levels on pea (Becwar

et al., 1982). Furthermore, pea and white clover are members of the Fabaceae, a plant family that for some time has been regarded as particularly sensitive to UV-B (Deckmyn and Impens, 1995; Singh, 1996; Adamse et al., 1997; Pal et al., 1997).

This chapter examines the morphological basis of UV -B responsiveness in white clover, ryegrass and pea. Measurements were recorded in two harvests for each species during summer and included leaf expansion and leaf initiation as well as whole plant biomass accumulation. Ryegrass was used to study solar UV-B effects on leaf senescence. In addition, examinations on white clover and pea tested UV-B effects on specific leaf mass, stem elongation and aspects of reproduction. It was hypothesised that natural UV-B levels would represent a limiting factor for growth or morphological development of temperate pasture and crop plants under New Zealand field conditions. It was expected that UV-B sensitivity would differ between white clover, ryegrass and pea. In the comparison of pasture plants it was hypothesised that this may result in lesser sensitivity for the monocotyledonous ryegrass. Furthermore, white clover was used as a model to test the hypothesis that younger plant tissue would be particularly sensitive to UV-B.

4.2. Materials and methods

The experiment was laid out as a randomised complete block design (Figure 4-1), with three replicates of the three different treatments outlined in section 3.2 on page 36. This resulted in a total of nine plots for each plant species examined. White clover (*Trifolium repens* L. cv. 'Kopu') and ryegrass (*Lolium perenne* L. cv. 'Embassy') plants were grown from seed from April 1995. Seeds of pea (*Pisum sativum* L. cv. 'Greenfeast') plants were sown early in December 1995 and in January 1996. The three plant species were grown separate from each other in monoculture (Figure 4-1). Originally, white clover and ryegrass were also planted in combination to examine UV-B effects on a mixture of the two species. However, this could not be assessed because of poor survival of white clover in the mixed plots. The experimental site was located at Massey University (Palmerston North, latitude 40°S, longitude 175°E) on an alluvial terrace next to the Turitea Stream. The soil is Te Arakura silt loam and soil test results for an adjacent paddock with similar fertiliser history have been described elsewhere (Kemp et al., 1994).

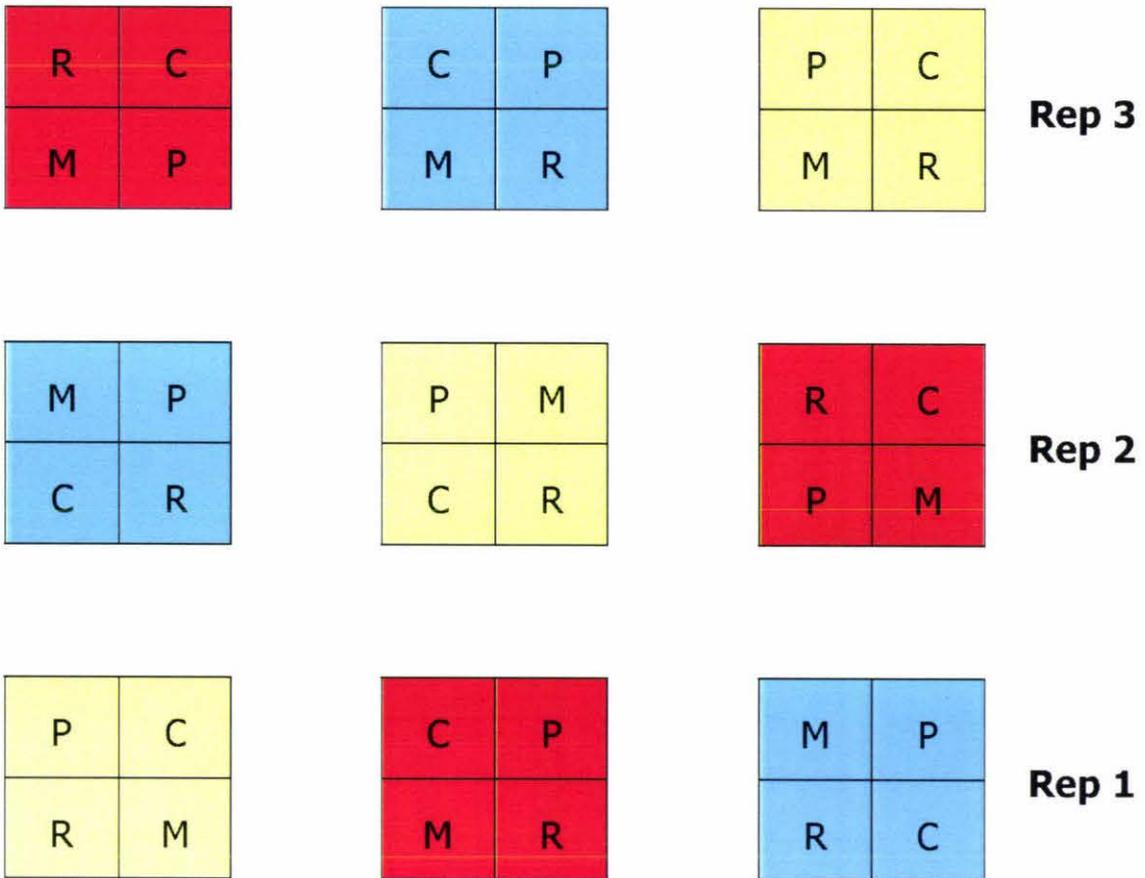


Figure 4-1. Diagram of the experimental layout. Yellow = open frames, red = UV-B-transmitting filters, blue = UV-B-absorbing filters. W = white clover, R = ryegrass, P = pea, M = mixed plots.

The size of each plot was 1500×1000 mm. A wooden frame of this size and with a height of 350 mm at the northeast side and 550 mm at the southwest side was placed over each plot. The filter frame was hinged and could be opened up for sampling (Figure 4-2). During sampling, a portable shelter was used on occasion as weather protection (Figure 4-2). Plants were usually harvested from the central regions in each plot. The UV-B-transmitting perspex filters were regularly cleaned, and the UV-absorbing polythene filters were replaced in midsummer. Condensation build-up in the morning under both filters was removed with a window wiper. Filter frames were fitted with four 180° microjet irrigation nozzles and 10 L water were provided to each plot per day. Natural precipitation was monitored regularly with a rain gauge and open plots were watered by hand to match water levels obtained by irrigation in the covered plots.



Figure 4-2. Photograph of the experimental set-up.

The white clover and ryegrass plants in the monoculture areas were defoliated at regular intervals to a simulated grazing height of 50 mm. After defoliation, ryegrass and white clover plants were marked to measure rates of plant development. Ten randomly selected advancing white clover stolons per plot were harvested in the third week after marking, on 12 January and 19 February 1996. Measurements at each nodal position included determination of leaf lamina area with a leaf area meter as well as measurement of petiole and internode length. In addition, the number of leaves, inflorescences and floral buds per stolon was recorded. Above-ground plant parts from both harvests were oven-dried for 48 h at 80 °C for biomass determination. Specific leaf mass (SLM) of the youngest fully unfolded leaf lamina was calculated as the ratio of leaf dry mass over leaf area.

Fifteen randomly selected ryegrass tillers in each plot were marked after defoliation, and from the second week of regrowth monitored to determine leaf elongation and senescence. The first series of these tissue turnover measurements was carried out during the week from 10 to 17 January 1996. A second series of measurements covered the period from 26 January to 9 February 1996. The rate of ryegrass leaf elongation was

calculated from measurements of leaf blade length during the two intervals. Similarly, leaf senescence was determined as leaf blade length reduction during these periods. Net leaf elongation rate was calculated as total leaf elongation minus length reduction due to senescence. Ryegrass leaf appearance rate was calculated from the number of newly formed leaves during each interval. Similarly, the number of leaves lost via senescence during the measurement periods was determined. Net leaf appearance was subsequently calculated from the difference between the rate of leaf appearance and leaf loss due to senescence. At the end of each harvest period, the ryegrass plants were dried at 80 °C for 48 h to determine above-ground plant biomass.

In the filter treatments, 30 pea plants per plot were harvested 4 weeks after establishment, on 8 January and 6 February 1996, respectively. In the open plots, a number of pea plants did not survive summer growth without canopies and sample sizes were 10, 18 and 30 per plot on 8 January and 10 per plot on 6 February. Measurements included leaf lamina area and petiole length of the youngest fully unfolded leaf and stem height. In addition, the number of leaves, inflorescences and floral buds was determined on 6 February. Above-ground plant material from both harvests was oven-dried for 48 h at 80 °C to determine plant biomass. The ratio of leaf dry mass over leaf area of the youngest fully unfolded leaf lamina was calculated to determine specific leaf mass.

In accordance with similar experimental layouts (Searles et al., 1999), the plots - rather than individual plants within the plots - were used as experimental units. The values from individual samples in each plot were averaged and the resulting plot means (3 means per treatment) analysed as randomised complete block design using the General Analysis of Variance (ANOVA) procedure in GENSTAT (Genstat, 1993). Treatment means were further compared using least significant differences at $P < 0.05$. In addition, two orthogonal sets of contrasts were defined to compare the effects under UV-B-absorbing filters with (1) those under the UV-B-transparent filters or (2) with the combined response measured under UV-B-transparent filters and open plots. Unless stated otherwise, the standard errors of individual means were used for the presentation of results. Discrete data from inflorescence counts (Table 4-4 and Table 4-6) were analysed using the GENSTAT Log-linear Modelling Procedure (Genstat, 1993). In line with most other UV-B filtration studies, the UV-B-absorbing treatment was chosen as the control for the presentation of results.

4.3. Results

4.3.1. White clover

4.3.1.1. Stolon morphology and growth

There were significant changes in white clover leaf area under UV-B, and these were dependent on leaf developmental stage. A particularly strong UV-B effect was found for the area of young leaves in January (Figure 4-3A and B). Compared with UV-B-absorbing filters, a 20% decrease in size was observed for the first fully unfolded leaf under UV-B-transmitting filters and in open plots ($P < 0.05$). Treatment comparisons in February also revealed decreases by 14% under the UV-B-transmitting filter ($P < 0.01$), while no size reduction was found in the open areas (Figure 4-3A). In the second mature leaf in January, overall leaf area reduction was about 18% ($P = 0.057$, Figure 4-3B). Size differences in February were non-significant for the second mature leaf (Figure 4-3B). Differences in size of the third mature leaf were not significant at both harvests (Figure 4-3C). Except for some small decreases in open plots, petiole elongation was generally not significantly affected by UV-B at any developmental stage or harvesting period (Figure 4-4A to C).

As with the leaf area observations, changes in stolon elongation were dependent on developmental stage and more pronounced at the first harvest. In January, internode length proximal to the youngest leaf was reduced by 40% under UV-B-transmitting filters and by 25% in open plots ($P = 0.056$, Figure 4-5A). A similar picture was observed for internode length behind the second mature leaf ($P < 0.05$, Figure 4-5B). Differences were non-significant for the internodes proximal to the third mature leaf in January (Figure 4-5C). Apart from a length reduction in the oldest internode section of uncovered plots, measurements of stolon elongation were generally not significantly affected in February (Figure 4-5A to C). Compared to the two younger stem sections, internodes behind the third unfolded leaf were generally shorter (Figure 4-5C), because in several plants the latter stolon sections were those behind the node of the most proximal (i.e. mature) leaf, which often was initiated close to the point of emergence from the ground.

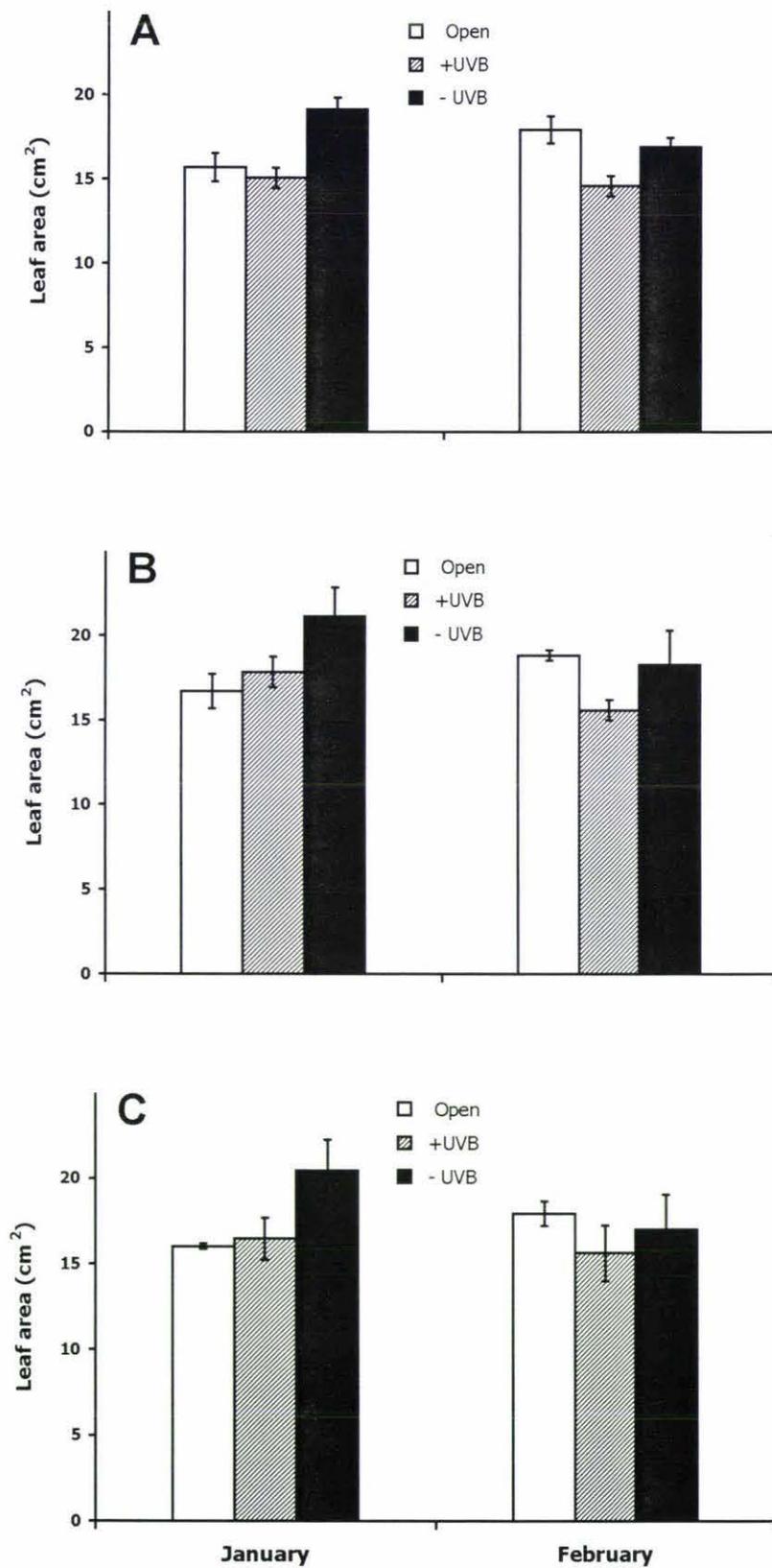


Figure 4-3. Area of the youngest (A), second (B) and third (C) unfolded white clover leaf (12 January and 19 February).

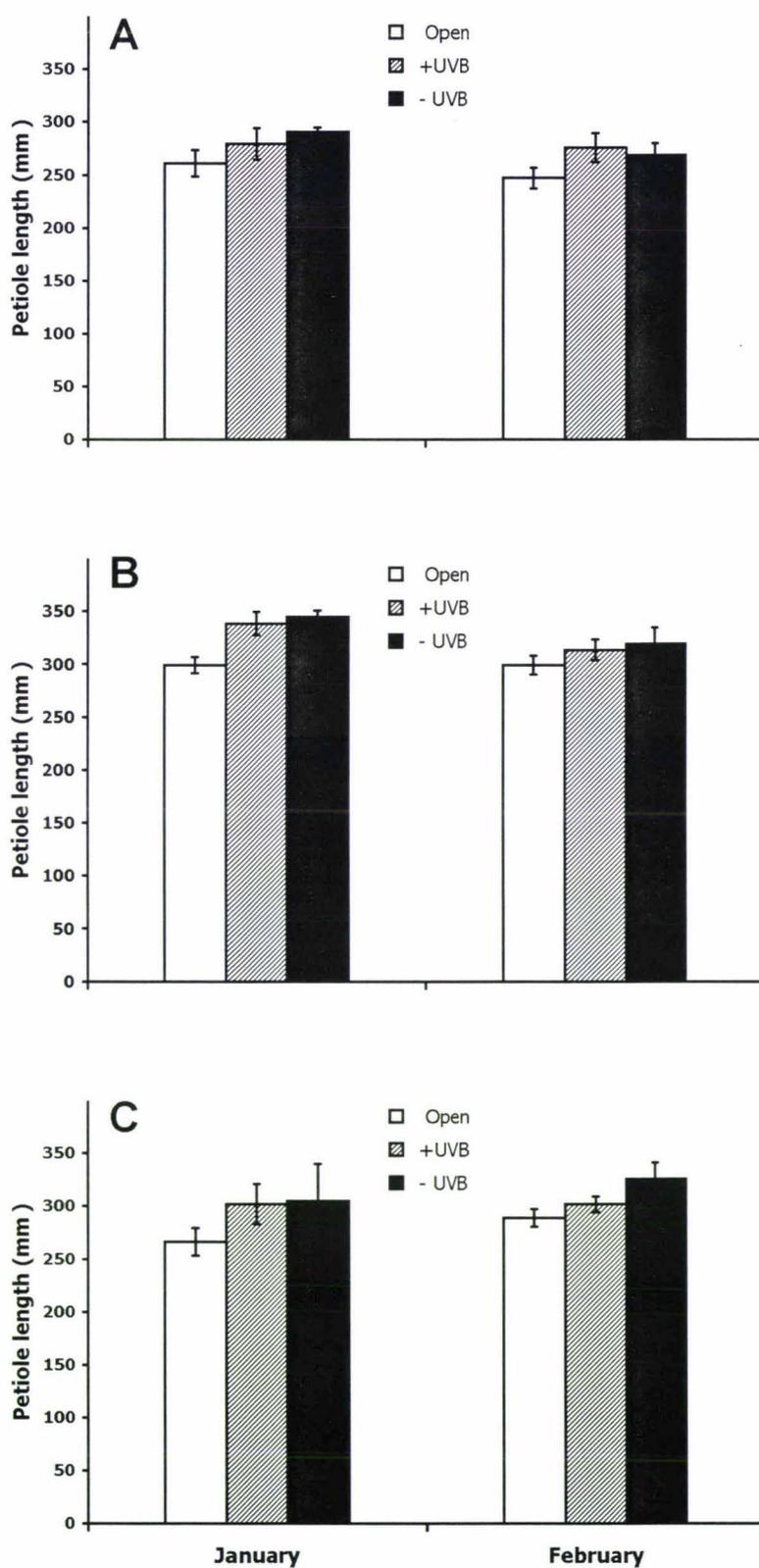


Figure 4-4. White clover petiole length of the youngest (A), second (B) and third (C) unfolded leaf (12 January and 19 February).

