White clover sensitivity to UV-B radiation – biochemical relationships and interaction with drought

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Abstract

During summertime in New Zealand, white clover experiences high levels of ultraviolet-B (UV-B) radiation. This frequently coincides with periods of summer drought. We investigated responses to UV-B and to the combination of UV-B and drought in various white clover populations, including New Zealand cultivars and ecotypes as well as overseas germplasm. The results were obtained under controlled environmental conditions in three independent trials. Overall, white clover growth was reduced by UV-B. The population comparisons indicated that low growth rate and adaptation to other forms of stress may be related to UV-B tolerance under well-watered conditions, but not during extended periods of drought. Flavonoid pigments that are involved in stress protection were strongly increased under UV-B and were further enhanced in the combination of UV-B and drought. The responses among these flavonoids were highly specific, with more pronounced UV-B-induced increases in quercetin glycosides, compared to their closely related kaempferol counterparts. UV-B tolerance of the less productive white clover populations was linked to the accumulation of quercetin compounds. In conclusion, these studies suggest (i) that slow-growing white clover ecotypes adapted to other stresses have higher capacity for biochemical acclimation to UV-B under well-watered conditions and (ii) that these biochemical attributes may also contribute to decreased UV-B sensitivity across white clover populations under drought. The findings alert plant breeders to potential benefits of selecting productive germplasm for high levels of specific flavonoids to balance trade-offs between plant productivity and stress tolerance.

Keywords: Drought, flavonoids, genetic variation, HPLC, kaempferol, quercetin, stress, *Trifolium repens* L., ultraviolet-B, white clover

Introduction

New Zealand has high natural levels of ultraviolet-B (UV-B) radiation compared to similar latitudes in the northern hemisphere (Seckmeyer & McKenzie 1992). Depletion of the stratospheric ozone layer during recent decades has further increased the amounts of UV-B reaching New Zealand (McKenzie *et al.* 1999). The important pasture legume white clover has shown sensitivity to New Zealand UV-B levels (Matthew *et al.* 1996). High UV-B levels often coincide with periods of drought during the New Zealand summer, warranting examination of the combined effects of UV-B and water stress for pasture plants.

Only limited information has previously been available on intraspecific differences in UV-B sensitivity for pasture plants such as white clover. Presence of such differences within a species would be important in discerning mechanisms of UV-B responsiveness for the development of stress-resistant cultivars. A key mechanism protecting plants against UV-B is the accumulation of flavonoid pigments which act as UV-B-screens, antioxidants and energy – dissipating agents (Kostina *et al.* 2001; Smith & Markham 1998). Controlled environment studies are particularly suitable for screening stress responses across numerous populations within a species and allow high precision in the application of stress (Corlett *et al.* 1997).

This paper provides a summary of some main findings from studies on UV-B responses of white clover populations under controlled environment conditions. The detailed results have been outlined in several recent publications (Hofmann *et al.* 2003a; Hofmann *et al.* 2003b; Hofmann *et al.* 2001; Hofmann *et al.* 2000; Lindroth *et al.* 2000). The studies aimed at investigating across three experiments whether white clover shows population-specific (intraspecific) differences in UV-B sensitivity. We further sought to examine whether white clover UV-B sensitivity is modified by drought and whether accumulation of flavonoids can be linked to UV-B protection in this species.

Materials and methods

Materials and methods have been described in detail previously (Hofmann *et al.* 2003a; Hofmann *et al.* 2003b; Hofmann *et al.* 2001; Hofmann *et al.* 2000; Lindroth *et al.* 2000). Main methodological features

Table 1 Comparison between UV-B application, plant conditions and UV-B sensitivity in three main experimental approaches studying UV-B effects on white clover.

