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 tempera ture, 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.

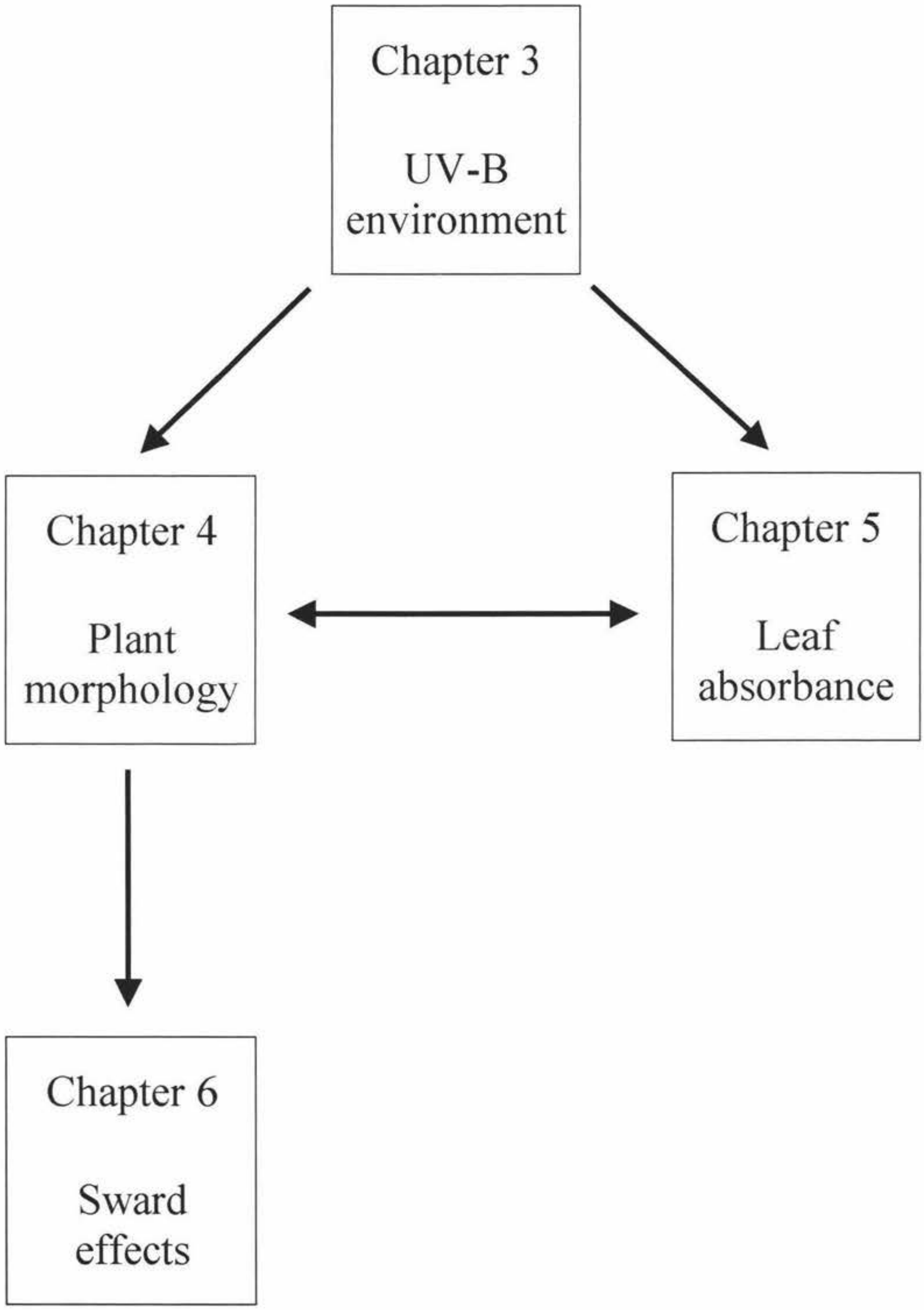

Figure 1-1. Flow diagram showing the main approaches used in this study.

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.