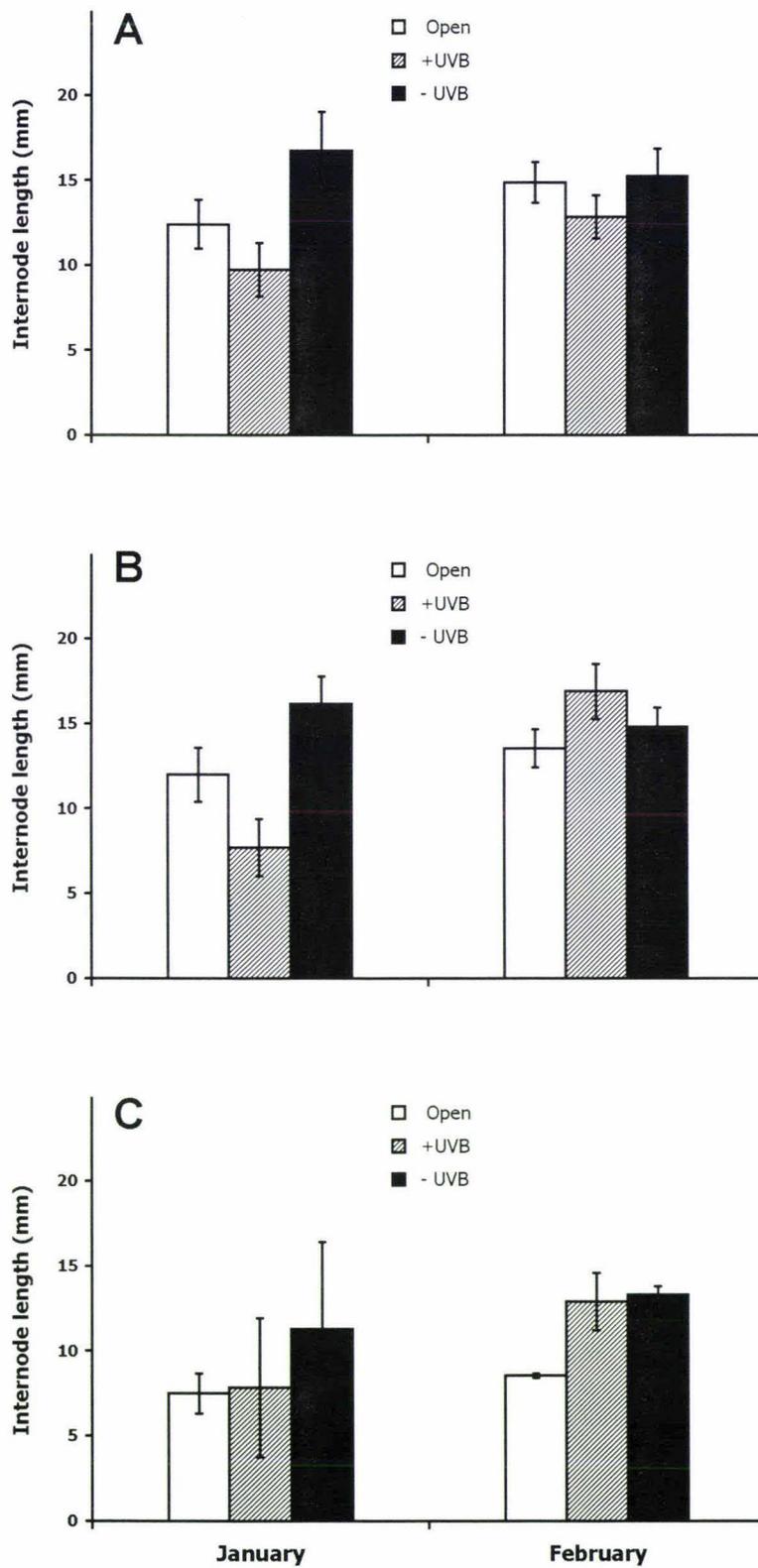


Figure 4-5. White clover internode length behind the youngest (A), second (B) and third (C) unfolded leaf (12 January and 19 February).

Lack of significance for leaf and stolon attributes at the February harvest also became apparent in integrated measurements across developmental stages (Table 4-1). In January, the mean leaf area decrease in uncovered plots and UV-B-transmitting filters was 20% (Table 4-1). Significance for reduced mean petiole height in January was due to a 10% decrease in open plots. UV-B-induced changes did not appear significant for total stolon elongation.

Table 4-1. Mean effects on white clover morphology. For statistical significance ** denotes $P < 0.01$, * denotes $P < 0.05$ and ns $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Leaf area (cm ²)					
12 January	16	16	20	1	*
19 February	18	15	17	1	ns
Petiole length (mm)					
12 January	277	309	312	4	**
19 February	276	301	304	10	ns
Stolon advance (mm d ⁻¹)					
12 January	1.6	1.2	2.2	0.3	ns
19 February	1.9	2.0	2.4	0.2	ns

The youngest mature leaf was further investigated to determine dry mass and specific leaf mass (Table 4-2). Although following a similar trend to that of leaf area (Figure 4-3A), changes in leaf dry mass were not statistically significant (Table 4-2). A similar picture emerged in the analysis of plant above-ground biomass (Table 4-3). There was no difference in specific leaf mass between treatments in white clover (Table 4-2).

Table 4-2. Effects on the dry mass and specific leaf mass (SLM) of the youngest mature white clover leaf; ns denotes $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Leaf dry mass (mg)					
12 January	42.4	38.2	53.1	5.7	ns
19 February	45.8	39.1	43.0	3.5	ns
SLM (mg cm ⁻²)					
12 January	2.69	2.52	2.77	0.26	ns
19 February	2.56	2.67	2.55	0.19	ns

Table 4-3. Effects on above-ground plant biomass in white clover; ns denotes $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Plant biomass (mg)					
12 January	312	320	400	35	ns
19 February	329	288	403	45	ns

4.3.1.2. Leaf appearance

The number of white clover leaves did not change during the first measurement period in January (Figure 4-6). However, after the second growth period, the number of leaves was 17% higher under the UV-B-absorbing filter, compared to UV-B-transmitting filters and open plots ($P < 0.05$, Figure 4-6).

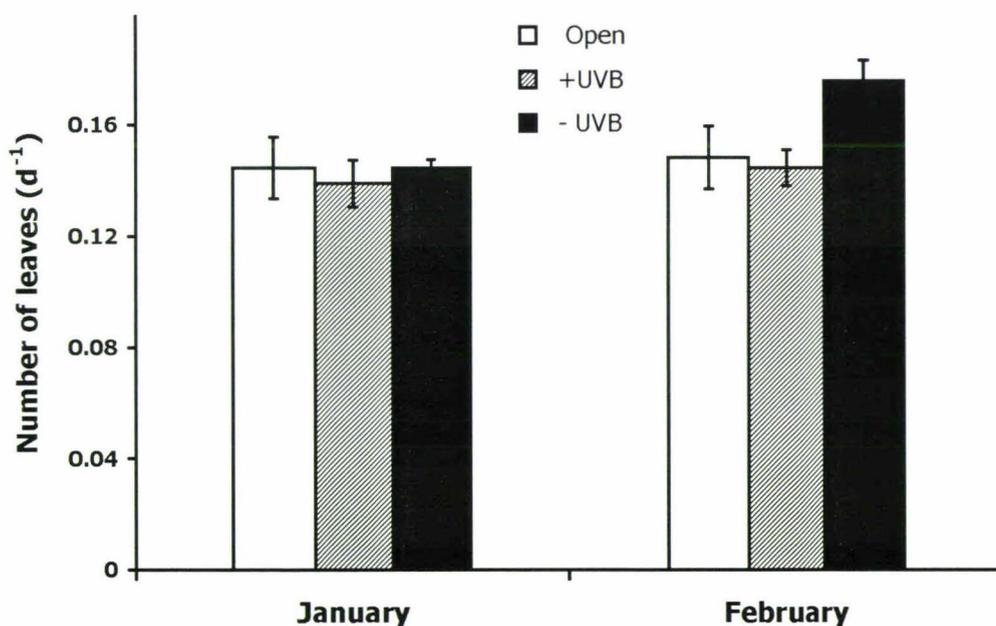


Figure 4-6. Leaf appearance in white clover (12 January and 19 February).

4.3.1.3. Inflorescence formation

White clover inflorescence numbers (buds and fully developed inflorescences) did not change significantly in January (Table 4-4). In February, however, there was a significant difference between treatments, with flowering only occurring under UV-B (Table 4-4).

Table 4-4. White clover inflorescence formation at the third week of regrowth. Numbers represent the sum of white clover inflorescences and buds per treatment. For statistical significance ** denotes $P < 0.01$, * denotes $P < 0.05$ and ns $P \geq 0.10$.

	Treatment			Deviance ratio	<i>P</i>
	Open	+ UV-B	- UV-B		
Open inflorescences					
12 January	3	7	9	0.77	ns
19 February	3	4	0	9.36	*
Buds					
12 January	12	4	8	1.62	ns
19 February	5	8	0	12.96	*
Total inflorescences					
12 January	15	11	17	1.45	ns
19 February	8	12	0	31.09	**

4.3.2. Ryegrass

4.3.2.1. Leaf elongation

Ryegrass leaf elongation rate in January showed an average decrease of about 25% in both UV-B-transmitting and open plots ($P < 0.05$, Figure 4-7). In February the reduction in open plots was similar at 29%, while less pronounced decreases of 15% were observed under UV-B-transmitting filters ($P = 0.092$, Figure 4-7). No significant differences could be found for leaf senescence rate in either harvest (Figure 4-8). In January, net leaf elongation (total leaf elongation minus length reduction due to senescence) was reduced by 27% in the UV-B-transmitting filters and by 39% in the open plots ($P = 0.05$, Figure 4-9). A similar pattern in February was outside statistical significance (Figure 4-9).

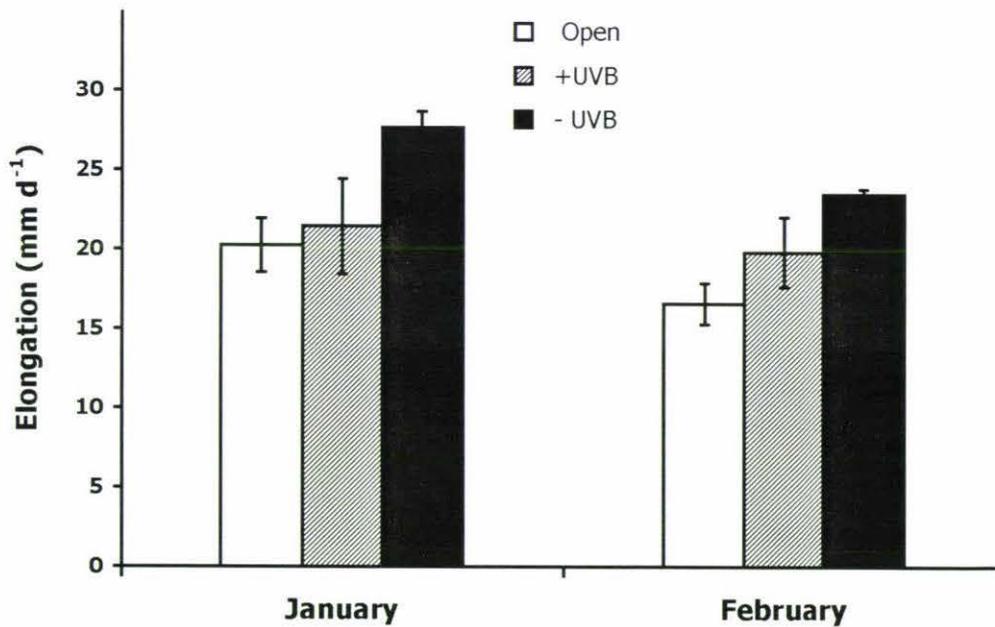


Figure 4-7. Ryegrass total leaf elongation per tiller per day during the week from 10 to 17 January and during two weeks ending 9 February.

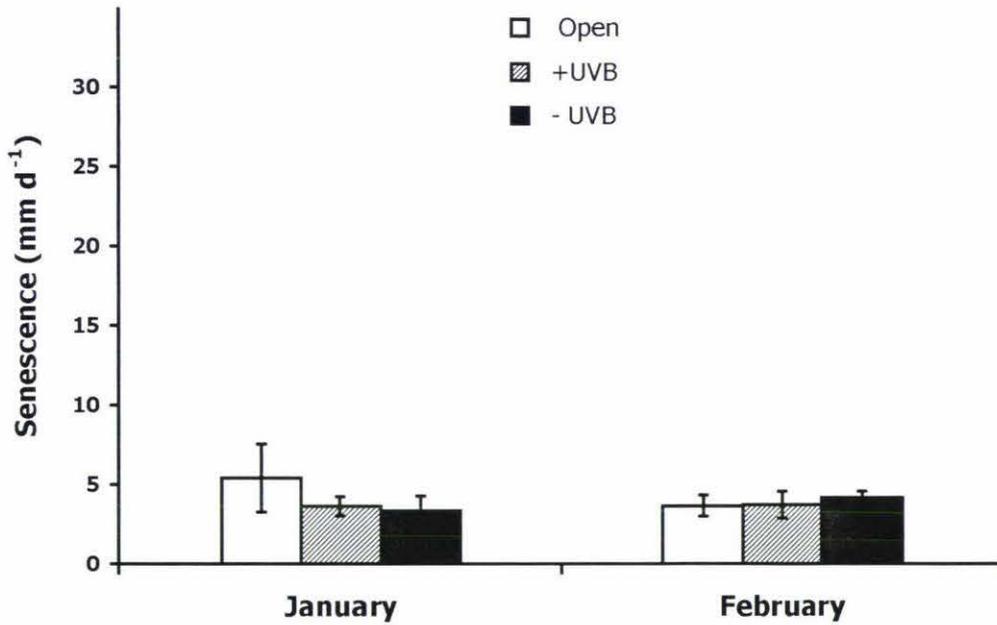


Figure 4-8. Ryegrass leaf senescence, measured as leaf length reduction per tiller per day during the week from 10 to 17 January and during two weeks ending 9 February.

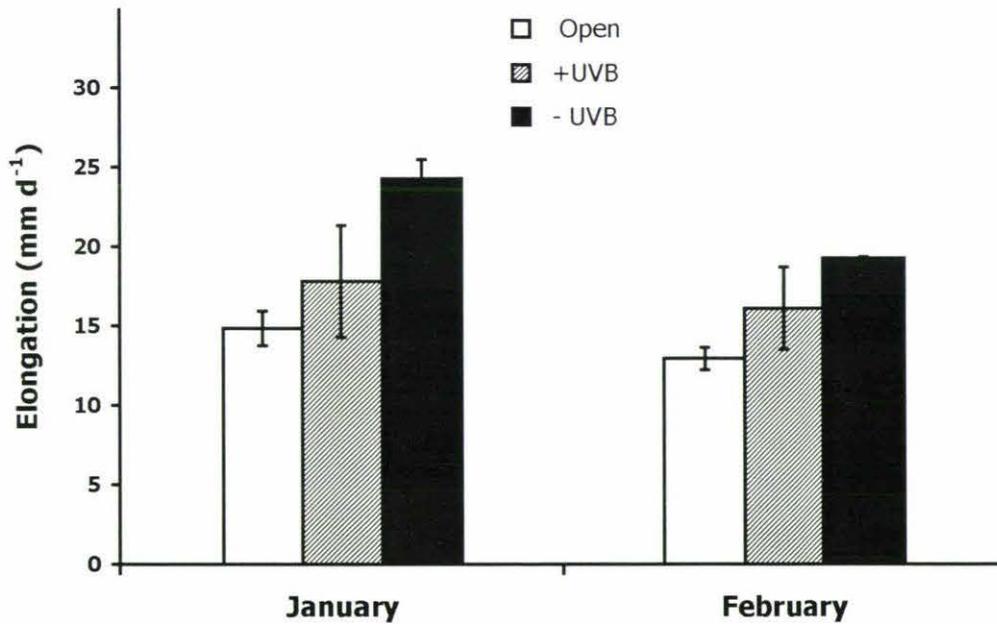


Figure 4-9. Ryegrass net leaf elongation (total leaf elongation minus length reduction due to senescence) per tiller per day during the week from 10 to 17 January and during two weeks ending 9 February.

4.3.2.2. Leaf appearance

There was a significant UV-B effect ($P < 0.01$) on the number of newly formed ryegrass leaves during the January measurement period (Figure 4-10). Compared to the UV-B-absorbing filter, the transmitting filters resulted in a 17% reduction in leaf appearance rate. This effect was twice as pronounced in the open plots. A similar but less marked trend was non-significant in February (Figure 4-10).

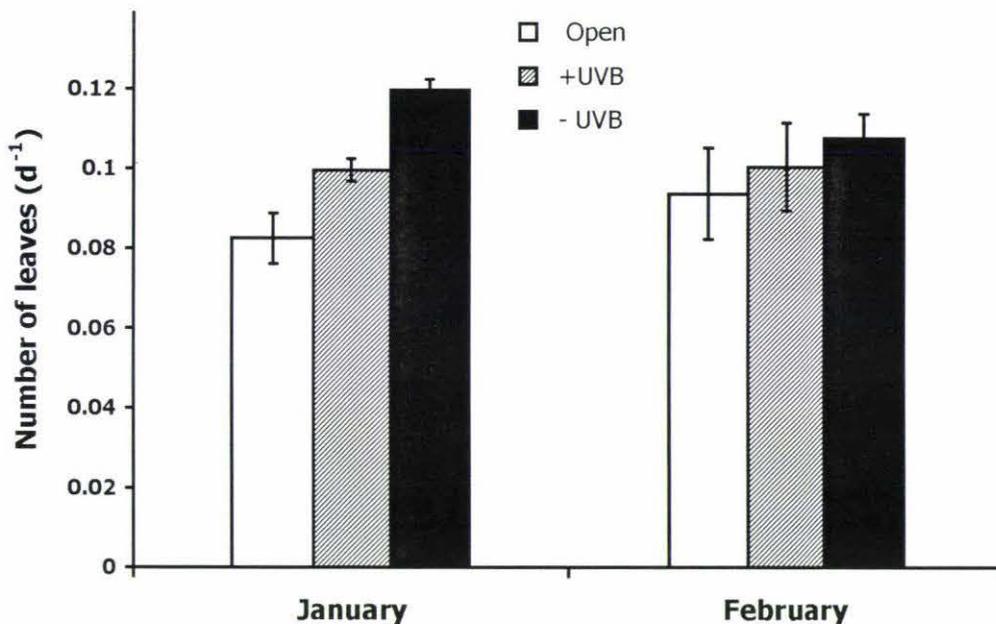


Figure 4-10. Number of ryegrass leaves per tiller per day during the week from 10 to 17 January and during two weeks ending 9 February.

The number of fully senescent leaves (sheaths with no green blades remaining) was lowest in UV-B-absorbing plots in January ($P = 0.086$, Figure 4-11). When this leaf loss due to senescence was subtracted from leaf appearance, net leaf appearance in January was reduced by one third under UV-B-absorbing filters and by 75% in open plots ($P < 0.05$, Figure 4-12). Differences in February were non-significant (Figure 4-11 and Figure 4-12). A generally higher number of fully senescent leaves in February (Figure 4-11) led to lower levels of net leaf appearance in all treatments in that month (Figure 4-12).

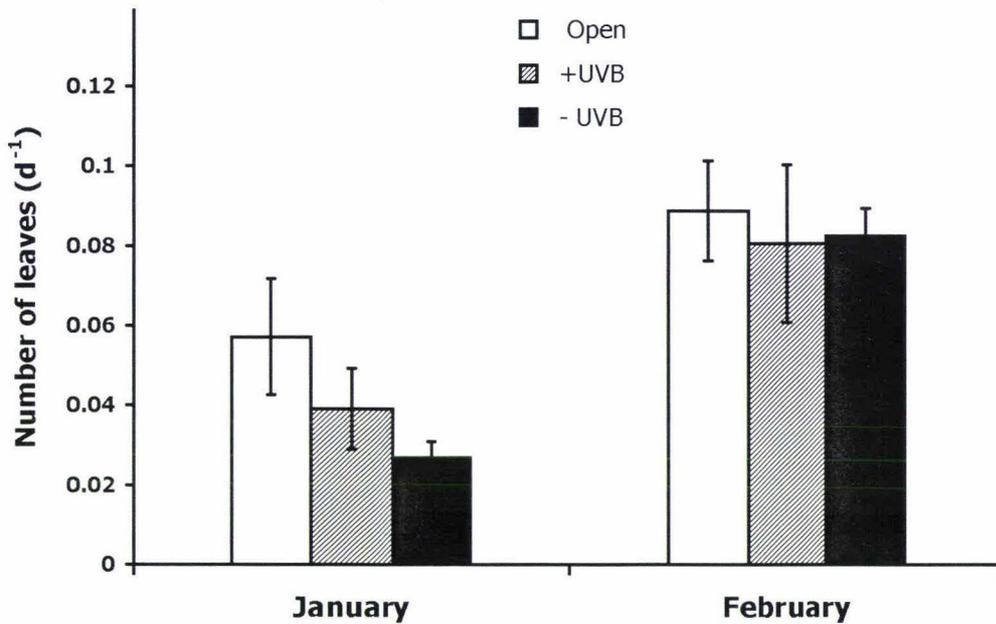


Figure 4-11. Number of fully senescent ryegrass leaves per tiller per day developing during the week from 10 to 17 January and during two weeks ending 9 February.

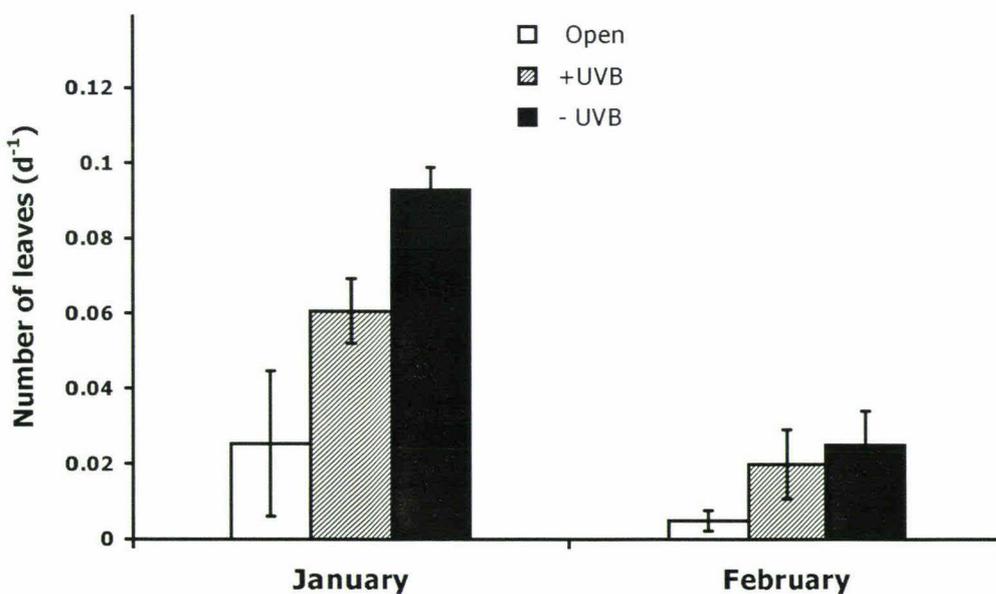


Figure 4-12. Net gain in the number of ryegrass leaves per tiller per day (difference in the number of leaves with attached blades between the beginning and the end of the measurement period) during the week from 10 to 17 January and during two weeks ending 9 February.

4.3.2.3. Plant biomass

Plant above-ground biomass in ryegrass on 17 January and 9 February was not significantly affected by solar UV-B (data not shown).

4.3.3. Pea

4.3.3.1. Shoot morphology and growth

In both pea harvests, plant growth attributes in uncovered plots showed significant reductions at $P < 0.05$, including leaf area and dry mass (Figure 4-13A and B) and petiole length (Figure 4-14). A similar picture was derived from measurements of plant biomass, height and leaf numbers in February (Table 4-5). However, many pea plants (one third in January and two thirds in February) did not survive growth without canopies during summer and results from open plots for pea therefore need to be regarded with caution. A marginally significant increase in specific leaf mass in February was also due to increases in open plots ($P = 0.080$, Figure 4-13C). Analysis of the contrast between the UV-B-transmitting and the UV-B-absorbing filter treatments did not reveal any significant differences for any of the parameters measured (Figure 4-13A to C, Figure 4-14, Table 4-5).

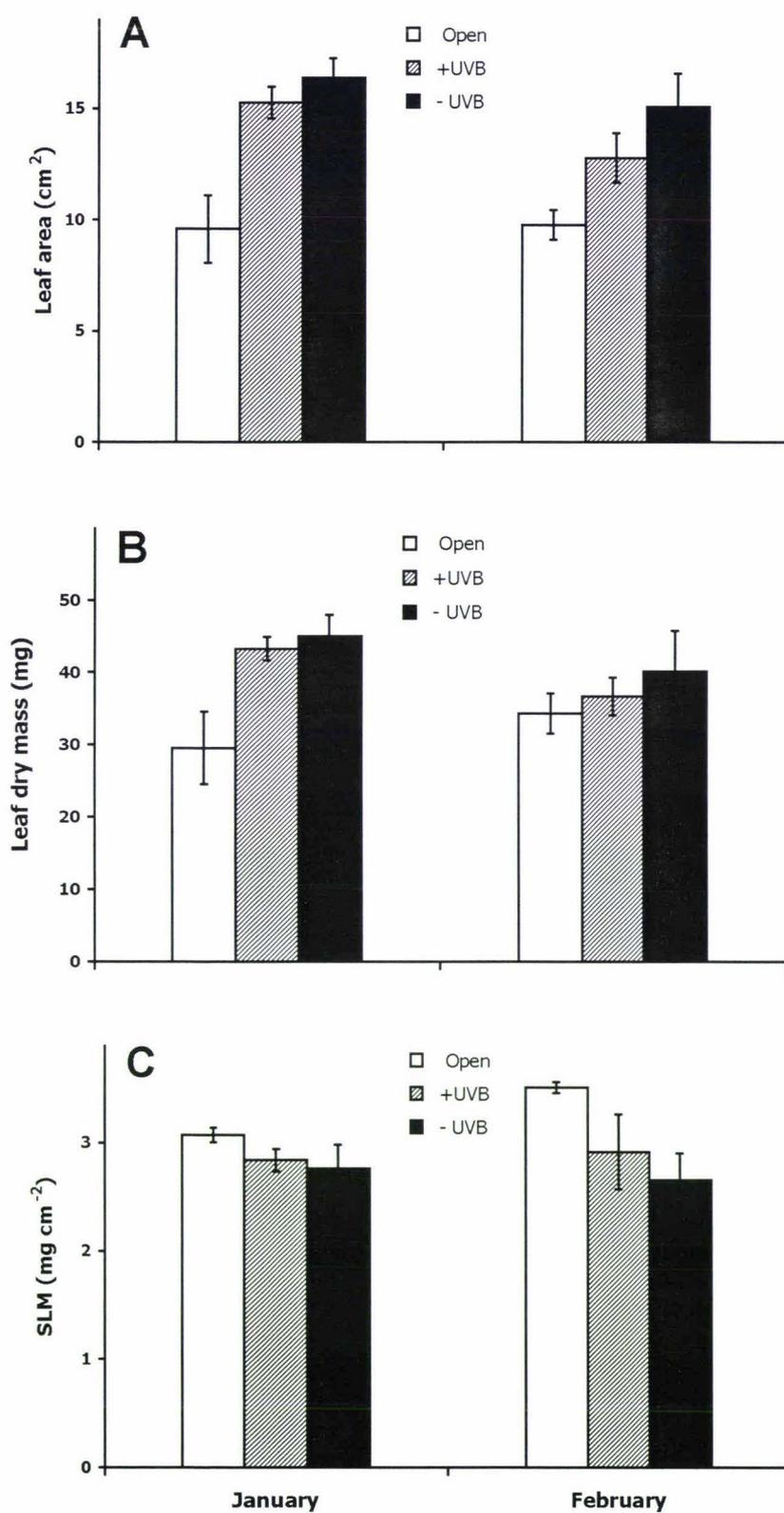


Figure 4-13. Area (A), dry mass (B) and specific leaf mass (C) of the youngest unfolded pea leaf (8 January and 6 February).

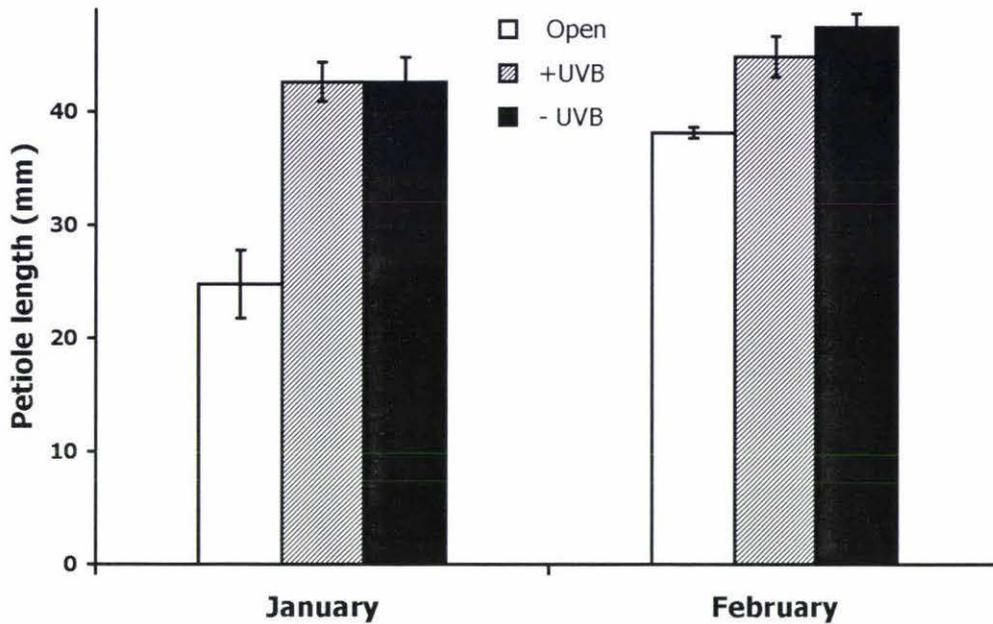


Figure 4-14. Petiole length of the youngest fully unfolded pea leaf harvested on 8 January and 6 February.

Table 4-5. Whole plant measurements in pea. ** denotes $P < 0.01$, * denotes $P < 0.05$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Plant biomass (mg)					
8 January	189	396	416	41	*
6 February	234	450	471	43	*
Stem height (mm)					
8 January	75.4	153.6	161.2	13.5	*
6 February	170.4	258.8	265.9	10.0	**
Leaf number					
6 February	6.4	8.0	7.7	0.3	*

4.3.3.2. Inflorescence formation

Surviving plants in the uncovered plots showed no signs of flowering, resulting in highly significant effects for all traits in the overall analysis (Table 4-6). In the contrast of the UV-B-absorbing and the UV-B-transmitting filter treatments, differences could also be found for inflorescence formation. Compared to UV-B-absorbing filters, there was an increase in open inflorescences by two thirds under UV-B-transmitting filters ($P = 0.057$). Furthermore, all inflorescences in the latter treatment had already developed into open inflorescences, while buds could still be observed in the reduced UV-B environment ($P < 0.001$). Taken together this led to an 18% increase in total inflorescence numbers under UV-B-transmitting filters, compared to UV-B-absorbing filters ($P = 0.085$).

Table 4-6. Inflorescence formation in pea plants on 6 February. Numbers represent the sum of pea inflorescences and buds per treatment. For statistical significance *** denotes $P < 0.001$.

	Treatment			Deviance ratio	<i>P</i>
	Open	+ UV-B	- UV-B		
Open inflorescences	0	78	47	226	***
Buds	0	0	19	45081232	***
Total inflorescences	0	78	66	1206	***

4.4. Discussion

4.4.1. Pasture species

To the knowledge of the author, this is the first study examining effects of solar UV-B on various vegetative, reproductive and developmental aspects in pasture and vegetable crop plants growing under field conditions in the southern hemisphere. The results show that present-day levels of solar UV-B in New Zealand can represent a limiting factor for the morphological development of important pasture plants. Despite recent increases in research activity, outdoor and filtration experiments are still less common to indoor studies. The findings from the present work are in accord with studies in controlled environments and the glasshouse, showing UV-B effects on white clover (Hofmann et al., 2000; Lindroth et al., 2000) and ryegrass (Deckmyn and Impens, 1999).

Most of the UV-B experimentation in the field has been conducted in the northern hemisphere. Furthermore, most of these investigations used UV-B supplementation, comparing effects under enhanced UV-B radiation to ambient UV-B levels. Studies examining effects of natural solar UV-B irradiation in the southern hemisphere are much less frequent. Such studies are of particular interest in regard to temperate plant species introduced from the northern hemisphere. Solar UV-B levels in New Zealand can be up to 40% higher than at comparable latitudes in the northern hemisphere (Madronich et al., 1998). This is likely to result in enhanced UV-B sensitivity compared to similar studies in the northern hemisphere (Papadopoulos et al., 1999). Studies at a mid-latitude site in Argentina on barley revealed that even the lower ambient UV-B levels in late winter and spring resulted in appreciable plant responses (Mazza et al., 1999a). One recently published study has reported the effects of solar UV-B in New Zealand (Lincoln) on native plants (Hunt and McNeil, 1999). That study included the same summer 1995/1996 season as the present work and results showed that ambient levels of UV-B can also affect the morphology and physiology of tree species native to New Zealand (Hunt and McNeil, 1999).

Contrary to what might have been expected, both pasture species were negatively affected by solar UV-B under New Zealand field conditions. While it has been suggested that monocotyledons could be more UV-B-tolerant than dicotyledons (Pal et al., 1997; Cybulski and Peterjohn, 1999; Musil and Wand, 1999), a number of field studies do indeed show susceptibility of monocotyledonous species to solar UV-B (Häder, 1996; Mark et al., 1996; Mark and Tevini, 1997; Mazza et al., 1999a; Xiong and Day, 2001). In fact, some of the morphological features of monocotyledons may not be of particular advantage for such species under field conditions. For example, the steeply inclined leaf orientation is seen only of advantage in supplementation experiments as these have a much higher direct UV-B component compared to the normally high isotropic diffuse UV-B component (Caldwell, 1981). Furthermore, while the DNA in meristematic regions of monocotyledons such as ryegrass may be shielded against UV-B, other damaging effects on growth can occur, e.g. due to inhibition of auxin production (Kulandaivelu et al., 1989).

White clover proved a suitable model to test the hypothesis that younger plant tissue is particularly sensitive to UV-B. Relatively few studies have examined developmental plant responses to solar UV-B and even less is known about sequential UV-B responsiveness along the plant stem. While there were similar trends between ontogenetic stages for leaf area (Figure 4-3A to C) – and to a lesser degree for internode length (Figure 4-5A to C) – statistically significant effects of solar UV-B were only observed in younger leaves and internodes. Lesser sensitivity in older plant parts was also reflected in the analysis of mean effects across developmental stages, with fewer and less significant effects (Table 4-1). Apart from an ontogenetic explanation, it is possible that less pronounced UV-B effects in mature plant parts could also be due to initiation at a time when UV-B levels were lower (December). On the other hand, UV-B effects were also lacking at the February harvest in mature white clover leaves (Figure 4-3C), which would still have been initiated at time of higher UV-B (January).

Results from other studies would support the suggestion that young plant tissues are sensitive to solar UV-B, and that this damage is transient (Deckmyn and Impens, 1995; Mark and Tevini, 1997; Saile-Mark and Tevini, 1997; Hunt and McNeil, 1999). In dwarf bean, leaf area of the highest (youngest) leaf layers was affected most by solar UV-B (Deckmyn and Impens, 1995). Under New Zealand field conditions, morphological changes in mountain beech under solar UV-B (decreased height and increased leaf number) were found at a developmental stage characterised by newly formed, rather than previously established leaves (Hunt and McNeil, 1999). From this it was suggested that early leaf appearance might be a particularly UV-B-sensitive developmental stage. These studies also showed temporarily increased photoinhibition in young, but not older leaves of mountain beech in response to solar UV-B in New Zealand (Hunt and McNeil, 1999).

In contrast to overall stem length or plant height, the length of the individual internodes has not been frequently studied in UV-B research. The results here show that non-significant effects on the length of the whole plant stem do not necessarily indicate non-significant effects on individual portions of the stem. The UV-B-induced shortening of young internodes in this study is remarkable, considering that in white clover horizontal stolon growth occurs close to the ground and is partially shielded by the leaf canopy. Furthermore, the stolon tip is well protected against UV-B by the developing leaves.

Rather than direct UV-B effects on the growing region in the stolon tip, this could suggest indirect responses resulting in internode shortening under solar UV-B. Studies in tomato have shown that inhibition of stem elongation has a maximum at 300 nm and is triggered by a specific photoreceptor (Ballare et al., 1995).

While the physiological mechanisms for decreases in stem elongation and in leaf area are not clear, it has been proposed that these particular changes could be due to altered synthesis of hormones (Tevini, 1994; Ros and Tevini, 1995; Krizek et al., 1997). The auxin indoleacetic acid (IAA) regulates elongation growth and can be destroyed or reduced by UV-B, while growth-retarding hormones such as abscisic acid can be enhanced (Zhang et al., 1994). UV-B-generated leaf area reductions can also be due to reductions in cell length or to redistribution of carbon to thick kness rather than expansion growth (Mark and Tevini, 1997).

In addition to the relationship with developmental stage, the present studies also indicated temporal dependency of UV-B sensitivity. Most morphological effects of solar UV-B in the two pasture species were observed during midsummer in January, with decreases of leaf area (Figure 4-3) and of internode elongation (Figure 4-5) in white clover, as well as reductions in leaf elongation (Figure 4-7), lower leaf numbers (Figure 4-10) and accelerated leaf loss by senescence in ryegrass (Figure 4-11).

In accordance with the present findings, leaf area was only reduced at early developmental stages in cultivars of maize and sensitivity of growth parameters decreased with increasing duration of exposure to UV-B during summer in Portugal (Mark et al., 1996). A filtration study in faba bean also demonstrated decreasing sensitivity of parameters such as leaf area and shoot length with subsequent harvests during mid to late summer in the Netherlands (Visser et al., 1997). Leaf length and plant height reductions in tropical plants exposed to solar UV-B were less pronounced with time (Searles et al., 1995). Under lower springtime solar UV-B in the northern hemisphere, no effects could be found on the morphology and growth of pot-grown ryegrass plants, but these parameters were significantly affected in summer (Deckmyn and Impens, 1999). This was explained by the fact that grass growth is usually already naturally reduced in summer, partly due to higher temperatures and lower water availability. While the ryegrass plants in the present study were watered daily, they

were exposed to higher temperatures in summer. It has been suggested that the latter could reduce general grass vigour, photosynthesis and repair mechanisms (Deckmyn and Impens, 1999).

The generally less pronounced morphological sensitivity of the plants in February could be related to acclimatisation to summer UV-B or may be due to lower UV-B doses received at the second harvest. However, for the second harvest on 9 February, leaf elongation and leaf appearance measurements in ryegrass were conducted during the two weeks preceding that harvest, when UV-B levels were still high. The tolerance of ryegrass to UV-B in this second harvest could therefore suggest that acclimatisation was the main factor. In tropical plants, acclimatisation to continued sun exposure resulted in reduced photosynthetic sensitivity to UV-B (Krause et al., 1999). Similarly, a study in the grass *Bromus* showed improvements in morphological responses to solar UV-B with time (Deckmyn and Impens, 1998a). These improvements were found during summer despite higher UV-B levels, also suggesting plant acclimatisation to UV-B rather than a mere dose response.

Features of senescence have only rarely been studied in examinations of solar UV-B effects on plants. Southern hemisphere solar UV-B did not affect the number of dead leaves in native vascular plants of Antarctica (Ruhland and Day, 2000). Recent findings in *Arabidopsis* showed that enhanced UV-B results in active stimulation of biochemical and molecular mechanisms of senescence (John et al., 2001). In the present study, ryegrass senescence was not significantly affected when measured as reduction in green leaf blade length (Figure 4-8). However ryegrass senescence measured as accelerated leaf loss was increased in January (Figure 4-11). This suggests that ryegrass leaves did not lose photosynthetic area via senescence-related reductions in leaf length, but by a faster rate of blade loss. Conversely, ryegrass plants under the UV-B-absorbing filters did not only form more and larger leaves, but also fewer were lost to senescence. The accelerated loss of older leaves under solar UV-B could also suggest an increase in litter production, which in turn could decrease decomposition rates and biogeochemical cycling, at least temporarily during midsummer.

Only a limited amount of research has separated effects of solar UV-B on leaf area per leaf from those on leaf number. A New Zealand study found UV-B reduced size per leaf

but increased leaf number in mountain beech due to solar UV-B (Hunt and McNeil, 1999). The findings in ryegrass are in accord with studies of the native grass *Deschampsia* in Antarctica, showing similar decreases in leaf elongation and reduced leaf numbers under solar UV-B (Day et al., 1999; Ruhland and Day, 2000; Ruhland and Day, 2001). Leaf number and leaf area were also decreased by solar UV-B in New Zealand spinach (Adamse et al., 1997) and UV-B-sensitive cucumber cultivars (Krizek et al., 1997).

In this study, decreases in white clover leaf number appeared to be delayed in comparison to decreases in leaf area. This may mean that the impact of UV-B on leaf initiation for white clover may take longer to come into effect than effects on leaf expansion. Leaf area development is a function of cell number formation and cell expansion. It is conceivable that the UV-B-induced reduction in cell expansion precedes that in cell division, e.g. by affecting the expansion of already formed – but not yet fully expanded – cells of emerging leaves. Leaf appearance rate in the well-shielded white clover stolon tip is mainly a function of cell division (Thomas, 1987), but could also be affected by indirect modes of UV-B action on cell division, e.g. via changes in plant hormone levels (Kulandaivelu et al., 1989). In addition to decreased initiation rates at the apex, reductions in leaf numbers could also be due to delays in leaf emergence from the stolon tip.

As with leaf appearance, inflorescence formation in white clover was affected by solar UV-B only at the late-summer harvest in February. Reproductive development was accelerated by solar UV-B and UV-B-induced increases in the number or biomass of reproductive structures have also been reported elsewhere, (e.g. Musil and Wand, 1994; Day and Demchik, 1996; Demchik and Day, 1996; Wand et al., 1996; Visser et al., 1997). Studies of a number of European heathland species also revealed stimulatory effects of UV-B on flowering, e.g. in *Vaccinium myrtillus* and *Cistus creticus* (Björn et al., 1997). The findings here are further substantiated by similar experiments with vascular native plants in Antarctica which were conducted during the same season as the present study (Day et al., 1999). Results from that work showed an increase in the development of open reproductive structures while vegetative growth was retarded by solar UV-B (Day et al., 1999). This was found particularly at the more advanced flower stages and with later harvests during the 1995/1996 summer season.

The results from white clover thus not only show decreases in the size of vegetative plant characteristics but also in the duration of vegetative development, represented by earlier inflorescence formation. These findings suggest a quantitative shift away from the formation of leaves towards formation of inflorescences. This could be mediated at the level of cell division and could be further investigated by detailed anatomical investigations at the stolon tip. The reason for this shift is not clear, but it has been suggested that the promotion of reproductive development could be a general stress response as a consequence – or at the cost – of the reduction in vegetative growth (Day et al., 1999). White clover leaf area and young stolon elongation was reduced at the first harvest, followed by increased reproductive development at the second harvest. This could suggest that some time was needed to translate the reduction in vegetative growth to the simulation of flowering.

Specific leaf mass in white clover was not significantly altered by solar UV-B (Table 4-2). Other studies show that UV-B tolerance can be related to increases in SLM (Deckmyn and Impens, 1999). However, no general trend towards increased SLM could be found across numerous field experiments using UV-B supplementation (Searles et al., 2001). Solar exclusion experiments also show a variety of SLM responses to ambient UV-B among legumes, including decreases in dwarf bean (Deckmyn and Impens, 1995), increases in cowpea (Lingakumar et al., 1999), and no change in faba bean (Visser et al., 1997). It is possible that the sensitivity of white clover morphology to solar UV-B in the present study may be due to a lack in the ability to increase SLM. In accordance with the findings here, SLM was not affected by solar UV-B in a number of UV-B-sensitive cucumber cultivars (Krizek et al., 1997).

Plant biomass was not significantly affected by solar UV-B in ryegrass and white clover (Table 4-3), suggesting that the overall measure of shoot biomass may not be sensitive enough to detect important UV-B-induced changes on a morphogenetic level, i.e. differential sensitivity within and between plant parts. A number of other UV-B filtration experiments have also found that morphological changes are not necessarily translated into a cumulative effect on overall biomass production (Deckmyn and Impens, 1995; Searles et al., 1995; Schumaker et al., 1997; Visser et al., 1997; Deckmyn and Impens, 1998a; Deckmyn and Impens, 1999; Hunt and McNeil, 1999).

For example, a northern hemisphere glasshouse study with pot-grown grasslands species showed no significant effect of springtime solar UV-B on ryegrass biomass, although leaf area and height were reduced (Deckmyn and Impens, 1999).

In the present work, solar UV-B affected some morphological attributes in white clover (e.g. the area of younger leaves, Figure 4-3), while other features were not affected (e.g. petiole elongation, Figure 4-4). Added together, less sensitive aspects and adaptive responses can mask more sensitive features, resulting in a non-significant overall carbohydrate cost. Similarly, taking the mean response across developmental stages (Table 4-1) showed fewer significant changes compared to the analysis of individual plant sections. The results from this study are further supported by meta-analysis of UV-B supplementation field research, showing that enhanced UV-B is less likely to result in damaging effects on overall plant biomass, but rather causes subtle shifts in plant morphology which in turn could translate into shifts in plant:plant relationships (Searles et al., 2001). Furthermore, the present study did not examine root biomass and it is possible that this could be differentially affected, compared with the above-ground biomass measurements.

4.4.2. Pea

To the author's knowledge, this is the first study examining in detail morphological responses of pea to solar UV-B radiation. An earlier more general study showed that solar UV-B at a similar northern hemisphere latitude (39°N in Colorado, USA) but at high elevation (3000 m altitude) did not affect biomass accumulation and height of pea (Becwar et al., 1982). The present findings support those from UV-B supplementation studies on pea, concluding no major sensitivity of vegetative growth or morphology to UV-B in the field, even under strongly enhanced UV-B radiation (Allen et al., 1999; Stephen et al., 1999). A contribution of SLM towards this UV-B tolerance in pea is not likely, because SLM values under UV-B-absorbing filters were similar to those in the UV-B sensitive white clover, and were not significantly increased under the UV-B-transmitting filters (Figure 4-13C).

UV-B tolerance in pea is further highlighted by the fact that – contrasting to the pasture plants which had been sown in autumn – the pea seedlings had only a few weeks to

acclimatise to UV-B and still showed no significant negative responses under UV-B-transmitting filters. The pronounced decreases in growth and morphological development in the open plots therefore could not be related to UV-B effects *per se*. It appears that field exposure without protective canopies represented the far greater stress for the young pea seedlings. A possible explanation for this could be exposure to intermittent desiccation during the day. Watering of the open plots by hand was usually done in the evening and it is possible that pea plants were more sensitive to temporary drought during daytime in summer. While not measured, it is likely that relative humidity was higher under the filters, maintaining and facilitating growth.

However, reproductive development did show significant differences between filter treatments in pea. A picture emerged similar to the results from white clover inflorescence formation in February, again suggesting acceleration of reproductive development under UV-B (Table 4-6). This is supported by the fact that all inflorescences in the UV-B-transmitting environments were already fully developed, with no floral buds remaining (Table 4-6).

In conclusion, the results from this study suggest interspecific differences in UV-B sensitivity between important pasture and crop species, independent of evolutionary relationship. Ryegrass and the legume white clover appeared UV-B-sensitive, while most characteristics in the legume pea generally displayed tolerance to near-ambient UV-B levels in New Zealand. In chapter 6 it will be examined whether the solar UV-B effects observed here on pasture species growing in monoculture also apply under actual sward conditions.

5. Interspecific differences in leaf absorbance

5.1. Introduction

An analytical review of UV-B field studies over the last 25 years showed that accumulation of UV-absorbing compounds is one of the most consistent plant responses to UV-B (Searles et al., 2001). The presence of higher levels of UV-absorbing compounds represents an important mechanism of plant defence against UV-B. This protection effect can be due both to high intrinsic (Gonzalez et al., 1996; Häder, 1996; Corlett et al., 1997) or high UV-B-elicited levels of such compounds (Murali and Teramura, 1986; Day and Demchik, 1996; Sato and Kumagai, 1997). UV-absorbing compounds have been demonstrated to protect leaf tissue against DNA damage from solar UV-B (Mazza et al., 2000). Studies with mutants lacking in these compounds show reduced plant growth under UV-B (Landry et al., 1995; Bieza and Lois, 2001).

UV-absorbing compounds encompass a number of plant molecules, including chlorophyll, proteins and a number of secondary compounds. The latter comprise the bulk of the UV-B-absorbing compounds, usually composed of soluble derivatives of the shikimic acid pathway, namely phenolic acids and flavonoids (Lavola et al., 1997). Accumulation of UV-B-absorbing compounds is wavelength-dependent and strongly induced by the UV-B portion of the solar spectrum (Mazza et al., 2000). A summary of responses in different plant groups from 102 indoor and outdoor studies showed that the largest taxonomic group with increased levels of UV-B-absorbing compounds or flavonoids was trees (30% of species tested), followed by grasses and shrubs (25%) (Gwynn-Jones et al., 1999b). In contrast, only 13% of the studies in herbaceous dicotyledons showed increases in these compounds.

Other findings have demonstrated increases in anthocyanin concentration under solar UV-B (Krizek et al., 1998). Anthocyanins are specific flavonoids that can act as antioxidants, scavenging active oxygen radicals resulting from UV-B radiation (Tsuda et al., 1996). While not UV-B-absorbing on their own, these pigments can also contribute to the absorption of UV-B in acylation with phenolic UV-absorbing

compounds (Woodall and Stewart, 1998). Results from UV-B supplementation studies suggest that accumulation of anthocyanins is related to UV-B tolerance (Stapleton and Walbot, 1994; Burger and Edwards, 1996).

Little is known about the effects of solar UV-B radiation in the field on the accumulation of UV-B-protective pigments in pasture species. Similarly for pea, all of the existing information on the formation of such compounds is based on UV-B supplementation studies. Furthermore, UV-B tolerance has most often been either linked to the accumulation of UV-absorbing compounds or to higher leaf thickness or SLM (Deckmyn and Impens, 1999). The results from the previous chapter showed no clear relationship between SLM and interspecific differences in UV-B sensitivity, warranting examination of the alternative hypothesis of a biochemical relationship with UV-B-protective pigmentation.

This chapter investigates the accumulation of UV-absorbing compounds and anthocyanins in white clover, ryegrass and pea. Examinations of UV-absorbing compounds included absorbance measurements across the UV range as well as more detailed investigations in the UV-B portion of the spectrum. It was hypothesised that there would be interspecific differences in the accumulation of UV-absorbing and anthocyanin pigments, with higher increases of UV-absorbing compounds in the grass species. It was also expected that intrinsic or UV-B-induced differences in UV-absorbing compound accumulation or anthocyanin levels would be related to the interspecific differences in UV-B sensitivity observed in the previous chapter.

5.2. Materials and methods

Five young fully unfolded leaves (white clover and pea) and eight young leaf blades (ryegrass) were collected and pooled from each plot (see also Figure 4-1 on page 53), resulting in triplicate samples for each species under each treatment. Collections were done on 12 January and 19 February (white clover), 17 January and 9 February (ryegrass) and 8 January and 6 February 1996 (pea). Samples were oven-dried and ground with a mortar and pestle. 15 mg ground material was extracted in darkness for 24 h in 1.2 mL MeOH:H₂O:HCl (79:20:1) (Mirecki and Teramura, 1984). During the

extraction period, each sample was shaken at regular intervals with a vortex. After centrifugation at 16 000 *g* for 5 minutes, 100 μL of the supernatant was diluted in 3 mL acidified MeOH using a quartz cuvette and analysed with a Hitachi U-2000 double beam scanning spectrophotometer.

Absorbances were measured in 5 nm steps in the UV range from 220 to 400 nm and in addition at 530 nm to estimate anthocyanin content. Effects on UV-B-absorbing compounds were examined at 300 nm. This wavelength is commonly chosen because of its location in the middle of the UV-B range and because the generalised plant action spectrum is standardised to 1 at this wavelength (Fiscus et al., 1999). For tests on leaf absorbance across several wavelengths in the UV spectrum, the mean absorbance level was calculated from all values recorded between 220 and 400 nm (for absorbance across the entire UV range) and between 290 and 315 nm (for absorbance across the UV-B range). Absorbance values were calculated on the basis of leaf dry weight. Additionally in white clover and pea, absorbance values were also calculated per unit area from the leaf area and corresponding dry mass of young unfolded leaves. Experimental layout, experimental units and use of ANOVA was identical to that described in section 4.2.

5.3. Results

5.3.1. Total UV absorbance

Analysis across the UV spectrum (220 - 400 nm) revealed interspecific differences in intrinsic levels of UV-absorbing compounds, independent of UV-B treatment. This is illustrated in Figure 5-1A and B, showing the total UV absorbances in both harvests under UV-B-absorbing filters. At both harvests, pea showed the highest levels of total UV-absorbing compounds, while overall differences between white clover and ryegrass were non-significant (Figure 5-1A and B). The same pattern was also observed for open ($P < 0.01$) and UV-B-transmitting ($P < 0.01$) plots in January as well as in February ($P < 0.05$ for open and for UV-B-transmitting plots).

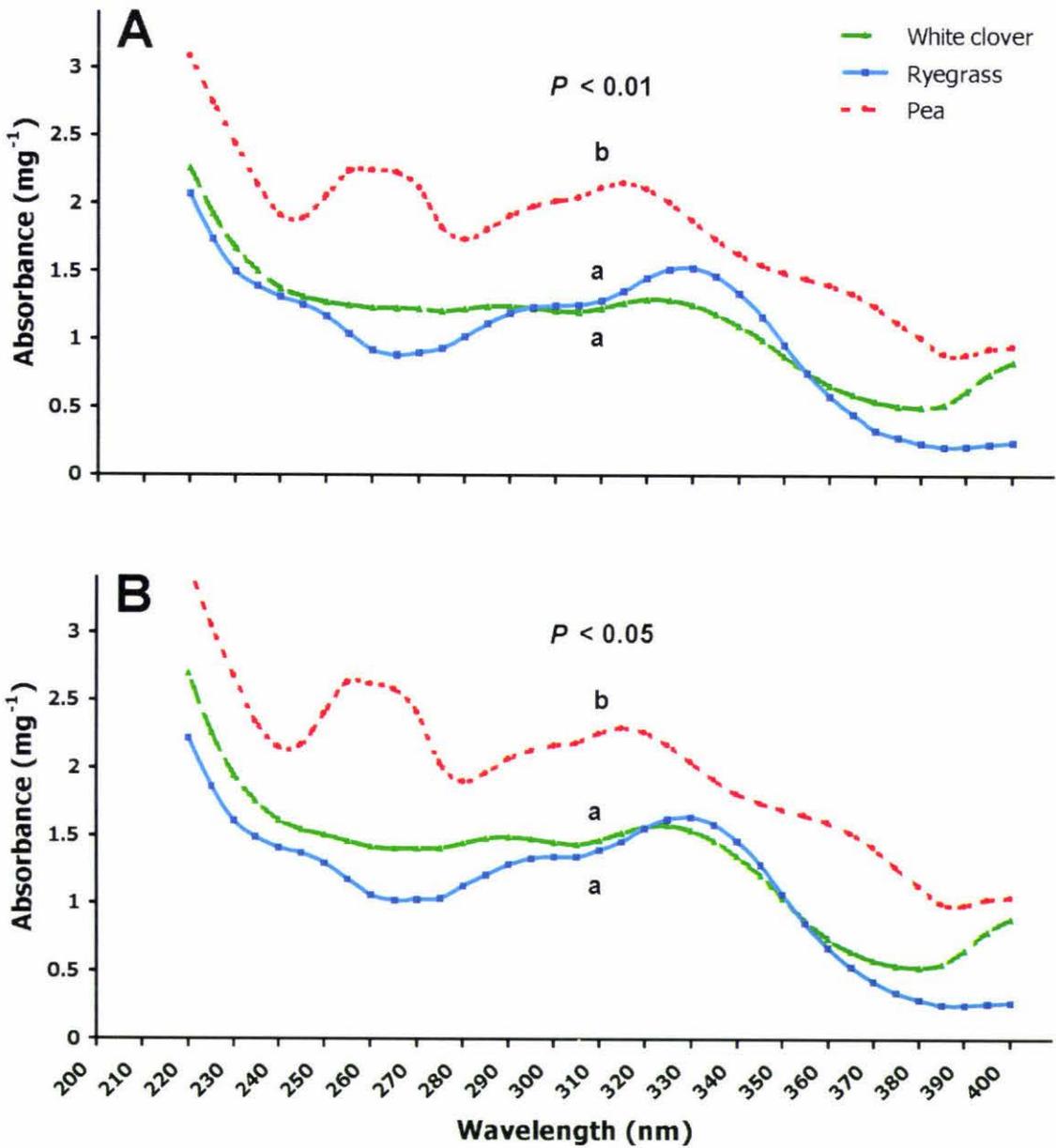


Figure 5-1. Total UV absorbance (per mg leaf dry weight) of methanolic leaf extracts of white clover, ryegrass and pea under UV-B-absorbing filters in (A) January and (B) February. Means are significantly different where letters are different.

5.3.2. UV-B-absorbing compounds

Close examination across the UV-B range (290 – 315 nm) revealed that under UV-B-absorbing (Figure 5-2A and B) and UV-B-transmitting filters (Figure 5-3A and B), absorbances in pea were one third to two thirds higher than for the other two species in both harvests. The distinction between pea and the two pasture crops was least

pronounced in open plots (Figure 5-4A and B). Significant differences in open plots were only detected between white clover and pea in January (Figure 5-4A), while UV-B absorbance did not differ between white clover and ryegrass in January, nor between all three species in February (Figure 5-4B).

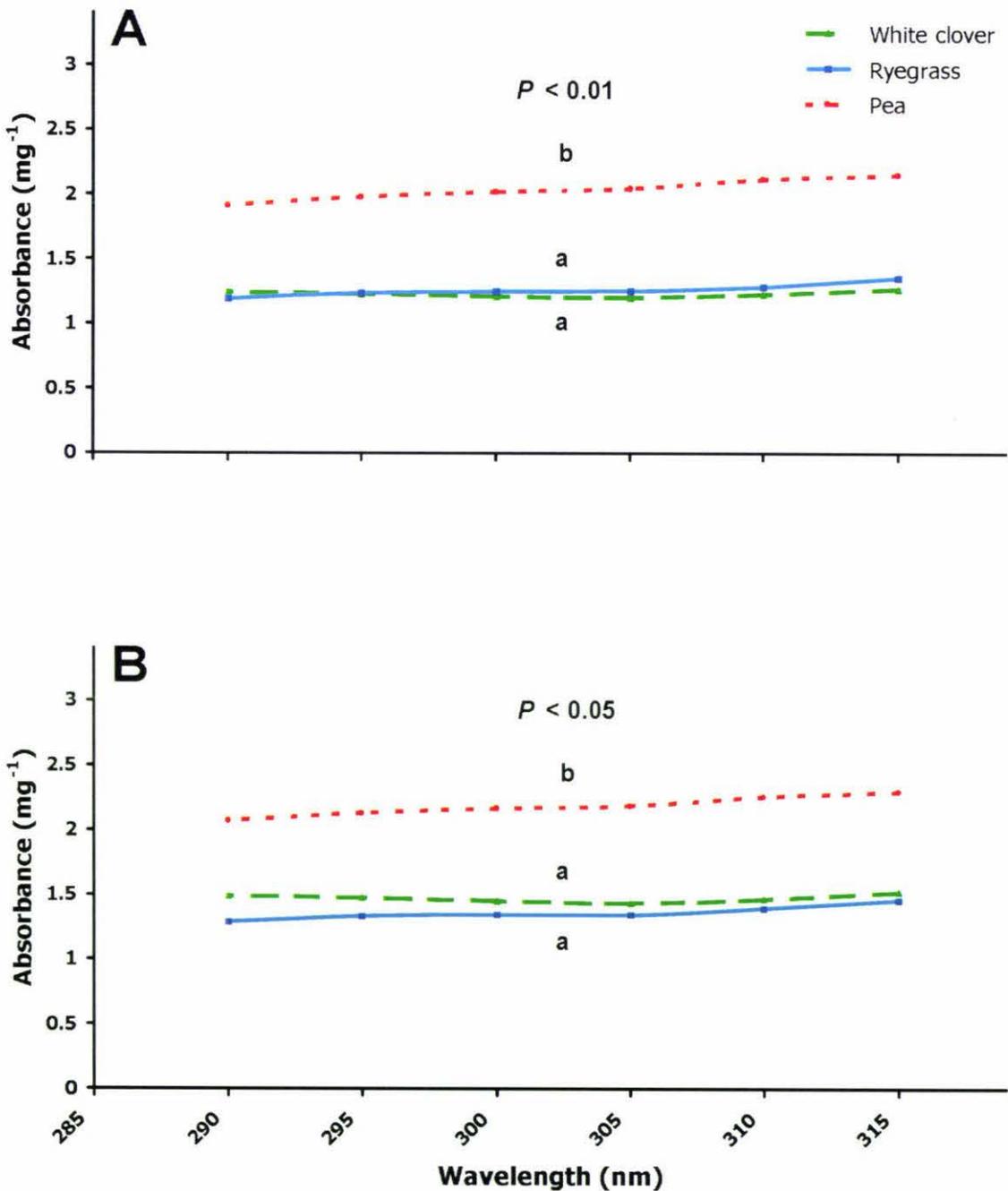


Figure 5-2. UV-B absorbance (per mg leaf dry weight) of methanolic leaf extracts of white clover, ryegrass and pea under UV-B-absorbing filters in (A) January and (B) February. Means are significantly different where letters are different.

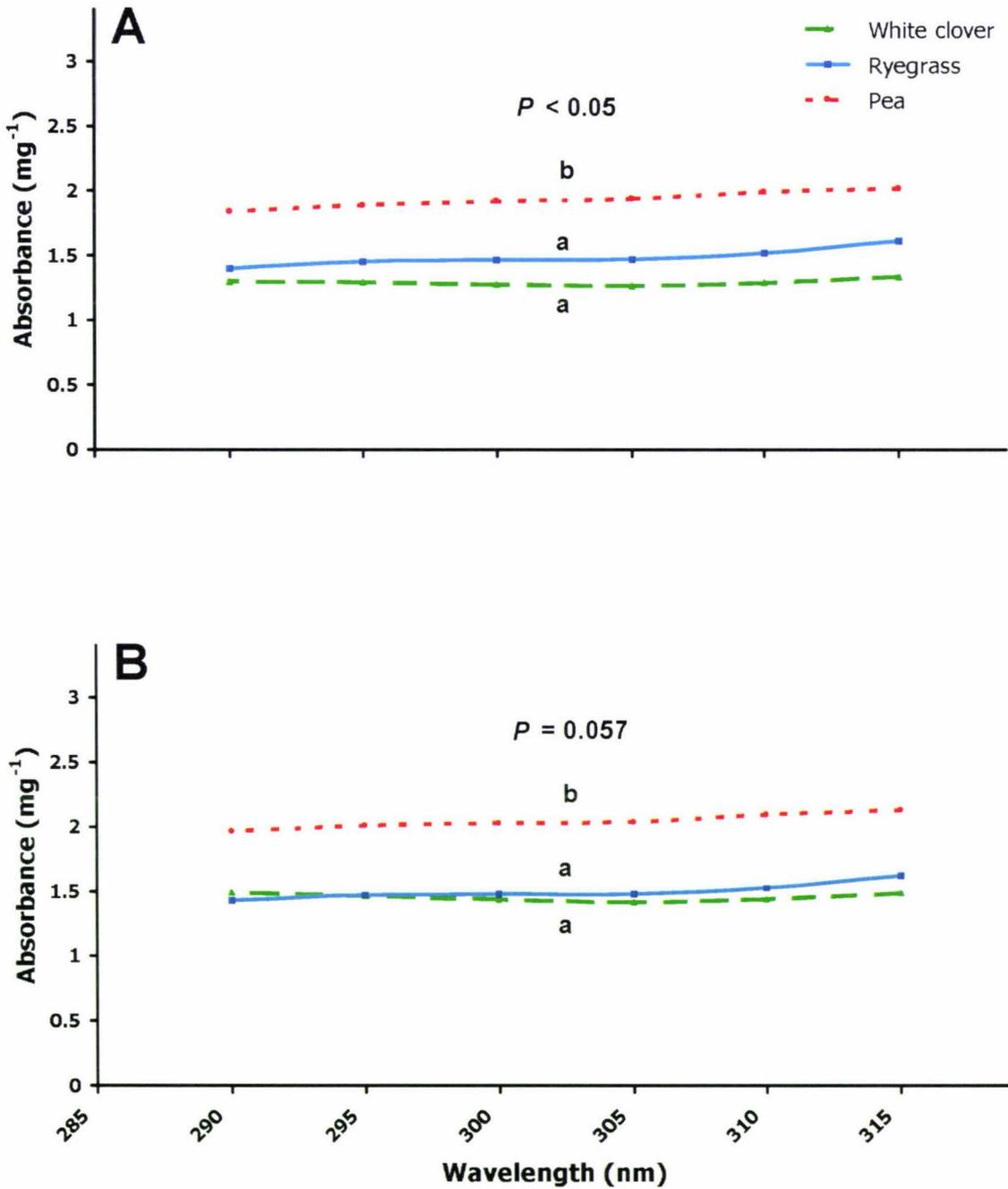


Figure 5-3. UV-B absorbance (per mg leaf dry weight) of methanolic leaf extracts of white clover, ryegrass and pea under UV-B-transmitting filters in (A) January and (B) February. Means are significantly different where letters are different.

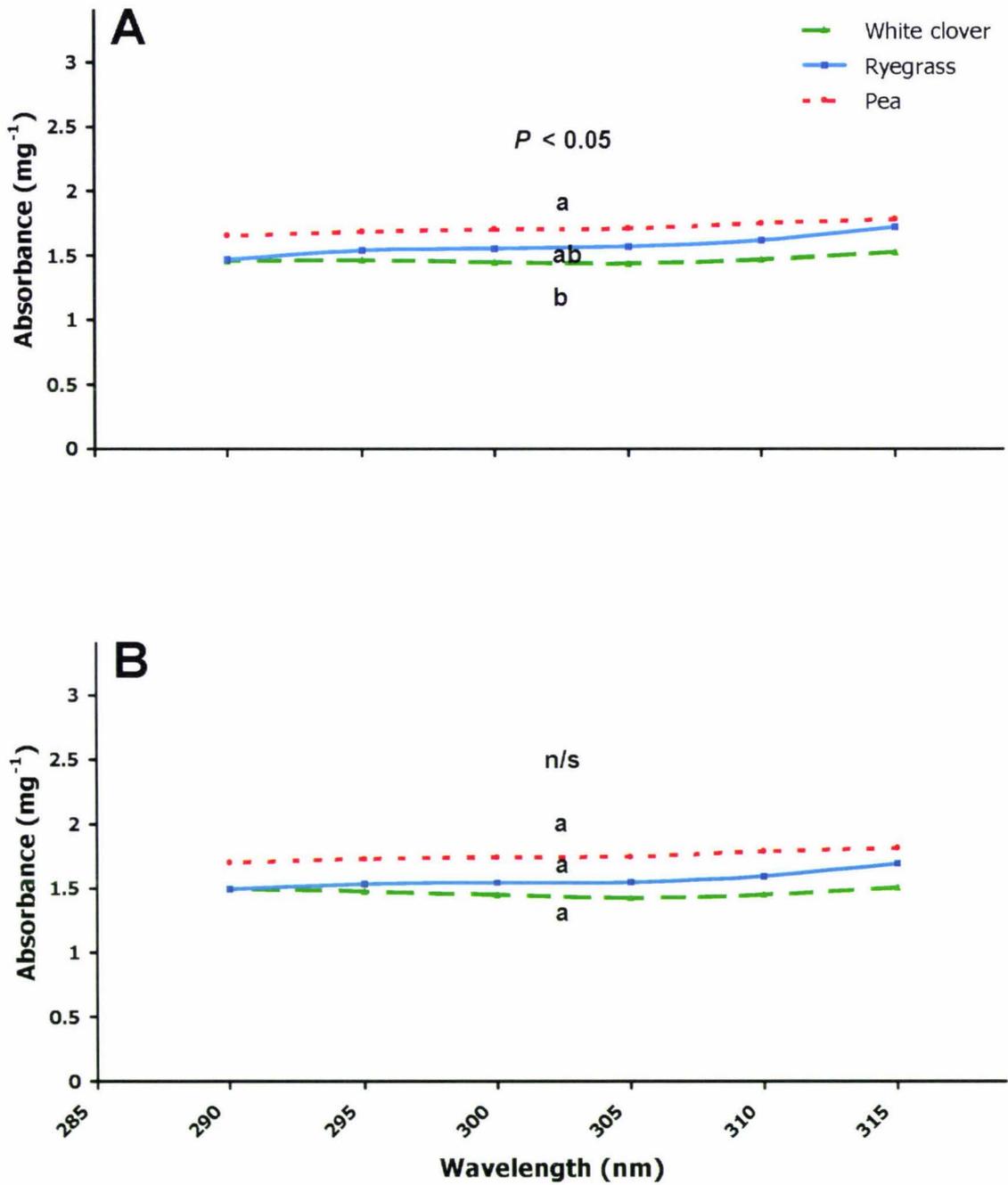


Figure 5-4. UV-B absorbance (per mg leaf dry weight) of methanolic leaf extracts of white clover, ryegrass and pea under open frames in (A) January and (B) February. Means are significantly different where letters are different.

Changes of UV-B absorbance in response to UV-B also showed differences within the individual plant species in January, while changes in February were generally non-significant. Results are presented here as absorbances at 300 nm and identical conclusions could be drawn from the analysis across the total UV-B range. In white clover in January, there was a non-significant increase in UV-B absorbance in the UV-B-transmitting plots and a 20% rise in open plots, compared to the levels under UV-B-absorbing filters ($P < 0.05$, Figure 5-5). In ryegrass, absorbances at 300 nm were on average 20% higher in both UV-B-transmitting treatments, compared to the absorbing filters in January ($P < 0.05$, Figure 5-6). In pea, UV-B absorbances did not change significantly between plastic filters, but were on average about 15% lower in the uncovered plots ($P = 0.074$, Figure 5-7). Changes in UV-B-absorbance were not significant when calculated on a leaf area basis (Table 5-1).

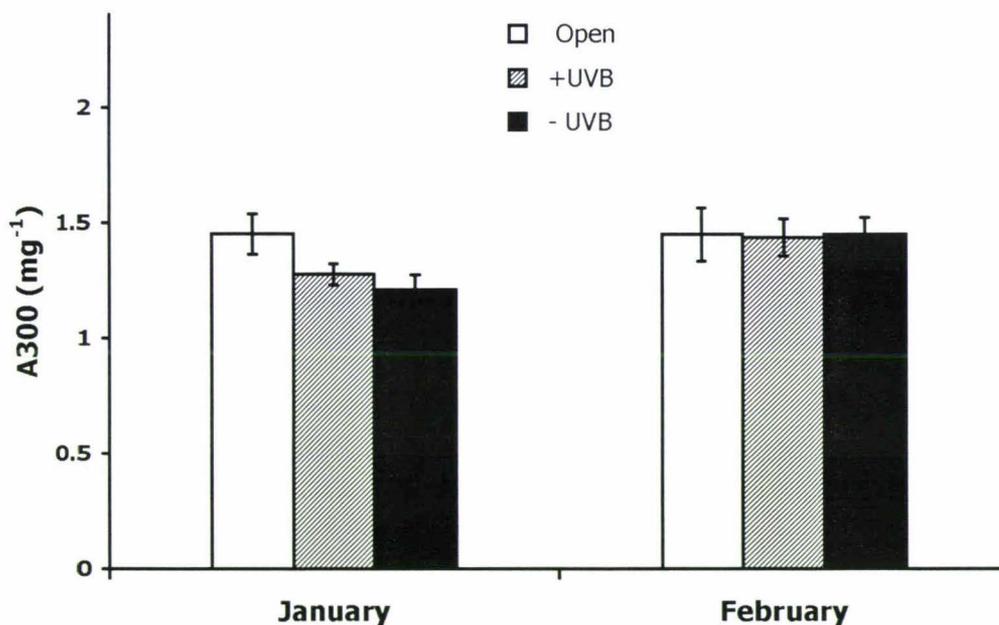


Figure 5-5. Absorbance at 300 nm of extracts of white clover leaves harvested on 12 January and 19 February.

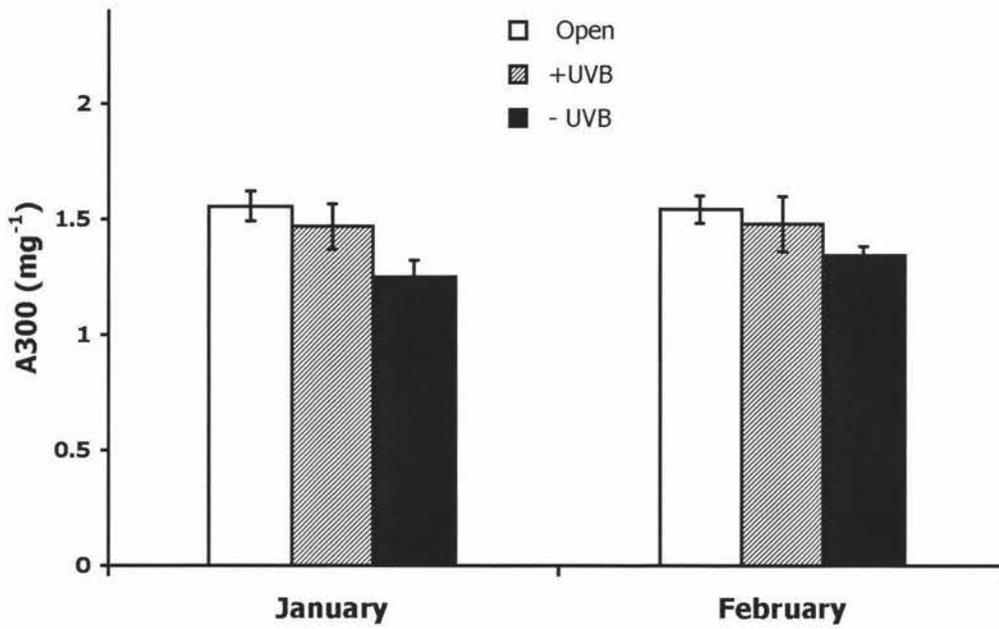


Figure 5-6. Absorbance at 300 nm of extracts of ryegrass leaves harvested on 17 January and 9 February.

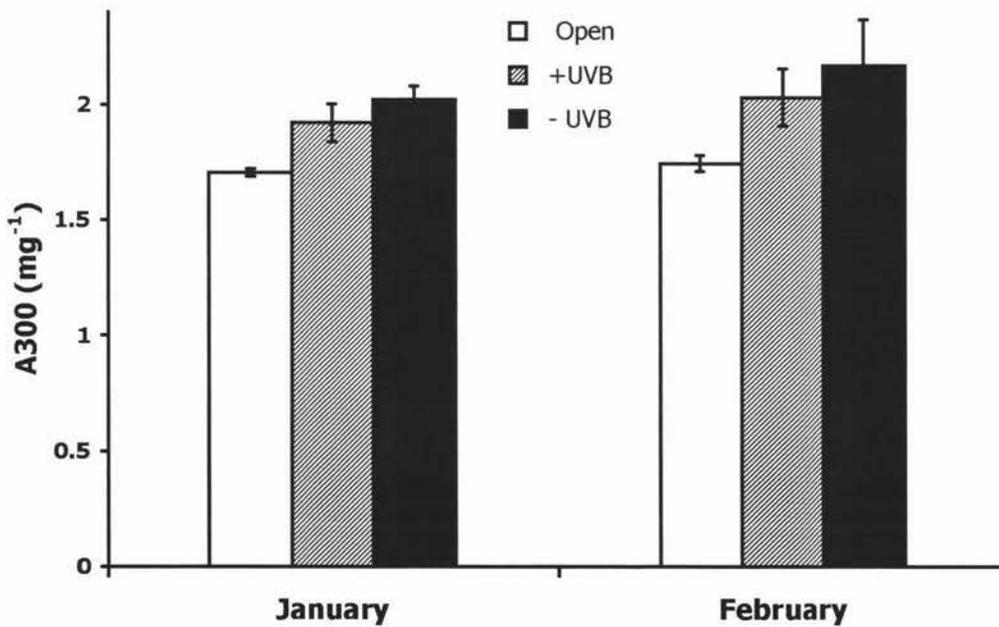


Figure 5-7. Absorbance at 300 nm of extracts of pea leaves harvested on 8 January and 6 February.

Table 5-1. Absorbance at 300 nm per unit area of white clover and pea leaves; ns denotes $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
White clover A300 cm ⁻²					
12 January	3.92	3.23	3.35	0.43	ns
19 February	3.74	3.82	3.68	0.29	ns
Pea A300 cm ⁻²					
8 January	5.24	5.44	5.60	0.31	ns
6 February	6.13	5.84	5.68	0.19	ns

5.3.3. Anthocyanins

Absorbances at 530 nm were used to estimate accumulation of anthocyanins. In all species, absorbances at 530 nm were more than an order of magnitude smaller compared to the absorbances in the UV-B range. Compared to ryegrass (Figure 5-9), absorbances at 530 nm in white clover (Figure 5-8) and in pea (Figure 5-10) were over three-fold higher across all treatments ($P < 0.05$). Absorbance levels at 530 nm between white clover and pea were not significantly different from each other in the UV-B-absorbing treatment in January and across all treatments in February (Figure 5-8 and Figure 5-10). In pea, there was a pronounced increase in absorbance at 530 nm in the UV-B-transmitting plots in January, more than one third that of the UV-B-absorbing filters ($P < 0.05$, Figure 5-10). In February, differences between plastic filters were non-significant for pea, but lower absorbance at 530 nm was noted in the uncovered plots ($P < 0.05$, Figure 5-10). Changes in absorbance at 530 nm were non-significant in white clover and ryegrass at both harvests. On a leaf area basis, changes in UV-B-absorbance were generally not significant (Table 5-2). However, when analysed as a contrast design (contrast of UV-B-absorbing versus transmitting filters), the increase in absorbance at 530 nm in pea under UV-B-transmitting filters in January was also noted on a leaf area basis ($P = 0.060$).

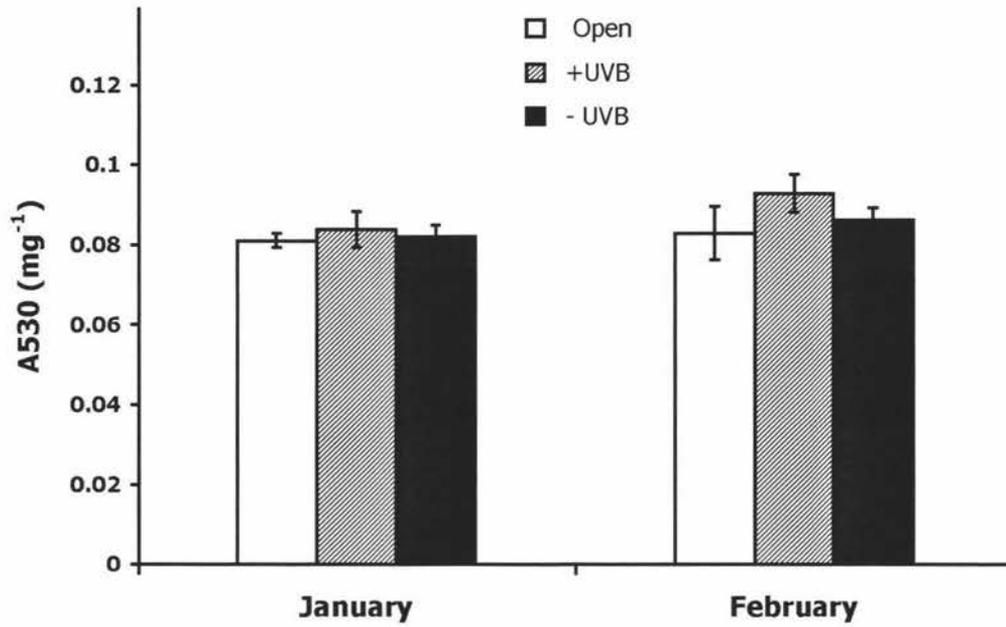


Figure 5-8. Absorbance at 530 nm of extracts of white clover leaves harvested on 12 January and 19 February.

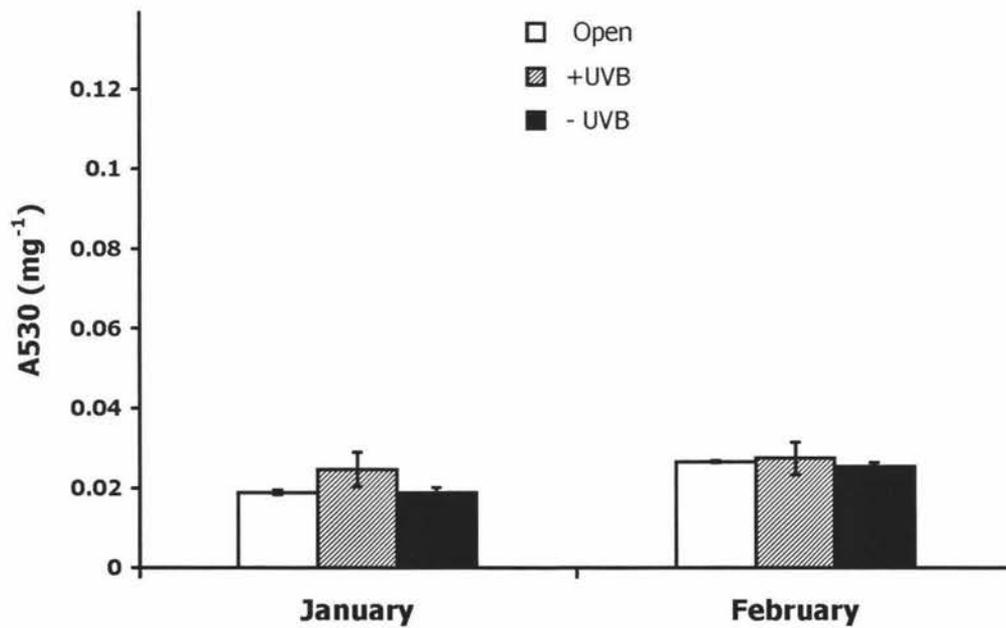


Figure 5-9. Absorbance at 530 nm of extracts of ryegrass leaves harvested on 17 January and 9 February.

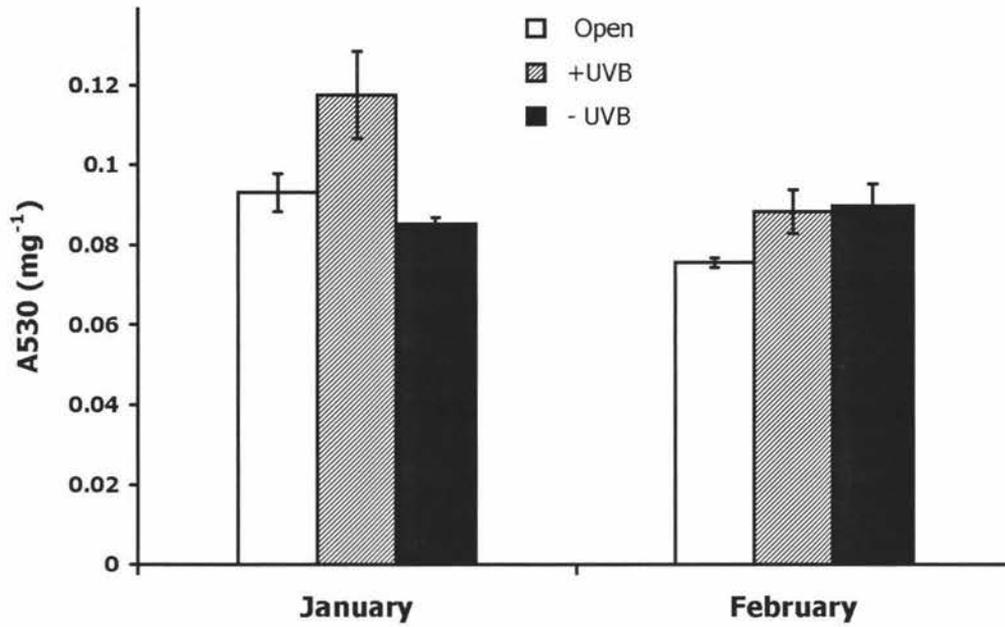


Figure 5-10. Absorbance at 530 nm of extracts of pea leaves harvested on 8 January and 6 February.

Table 5-2. Absorbance at 530 nm per unit area of white clover and pea leaves; ns denotes $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
White clover A530 cm ⁻²					
12 January	0.219	0.209	0.228	0.02	ns
19 February	0.214	0.247	0.219	0.02	ns
Pea A530 cm ⁻²					
8 January	0.286	0.333	0.236	0.03	ns
6 February	0.265	0.254	0.236	0.01	ns

5.4. Discussion

5.4.1. UV-B-absorbing compounds

The results from this study suggest that interspecific differences in UV-B sensitivity in the three plant species are linked to the absolute amounts of UV-absorbing compounds, rather than how much these can be increased by. Pea showed significantly higher levels of UV-absorbing compounds compared to the UV-B-sensitive pasture species white clover and ryegrass (Figure 5-1). UV-B-induced increases in UV-absorbing compounds could only be observed in the pasture species, particularly in ryegrass.

A number of studies have related differential UV-B responsiveness to intrinsic (rather than UV-B-elicited) levels of such compounds and the results from the present study are in accord with observations showing that plant species with high intrinsic levels of UV-absorbing compounds often do not increase these levels further under UV-B (Ballare et al., 1999). UV-B supplementation studies have shown that pea plants with higher intrinsic levels of UV-absorbing compounds were UV-B tolerant (Gonzalez et al., 1996; Corlett et al., 1997). In studies of solar UV-B effects on wheat, Chilean cultivars contained higher intrinsic amounts of UV-absorbing compounds and were less sensitive to the solar UV-B levels of northern Chile (22° S) compared to German cultivars which had lower amounts of UV-absorbing compounds (Häder, 1996). The latter cultivars nevertheless showed higher UV-B-induced levels of UV-absorbing compounds, demonstrating that an increase in these substances is not necessarily linked to UV-B sensitivity.

This is in agreement with the results from the present work, showing increased UV-B-absorbing compounds during midsummer in ryegrass (Figure 5-6) and to a lesser degree in white clover (Figure 5-5), concomitant with morphological UV-B sensitivity during that period (chapter 4). UV-B-absorbing compounds also increased in pot-grown ryegrass plants under solar northern hemisphere UV-B levels in the glasshouse (Deckmyn and Impens, 1999). That study also showed that increased production of UV-B-absorbing compounds occurred in more sensitive grass species such as ryegrass. The findings from the present work suggest that the increases in the pasture species were

still insufficient to provide effective UV-B protection, because under the UV-B-transmitting filters these levels never reached those of pea (Figure 5-3). The possibility of a threshold level is further indicated by the examination of UV-B-absorbing compound levels in the different treatments (Figure 5-2 to Figure 5-4). Only in the open plots were levels of these compounds in pea similar to the other two species, and in turn pea plants in the open plots showed reduced growth (Figure 4-13A and B, Figure 4-14).

Filtration studies on barley in South America showed that reductions in growth parameters – despite increases in UV-absorbing compounds – were due to DNA damage and oxidative stress (Mazza et al., 1999a). Alternatively, the negative morphological effects in the pasture species may even be due to the accompanying increases in UV-absorbing compounds. Restricted leaf development (e.g. expansion) has been related to reallocation of carbon resources towards UV-absorbing compound formation and other competing carbon sinks in response to solar UV-B (Schumaker et al., 1997).

In the comparison between filter treatments, there was no significant change in UV-B-absorbing compound formation for pea (Figure 5-7). No effects of ambient solar UV-B on UV-absorbing compound formation have also been recorded in other studies, e.g. in *Bromus* (Deckmyn and Impens, 1998a), cucumber (Krizek et al., 1997), faba bean (Visser et al., 1997), *Gumnera* (Rousseaux et al., 1998) and maize (Mark et al., 1996). This could be due to transfer of carbon to other protective functions such as antioxidant formation, or because of species-specific threshold levels of UV-B necessary to induce further production of these compounds (Krizek et al., 1997). The levels of UV-absorbing compounds in this study were similar to those observed for other herbaceous plant species investigated in examinations of solar UV-B filtration, e.g. in cucumber (Krizek et al., 1997) and lettuce (Krizek et al., 1998).

It was of further interest to note a decrease in UV-B-absorbing compounds in the open plots for pea (Figure 5-7). As discussed in the previous chapter, effects on pea in the open plots could not be attributed to a UV-B effect *per se*. The biochemical information obtained here shows that the additional stress experienced by these plants was also reflected in reduced accumulation of protective pigments. Other studies show that decreases in UV-absorbing compound levels can result in decreased growth and

morphological development, e.g. in cucumber (Krizek et al., 1997), soybean (D'Surney et al., 1993; Adamse et al., 1997) and tropical plants (Krause et al., 1999). The depletion of these compounds could represent damage to biosynthesis or turnover, or of transfer to other functions such as radical scavenging (Krizek et al., 1997).

While expression of the absorbance values per unit area did not change the overall interpretation of the weight-based data, some additional information could be gained from this measurement. The pronounced decrease in UV-B-absorbing compounds of pea plants in the open plots (Figure 5-7) was accompanied by increases in SLM in that treatment (Figure 4-13c). The measurement of UV-B-absorbing compounds on a leaf area basis represents a multiplication of weight-based absorbance levels (Absorbance mg^{-1}) with SLM (mg cm^{-2}). The multiplicative effect therefore meant that the increase in SLM partially compensated for the decrease in UV-B-absorbing compounds, so that on a leaf area basis this effect was statistically non-significant (Table 5-1).

However, if SLM - as commonly assumed - is related to leaf thickness, this result would also demonstrate even more pronounced decreases of UV-B-absorbing compounds per cross-section of the leaf (amount of UV-absorbing compounds *divided* by SLM). Thus while the leaves may have become thicker under stress exposure in the open plots, the amount of UV-B-absorbing compounds could not follow suit and even declined. While the nature and distribution pattern of UV-B-absorbing compounds in these leaves was not determined, it could be speculated that the morphological response could have even worsened the decline in biochemical protection as UV-B-absorbing compounds may have been distributed away from epidermal layers to the newly developed internal regions. These reflections further contribute to the suggestion that the observed decline in the number and growth of pea plants in open plots was related to pronounced biochemical deficiencies caused by stress exposure in the open plots.

5.4.2. Anthocyanins

Compared to the concentration of UV-absorbing compounds, anthocyanin levels were more than an order of magnitude lower in all plant species. A similar degree in difference has also been observed in other studies, e.g. in barley (Mazza et al., 1999a). In contrast to the absorbance readings at 300 nm, values at 530 nm were lowest in

ryegrass (Figure 5-9), while there was little difference between white clover (Figure 5-8) and pea intrinsic anthocyanin levels (Figure 5-10).

Unlike the observations from UV-B-absorbing compounds, the two pasture species showed no significant UV-B-induced changes in anthocyanin levels, while levels were markedly increased in pea plants at the midsummer harvest under UV-B-transmitting filters (Figure 5-10). The interspecific difference in this response may have contributed to UV-B tolerance of pea during high UV-B periods in summer. Anthocyanins can increase UV-B absorbance in combination with phenolic UV-absorbing substances (Woodall and Stewart, 1998) and this may have been facilitated by relatively high abundance of other UV-B-absorbing compounds (Figure 5-7). Moreover, this could lead to higher antioxidant capacity, further contributing to increased UV-B tolerance (Tsuda et al., 1996). Anthocyanin levels in pea were significantly reduced in the open plots, compared to the UV-B-transmitting filter treatment. This supports the findings from UV-absorbing compound accumulation, further indicating that these plants experienced marked damage to protective biochemical functions linked to secondary metabolism.

In conclusion, this study reveals possible biochemical explanations for the interspecific differences in morphological responses to solar UV-B observed in chapter 4. The findings suggest that higher levels of UV-B-absorbing compounds could provide an effective intrinsic shield against solar UV-B in pea. This could be further augmented by the capability to increase specific flavonoids such as anthocyanins in response to higher solar UV-B levels in summer.

6. Effects of solar UV-B radiation on white clover and ryegrass in a mixed sward

6.1. Introduction

During their evolution, New Zealand native plants were exposed to the higher UV-B levels of lower latitudes during the drift of the landmass south after the break-up of Gondwanaland. In contrast, the New Zealand pasture plants investigated here originate from temperate northern hemisphere latitudes and may be more sensitive to enhanced UV-B levels than native plants. Nonetheless, studies have shown that native New Zealand plants can be affected by present-day levels of UV-B in New Zealand (Hunt and McNeil, 1999). This observation and the results presented in chapter 4 suggest that UV-B sensitivity in swards could also be expected under higher UV-B levels in New Zealand.

Only a limited number of studies have measured effects of UV-B radiation on whole ecosystems *in situ*. There is little information on solar UV-B effects for pasture grasslands. Studies in the southern hemisphere are of particular value in this regard, as they allow testing of UV-B sensitivity of swards under naturally higher ambient UV-B levels. In addition, such field examinations allow inclusion of other pasture-specific factors that rarely are represented in UV-B studies, e.g. grazing by ruminants.

Species responses to solar UV-B in swards may differ from effects observed in monoculture as there are indications that UV-B might affect competitive interactions between plant species (Barnes et al., 1996). A field study on the effects of supplemental UV-B on the competition of several agricultural crops with their associated weeds included red clover, grown in tandem with the grass *Setaria glauca* (Fox and Caldwell, 1978). Plants in that study were grown in pots and subjected to strongly enhanced UV-B, equivalent to about 40% ozone depletion at temperate northern hemisphere latitudes. Findings showed that in situations of strong competition between the two species, the density of red clover was reduced by UV-B (Fox and Caldwell, 1978). This would be of further interest to investigations on the New Zealand pasture association, which also

represents the combination of a grass and a species of *Trifolium*. Compared to dicotyledons, monocotyledonous species such as grasses have distinctly different morphological and physiological characteristics, which could confer competitive advantages to ryegrass in the UV-B-exposed sward (He et al., 1993; Pal et al., 1997; Cybulski and Peterjohn, 1999; Musil and Wand, 1999). Conversely, sensitivity of both white clover and ryegrass under monoculture conditions (chapter 4) could suggest UV - B-induced decreases in overall sward productivity, rather than shifts in susceptibility between the two plant species under sward conditions.

There is a general lack of studies that compare solar UV-B responses of different plant species grown in monoculture with those grown in association. The present study on the pasture association of white clover and ryegrass was conducted during the same season and used a similar set-up to that employed in the investigations on these species grown in monoculture (chapter 4). The trial was conducted in an established sward that was regularly grazed by sheep. Measurements included whole sward growth parameters as well as individual morphological features of the two pasture species.

This chapter examines whether the two pasture species white clover and ryegrass growing *in situ* in their usual pasture association are affected by the presence of solar UV-B in New Zealand. A particular aim was to test whether the effects of natural solar UV-B levels on the morphology of pasture species growing in monoculture (chapter 4) also apply under actual sward conditions.

6.2. Materials and methods

The experiment was performed using a long -established sward that was located in close proximity to the site described in chapter 4 (section 4.2). The sward was a ryegrass-dominant perennial pasture with white clover as the main legume species and a small percentage (not quantified) of other grasses and herbs. The experimental layout was a randomised complete block design, comprising four replicates of the three treatments described in section 3.2 on page 36 (twelve plots). The size of the wooden frames over each plot was 1800 mm × 1500 mm, with a height of 250 mm at the northeast side and 450 mm at the southwest side. Filters were placed in the field in September 1995. At

regular intervals, typically every three to four weeks, frames were temporarily removed to allow grazing by sheep. An exploratory harvest examined herbage height with a rising plate meter on 4 October 1995. These measurements were repeated on 13 December 1995 and 6 January 1996. On 6 January, herbage mass and accumulation was determined by cutting plots to a simulated grazing height of 50 mm. The plant material was subsequently dried at 80°C for 24 hours before weighing.

Twelve randomly selected ryegrass tillers and 12 young daughter tillers under each treatment were marked after defoliation on 6 January 1996 and monitored to determine rate of leaf elongation and senescence. These tissue turnover measurements were carried out during the second week of regrowth and repeated for a second time during the third and fourth week of regrowth. In each treatment, 12 advancing white clover stolons were marked at the beginning of the first ryegrass observation period and harvested at the end of January 1996 to determine stolon elongation as well as mean leaf area and petiole length. On 13 December 1995, grass:clover ratios were determined with a point quadrat apparatus as percentage of 100 clover hits per plot. Grass:clover biomass ratios on 6 January and 6 February 1996 were determined by dissection of herbage samples. Experimental units and use of ANOVA for this study were identical to that described in section 4.2.

6.3. Results

6.3.1. White clover stolon morphology

White clover leaf area and petiole length were similar between open and UV-B-transmitting filters but showed higher values under UV-B-absorbing filters (Table 6-1). Compared to the latter, there was a decrease by about 20% for petiole length in both UV-B-transmitting treatments. While the overall analysis of variance of white clover leaf area was non-significant, analysis as an orthogonal contrast design showed an average 13% decline (at $P < 0.10$) for open and UV-B-transmitting plots, compared to the UV-B-absorbing filters. The rate of white clover stolon elongation in the sward was not significantly affected by UV-B (Table 6-1).

Table 6-1. White clover stolon morphology measured at the end of January. For statistical significance * denotes $P < 0.05$ and ns $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Leaf area (cm ²)	4.3	4.2	4.9	0.3	ns
Petiole length (mm)	131	124	158	8	*
Stolon advance (mm d ⁻¹)	1.8	1.7	1.8	0.4	ns

6.3.2. Ryegrass tissue turnover

Decreases in ryegrass leaf elongation were more pronounced in young tillers (Table 6-2). A non-significant trend two weeks after grazing was significant three to four weeks later. During that second measurement period at the end of January, UV-B resulted in average reductions of leaf elongation by more than 20% in young tillers and by about 13% in adult plants (Table 6-2). Ryegrass senescence, measured as leaf length reduction was generally not affected by UV-B, but there were some decreases in senescence under both filters, compared to the open plots.

Table 6-2. Ryegrass leaf elongation and senescence (leaf length reduction) per tiller per day for adult and daughter tillers in January. Period 1 denotes measurements made during the second week of regrowth following defoliation and Period 2 denotes measurements made during weeks 3 and 4. For statistical significance ** denotes $P < 0.01$, * denotes $P < 0.05$, + denotes $P < 0.10$, and ns $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Adult tillers (mm d ⁻¹)					
Elongation, period 1	18.1	17.4	16.7	0.9	ns
Elongation, period 2	18.7	19.7	22.2	0.8	+
Senescence, period 1	3.2	2.2	2.0	0.3	+
Senescence, period 2	6.1	2.8	4.8	0.7	**
Daughter tillers (mm d ⁻¹)					
Elongation, period 1	13.9	14.5	17.7	1.0	ns
Elongation, period 2	14.5	16.4	20.1	1.0	*
Senescence, period 1	1.0	0.4	1.1	0.5	ns
Senescence, period 2	6.3	3.2	3.9	1.0	ns

6.3.3. Grass:clover balance

Both filter types reduced seasonal clover content in the sward and increased the ratio of ryegrass to white clover (Table 6-3). There was no consistent direction of change in the comparison of the two UV-B-transmitting treatments with the UV-B-absorbing treatment.

Table 6-3. White clover content in the sward and sward grass:clover ratio. For statistical significance * denotes $P < 0.05$, + denotes $P < 0.10$, and ns $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Sward % clover					
13 December	41	26	29	4	*
6 January	40	16	27	8	ns
6 February	12	6	9	2	+
Log grass:clover ratio					
6 February	0.14	0.65	0.41	0.11	*

6.3.4. Sward height and herbage mass

Sward height was significantly increased under both filters at the first harvest after placing frames on the plots in October (Table 6-4). Differences between filters became significant on 6 January (Table 6-4). Compared to the UV-B-absorbing treatment, sward height was decreased by 15% under UV-B-transmitting filters and by a further 10% in open plots. Measurements of herbage accumulation on 6 January confirmed this effect with reductions by 20% under transmitting filters and a further 13% in open plots (Table 6-4).

Table 6-4. Sward height and herbage mass. For statistical significance ** denotes $P < 0.01$, * denotes $P < 0.05$ and ns $P \geq 0.10$. S.E. = standard error of the mean.

	Treatment			S.E.	Significance
	Open	+ UV-B	- UV-B		
Sward height (mm)					
4 October	88	115	116	6	*
13 December	131	156	168	14	ns
6 January	117	133	154	4	**
Herbage mass (kg DM ha ⁻¹)					
6 January	1040	1200	1490	88	*

6.4. Discussion

This study investigated solar UV-B effects *in situ* on an agricultural pasture ecosystem. Furthermore, this study can be used to compare solar UV-B responses of plant species grown in association to those grown in monoculture (chapter 4). The results here fully support the conclusions of general morphological UV-B sensitivity that were drawn from the more detailed investigations of the pasture species in monoculture. The findings also suggest UV-B sensitivity irrespective of whether the pasture plants were grown from seed (chapter 4) or as components of an established sward.

6.4.1. Species responses

As with the observations of plants grown in monoculture (Table 4-1), mean white clover leaf area was reduced and mean stolon length unaltered in the sward in response to summer UV-B (Table 6-1). The previous results from internode elongation (Figure 4-5) show however, that younger stolon sections in the sward could still have been affected by solar UV-B. Under monoculture conditions, a decrease in white clover petiole elongation could particularly be observed in open plots (Table 4-1), and this was also reduced under UV-B-transmitting filters in the sward (Table 6-1). Reductions in petiole elongation under solar UV-B have also been observed in other studies (Adamse et al., 1997; Krizek et al., 1997). Due to the upright orientation of the petioles in white

clover, this essentially represents a decrease in vertical growth. The same can be said for the reduction in ryegrass leaf length (Table 6-2), confirming another response observed under monoculture conditions (Figure 4-7). The more pronounced decrease of ryegrass leaf elongation in younger tillers (Table 6-2) further illustrates the developmental dependency of UV-B responsiveness observed for white clover in chapter 4. As for monoculture conditions, ryegrass leaf senescence measured as leaf length reduction was not affected by solar UV-B (Table 6-2). It cannot be excluded, however, that senescence was affected via increased leaf loss, as shown in the more detailed studies in chapter 4 (Figure 4-11).

The few differences to the monoculture studies in chapter 4 (e.g. more pronounced reduction of petiole elongation under the UV-B-transmitting filters) could be due to different harvesting times or because of sward effects. Competition between plant species can be altered by morphogenetic shifts that change light interception (Barnes et al., 1988; Ryel et al., 1990). However, such effects are less likely for the sward system investigated here, as both white clover and ryegrass showed reductions in vertical growth under solar UV-B.

Previously, there had been only limited research on UV-B effects on pasture species, particularly under solar UV-B and using established mixed swards. Studies on white clover showed sensitivity in response to enhanced UV-B under controlled environmental conditions (Hofmann et al., 2000; Lindroth et al., 2000) and morphological changes along an elevational gradient in the tropics (Rozema et al., 1997a). While there are reports of sensitivity in pot-grown ryegrass plants to northern hemisphere solar UV-B (Deckmyn and Impens, 1999), a number of UV-B enhancement studies found no such susceptibility for this species (Newsham et al., 1998; Norton et al., 1999; Xue and Hartikainen, 2000). In addition, it has been shown for ryegrass that differences in UV-B responsiveness may also relate to the availability of trace elements. Studies with UV-B supplementation showed that the presence of Selenium in the soil protected ryegrass plants against UV-induced oxidative stress by increasing the activity of antioxidative enzymes (Xue and Hartikainen, 2000). Detailed soil analyses could be used to test whether the observed sensitivity of ryegrass in the present study may at least partly be due to the lack of such trace elements in the soil of the experimental site.

Thus far there has been a lack of published reports on UV-B effects in pasture plants that are exposed to regular defoliation events. In this work, morphological effects on the pasture species were observed under conditions of intermittent grazing by sheep. Such defoliation results in regular removal of leaves before they reach maturity and it is possible that this puts more stress on the re-emerging younger leaf developmental stages. In a study of northern hemisphere, pot-grown grass species in the glasshouse were regularly cut and this was related to enhanced sensitivity to solar UV-B (Deckmyn and Impens, 1999). Results from chapter 4 show higher UV-B sensitivity particularly for younger leaf developmental stages (Figure 4-3) and it is possible that lower UV-B sensitivity could be found in swards that are less frequently defoliated.

6.4.2. Sward responses

Assessments of the grass:clover balance suggest that effects of solar UV-B on the two pasture species are similar enough to not markedly change the overall pasture composition (Table 6-3). The main effect on the grass:clover balance was a shift towards ryegrass under both filters. This could be linked to the decreased rates of ryegrass leaf senescence which was also observed under both filters (Table 6-2). Reduced ryegrass leaf senescence and improved ryegrass content (relative to white clover) would be consistent with increased nitrogen mineralisation, which could be effected by higher temperatures under the filters. This suggests merit for investigations of herbage and soil nitrogen levels in future UV-B studies.

The results from the species comparisons further suggest that the reduction in sward height (Table 6-4) can be attributed to both a decrease in white clover petiole length as well as to reduced ryegrass leaf elongation. The increase in sward height under both filters at the beginning of the trial period (Table 6-4) was probably due to the higher temperatures under the filters at a time of the year when growth is temperature-limited (Butler et al., 1990). Future studies on sward height and herbage weight could also examine whether the gradual decrease in sward growth (Table 6-4) would be reversed during subsequent harvests later in summer.

Yield per unit ground area (i.e. per plot rather than per plant) is the relevant measurement in the assessment of UV-B effects on agricultural yield (Corlett et al.,

1997). Results in chapter 4 have shown that reductions in biomass per plant were not significant for both ryegrass and white clover (Table 4-3). The findings from reduced herbage accumulation (Table 6-4) may mean that solar UV-B also results in reductions in the number of plants per plot.

While previously there had been a general lack of studies of solar UV-B effects on temperate agricultural pastoral ecosystems, one series of examinations investigated UV-B effects on a temperate dune grassland ecosystem in the Netherlands (Tosserams et al., 1996; Rozema et al., 1999b). In general those investigations found UV-B tolerance for the dune grasslands species. However, native species are often exposed to other environmental stresses and often possess morphological attributes that may also confer UV-B resistance (e.g. thicker leaves or more vertical leaf orientation) (Rozema et al., 1997d). Agricultural plants are generally more UV-B sensitive than native plants and this can be partly due to the process of breeding (Rozema et al., 1997d). During breeding, plants are often grown in glasshouses that are opaque to UV-B and selected for high productivity, and there can also be selection against morphological (e.g. leaf hairs) or biochemical features (e.g. flavonoids that affect taste) that could provide protection against UV-B (Rozema et al., 1997d).

In conclusion, the results presented in this chapter are in general agreement with findings from simultaneously conducted monoculture studies, showing morphological sensitivity of both white clover and ryegrass to solar southern hemisphere UV -B levels. This in turn was reflected in decreases in whole sward growth attributes, suggesting merit for future studies to test these findings under long-term UV-B exposure in New Zealand.

7. General discussion

This study investigated a number of questions relevant to the field of UV-B research in plants. What are the effects of solar UV-B on important pasture plants growing under field conditions? Do these responses differ depending on whether the pasture species are grown in association or in monoculture? Is the monocotyledonous ryegrass more UV -B-tolerant than the dicotyledonous white clover? How do these responses compare to pea, a vegetable crop for which information was extensive in regard to its sensitivity to supplemental UV-B, but only very limited in regard to effects of ambient solar UV-B. Are young plant parts more sensitive to UV -B than more mature plant parts? Does near-ambient southern hemisphere UV-B affect senescence or reproductive development? Are responses confined to periods of high UV-B exposure, or can carry-over effects also be observed? Could intraspecific differences in UV-B tolerance be related to intraspecific differences in UV-B absorbance?

The findings show that solar UV-B levels in New Zealand can represent a limiting factor for the growth or morphological development of pasture plants. This holds true both for plants growing in monoculture and in their usual pastoral association in a grazed sward, and irrespective of their cotyledonous nature. UV-B sensitivity was most pronounced in the young leaves (Figure 4-3) and internodes (Figure 4-5) of white clover plants and in young ryegrass tillers (Table 6-2) and appeared to be transient, with effects largely recorded during midsummer in periods of higher UV-B incidence. Aspects of leaf senescence in ryegrass (Figure 4-11) and reproductive development in pea (Table 4-6) and white clover (Table 4-4) were accelerated by solar UV-B, with the latter effect occurring later in summer. Vegetative growth in pea appeared tolerant to near-ambient UV-B and the findings suggest that the species-specific differences in UV-B sensitivity may be explained by the presence of higher levels of UV -absorbing compounds in pea.

In addition to a general discussion on the findings that were discussed in more detail in the individual chapters, this chapter gives an overview of possibilities for future studies that could follow on from this work.

7.1. UV-B environment

The results in this study were observed under solar UV-B radiation levels of the southern hemisphere, which are elevated in comparison to similar northern hemisphere latitudes due to a combination of natural and anthropogenic causes. Other more recently published studies from the same southern hemisphere summer season 1995/1996 showed several comparable effects of solar UV-B on native trees in New Zealand (Hunt and McNeil, 1999) and on vascular native plants in Antarctica (Day et al., 1999). In accordance with numerous other UV-B exclusion studies, the present investigation revealed a number of significant UV-B effects. This is in contrast to the view of – in general – relative insensitivity of plants to UV-B supplementation (Searles et al., 2001). Such difference could suggest that plants are affected by UV-B *per se* and that further enhancement in general does not increase this effect.

Given the strong plant responses often found in filtration studies, it could be argued that this sensitivity is due to the strong reduction in UV-B usually achieved in experiments conducted with exclusion filters (Figure 3-8 and Figure 3-9). However, filtration studies that have used much smaller reductions in ambient UV-B radiation typically show effects on plant growth and physiology of similar magnitude to those reported here (Deckmyn and Impens, 1995; Ballare et al., 1996; Mark and Tevini, 1996; Mark and Tevini, 1997; Deckmyn and Impens, 1998a; Hunt and McNeil, 1998). Furthermore, studies of plant responses to solar UV-B not only help understand effects of stratospheric ozone depletion but also provide information towards wider biological questions such as variation of UV-B effects within plant canopies, or between sites exposed to differential shading, clouding or altitude (Paul, 2001). Depending on the presence of clouds, days of low UV-B irradiance can be followed by levels that are enhanced by more than double (e.g. Figure 3-3 to Figure 3-5). Moreover, periods of rapidly enhanced UV-B levels in New Zealand can result from direct overpass events of ozone-depleted air from the Antarctic ozone hole (Brinkma et al., 1998). The same holds true for management practices such as removal of upper canopies exposing plant regions underneath to strongly increased levels of UV-B. From this it has been argued that strong differences in exposure to ambient UV-B are part and parcel of plant life and warrant closer examination using filtration studies (Paul, 2001).

As stated in chapter 2, exclusion of UV-B using filters has limitations as an experimental method. Some of the disadvantages of UV-B filtration include canopy effects such as increased temperature, humidity or interception of precipitation under the filters (Table 2-2). While these effects can be reduced (e.g. by ventilation and compensatory watering under the filters), the inclusion of an 'open' control, as in the present experiment, can be used to test for passive warming and interpret the findings from the near-ambient treatment. Thus far only a minority of studies have used open controls and the findings from this study strongly suggest merit for increased use of this approach. The comparison between covered and open frames showed some effects on the plants of filters *per se*, e.g. decreases in petiole length of white clover (Figure 4-4) and of several growth parameters in pea (Figure 4-13 A and B, Figure 4-14, Table 4-5), as well as increased senescence in ryegrass under sward conditions (Table 6-2). Other UV-B filtration studies using open controls have related this to canopy effects such as passive warming (Day et al., 1999; Ruhland and Day, 2001).

The currently enhanced UV-B levels are predicted to be maintained in the near future, and future studies should therefore increasingly concentrate on the effects of the recent increases in UV-B due to ozone depletion. Studies are needed that create controls that transmit UV-B radiation at levels comparable to pre-depletion values. This could be done by using filters that differ only slightly in UV-B transmission, e.g. by using various ozone amounts in ozone cuvettes (Mark and Tevini, 1996; Mark and Tevini, 1997), or by using UV-B-absorbing filters of differential thickness or composition (Deckmyn and Impens, 1995; Ballare et al., 1996; Saile-Mark and Tevini, 1997; Deckmyn and Impens, 1998a; Deckmyn and Impens, 1999). A further approach has been conducted in a New Zealand study, using 80% transmission of ambient UV -B as a control, supplemented with 25% higher UV-B levels in the treatment, thus simulating UV-B increases that were comparable to those under ozone depletion from 1979 to 1999 (Hunt and McNeil, 1998). Further possibilities using filter techniques could include experimentation in northern New Zealand, where climatic conditions are favourable to pasture growth but natural UV-B levels are higher than at more southern latitudes (McKenzie et al., 1996).

7.2. Species responses

A recently published Canadian study reported findings on the effects of solar UV-B on the yield of some forage crops from 1994 to 1996 (Papadopoulos et al., 1999). That study revealed no major effects of relatively low northern hemisphere UV-B levels on the yield of most species, including white clover (Papadopoulos et al., 1999). Plant responses were examined under simulated, rather than actual sward conditions and ryegrass was not included. Furthermore, only yield (above-ground biomass) was measured (Papadopoulos et al., 1999). As became obvious in the present study, a non-significant effect of solar UV-B on above-ground biomass in the New Zealand pasture cultivars can nevertheless be accompanied by other important morphological and biochemical changes under southern hemisphere solar UV-B. In addition to lower UV-B levels, a differential result compared to the present study may also be due to cultivar differences. Under laboratory conditions, New Zealand white clover cultivars have shown susceptibility to UV-B, while less productive northern hemisphere cultivars were UV-B-tolerant (Hofmann et al., 2000). This also suggests a case for breeding or selecting for more UV-B-tolerant white clover cultivars.

The observed effects of solar UV-B on the pasture plants warrant further investigations into specific aspects of the physiology of responses in these species. In other legumes, solar UV-B has been shown to reduce root nodulation (Lingakumar et al., 1999). This would have to be investigated for white clover, serving as an important source of nitrogen to New Zealand pastures. In ryegrass, elevated UV-B can decrease the fertility of plants containing the endophytic fungus *Neotyphodium lolii* (Newsham et al., 1998). This sensitivity was attributed to the presence of the fungus, as endophyte-free ryegrass plants were not UV-B-sensitive. Thus, it remains to be tested whether solar UV-B levels in New Zealand could negatively affect the population dynamics in ryegrass, depending on presence of the fungus. This however may be offset by protection against herbivory by the endophytic fungus and by the fact that ryegrass predominantly propagates vegetatively (Newsham et al., 1998).

The effects of UV-B on the morphology of the pasture species were observed under irrigation in this experiment. UV-B effects are strongly dependent on water availability (Gwynn-Jones et al., 1999a) and it is possible that drier conditions in the field could

have masked UV-B responses (Petropoulou et al., 1995; Manetas et al., 1997). Conversely, there is also evidence showing worsening of UV-B stress by drought (Björn et al., 1997; Drilias et al., 1997). Future experiments should therefore include drought treatments to evaluate the direction and extent of a possible interaction of UV-B with drought on the New Zealand pasture association. Furthermore, there is a lack of studies combining UV-B filtration with controlled application of water stress. The filters in UV-B exclusion studies can conveniently be used as rainout -shelters to expose plants to extended periods of drought in the field.

There also is a need for long-term studies on the effects of solar UV-B as plant responses can accumulate over longer growing periods. For example, growth in *Deschampsia* was not affected by solar UV-B on the Antarctic Peninsula in the first year of investigation, but showed sensitivity in the subsequent season (Day et al., 1999). Some findings point at negative effects when plants are exposed to long-term UV-B irradiation (Sprtova et al., 2000), while others indicate no change (Papadopoulos et al., 1999) or even improvements over time (Visser et al., 1997). UV-B supplementation studies also show that even effects of high doses of UV-B can be reversible (Hunt et al., 1996). While the observations from the present work suggest that many of the effects are transient, only long-term studies can provide information on whether or not this could lead to cumulative responses for specific pasture growth processes such as leaf elongation and senescence.

Pea appeared UV-B-tolerant in comparison to the pasture plants. Compared to the latter, pea plants were harvested at a younger age when measured from seed establishment. The findings from this thesis and a number of other experiments suggest that UV-B sensitivity in plants often occurs at younger, rather than more mature stages of plant development. It is therefore possible that the observed interspecific contrast in UV-B responsiveness between the tolerant pea on the one hand and the sensitive pasture plants on the other would have been even more pronounced in pasture plants of similar age or physiological maturity to that of the pea plants.

7.3. Trait responses

This study investigated a number of important traits for the assessment of plant responses to UV-B, including plant biomass, leaf area, SLM and UV-absorbing compounds. A consistent trend emerged across plant species and experiments, generally showing highest growth under UV-B-absorbing filters. The magnitude of the UV-B effects observed in the pasture species was similar to that established for growth responses in a number of other UV-B filtration studies, ranging around the 10-20% mark (Ballare et al., 1999). The plant species were harvested at different times during the summer period and there were marked differences in day -to-day UV-B irradiance at several occasions during the experimental period. A number of plant responses can be influenced by short-term changes in UV-B dose, including damage to DNA and photosystem II proteins and flavonoid synthesis (Ballare et al., 1996; Hada et al., 1996; Jansen et al., 1996; Rousseaux et al., 1999). However, changes in overall plant growth and morphology represent integrated responses to UV-B, accumulated during ontogeny (Stapleton et al., 1997).

A number of UV-B field studies have been criticised for a lack of true replication, often paired with the use of pots rather than plots or very small plot sizes, thus making extrapolations to the actual field situation difficult (Corlett et al., 1997; Searles et al., 2001). In this study, all parameters were analysed using data from replicated plots (within blocks), rather than using individual plants from within a plot as replicate units. Other studies have also used this approach (Xiong and Day, 2001), which is strongly supported by a recent review showing that leaf area reductions under UV-B supplementation in the field on a per-plant basis were generally non-significant on a per-plot basis (Searles et al., 2001). It was argued that although reducing variance, individual plants within a plot do not represent independent experimental units and that only plots constitute the correct treatment replicates (Searles et al., 2001). While more conservative, the latter approach however has the disadvantage of lower statistical power. This could be improved by larger replication of plots in future UV-B field experimentation (Searles et al., 2001).

The effects on white clover leaf area and ryegrass leaf elongation observed here are consistent with conclusions from a number of other UV-B exclusion studies, indicating

that decreases in leaf expansion represent a common plant response to solar UV-B (Ballare et al., 1999). Epidermal cell size and cell number determine leaf area per leaf, and UV-B-induced decreases in leaf expansion are likely to result from detrimental effects on cellular morphology (Dale, 1992; Ruhland and Day, 2000). Southern hemisphere solar UV-B has been shown to reduce the size of epidermal cells in native vascular plants of Antarctica, particularly at the leaf tips (Ruhland and Day, 2000). Other studies have also demonstrated reduction in cell number accompanying leaf area decreases (Nogues et al., 1998). In the present work, significant effects could be found for young white clover leaves. Since cell division has stopped at the more mature leaf developmental stage, this could imply that cell expansion was to some degree able to compensate for the earlier damage. This could mean that cell division may be more affected by UV-B than cell expansion in these plants. Future studies could test this hypothesis, e.g. by comparing effects of UV-B on cell division rates or on DNA damage in developing white clover leaves with effects on cell expansion rates and hormone levels.

The present study shows that effects of solar UV-B on vegetative and reproductive growth often differ. The results of increased reproductive development in pea could suggest an increase in pod number per plant under UV -B. However it would have to be determined whether there are no negative effects on pod size or quality. The findings in white clover not only show decreases in the size of vegetative plant characteristics but also in the duration of vegetative development. Further studies at the stem apex could investigate at the anatomical level whether a possible shift from leaf to inflorescence formation is mediated at the level of cell division. Such studies could also examine whether the decrease in white clover leaf numbers is due to decreased initiation rates at the stolon tip or caused by a delay in the emergence of preformed leaf primordia.

Together with the accumulation of UV-absorbing compounds, increases in specific leaf mass or in leaf thickness have been recognised as important mechanism of plant defence against UV-B (Deckmyn and Impens, 1999). While a relationship between SLM and UV-B tolerance was not apparent in this study, it would still be of interest to include examinations on leaf thickness in future examinations. Furthermore, SLM was not investigated in ryegrass and other findings indicate that decreases in SLM can contribute towards morphological UV-B sensitivity in grasses (Deckmyn and Impens,

1999). Plants sensitive to UV-B can experience increases in genetic instability and this can also be accompanied by decreases in SLM (Musil and Wand, 1999).

Daily UV-B irradiance was highly variable (Figure 3-3 to Figure 3-5) and it is not known whether UV-absorbing compound accumulation responded to these short-term fluctuations. This study did not investigate the kinetics of UV-B-absorbing compound and anthocyanin formation and it would be interesting to investigate whether the plant species differ in their ability to accumulate these substances in response to daily changes in UV-B irradiance. However, even if the increase in UV-absorbing compounds in ryegrass (Figure 5-6) did reflect higher solar UV-B levels preceding the harvest of these plants, the levels were nevertheless much lower than the intrinsic levels in pea (Figure 5-7).

Subtle overall increases in UV-absorbing compounds often are accompanied by greatly amplified changes in individual UV-absorbing compounds, e.g. flavonoids (Meijkamp et al., 1999). The differential interspecific UV-B effects on the levels of these compounds in this study could also reflect differential accumulation of specific UV-absorbing compounds. This could be followed up in further experiments, using high performance liquid chromatography (HPLC) and other chromatographic techniques to examine in detail the nature of the UV-B-absorbing compounds detected here. Such studies could also examine whether increases in specific UV-absorbing compounds are due to renewed synthesis or also because of reduced degradation rates. Furthermore, while UV-absorbing compound measurements of whole leaves provide a useful overall measure of possible UV-B protection, they do not offer information on response patterns and localisation of such substances within the leaf. There are distinct distribution patterns for flavonoids and hydroxycinnamic acids in layers of the leaf and changes in these would be of particular relevance for UV-B protection (Bornman et al., 1997). Localisation studies could also compare the concentration of such UV-absorbing compounds to the transmittance of UV-B through epidermal cells and anticlinal walls. This could be done using epidermal peels and fibre optic microprobes (Day et al., 1999).

Increases in UV-absorbing compounds also warrant further work on trophic interactions as these substances include a number of secondary plant compounds which can affect

insect herbivory and litter decomposition (Moorhead and Callaghan, 1994). This could be studied in conjunction with investigations on reproductive development, as increases in flowering can be related to increases in particular UV-absorbing compounds under UV-B (Musil et al., 1999).

7.4. Conclusion

Ozone-destroying precursor substances are continually phased out and this is seen to improve the recovery of the stratospheric ozone layer. However, this may be counteracted due to the interrelationship of ozone depletion with global warming. Climate change will have a strong impact on UV-B received by plants, by causing stratospheric cooling and thus contributing to ozone depletion and also by changing weather patterns including cloud formation. UV-B filtration studies such as the present work can be used to examine whether such changes in solar UV-B will be translated into effects on agricultural plants and ecosystems. This will also facilitate predictions about UV-B responsiveness of northern hemisphere pastures growing under potentially elevated future UV-B levels similar to those already present in New Zealand.

The findings from this work illustrate that white clover and ryegrass can both be affected by summer UV-B levels in New Zealand. Based on these results, it would be interesting to select for UV-B tolerance at the plant level in these species. This could be done by measuring key traits such as leaf expansion and UV-B-absorbing compound formation under UV-B, and subsequently test the progeny at sward level for higher UV - B tolerance.

8. References

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