Feature	Experiment I: Large-scale screening	Experiment II: Stress interaction studies	Experiment III: Bioassay studies
Duration of UV-B treatment	2½ weeks	12 weeks	16 weeks
Factorial design	2 UV-B levels × 26 populations	2 UV-B levels × 2 water levels × 9 populations	2 UV-B levels × 2 populations
Plant material	16 ecotypes, 8 cultivars, 2 breeding lines	3 ecotypes, 4 cultivars, 2 breeding lines	Ecotype 'Tienshan' and cultivar 'Grasslands Huia'
Plants grown from	Stolon cuttings	Seedlings	Stolon cuttings
Growth medium	Sand	Sand	Soil
Defoliation	No	Infrequent	Frequent
UV-B sensitivity (growth reduction)	Yes	Yes	Yes
Differences in UV-B sensitivity among white clover populations	Yes	Yes	Yes
UV-B tolerance for ecotypes	Yes	Yes	Yes

are summarised in the following (see also Table 1). Three independent trials were conducted in large growth chambers of the National Climate Laboratories in Palmerston North. Biologically effective levels of UV-B (Caldwell 1971) were 13.3 kJ/m^2/d, equivalent to about 25% mid-summer ozone depletion above Palmerston North. This facilitated comparability with a number of other studies using similar levels of UV-B enhancement. White clover commonly propagates via stolons in the field, while most studies of UV-B effects on plants are conducted on seedlings. Both types of starting material were used in our experiments (Table 1). Plants were grown in pots and comprised a number of white clover populations, including cultivars selected for agronomic value in breeding programmes, ecotypes (populations collected in the wild) and breeding lines (unmultiplied selections) (Table 1).

Experiment I screened in a short-term trial UV-B responses of 26 New Zealand and overseas white clover populations. In experiment II, drought was applied during the last 4 weeks of a 12 week UV-B-supplementation period by withholding water and soil moisture was monitored gravimetrically (Barbour *et al.* 1996). To prevent plants exceeding pot size, leaves in the pots were clipped on average every 3 weeks. Much stronger defoliation pressure (daily intervals) was applied in experiment III, as a result of simultaneous long-term studies examining UV-B effects on insect herbivory (Lindroth *et al.* 2000). In experiment II, biochemical investigations examined flavonoid responses to UV-B and drought in white clover leaves, using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) (Hofmann *et al.* 2000). At the end of each experiment, aboveground plant biomass was dried at 80°C for 48 h to provide measures of plant productivity. Analysis of variance of main and interaction effects and regression analysis was performed with the SAS (SAS 1996) and Genstat (Genstat 1993) statistical packages.

Results and discussion

Averaged across populations, white clover was sensitive to UV-B in all three experiments (Table 1). Under well-watered conditions, plant dry matter production decreased by 26% in experiment I ($P < 0.001$), by 20% in experiment II ($P < 0.001$) and by 11% in experiment III ($P = 0.051$). There were differences in this UV-B sensitivity among the white clover populations, with decreased dry matter production ranging from 0% to 40% (Hofmann *et al.* 2003b). Slow-growing ecotypes that are adapted to higher ambient UV-B levels and to other forms of stress in their natural habitat (e.g. drought, low temperature) were significantly less sensitive to UV-B than cultivars bred for agricultural performance (Hofmann *et al.* 2003b; Hofmann *et al.* 2001).

This is exemplified here by a comparison from experiment II of the New Zealand cultivar 'Grasslands Kopu' and the population 'Tienshan', a high-altitude ecotype from China adapted to a number of limiting environmental conditions. In well-watered plants ('WW' in Figure 1), 'Grasslands Kopu' showed a UV-B-induced reduction in productivity of 30%, while 'Tienshan' was UV-B-tolerant. These results are in accord with ecological models predicting higher stress tolerance for plants from low habitat and low plant productivity (Grime 2001). Under drought conditions ('DR' in Figure 1), UV-B sensitivity was less pronounced across white clover populations. Mitigating effects of drought for UV-B sensitivity have also been reported for other crops, e.g. soybean (Teramura *et al.* 1990) and cowpea (Balakumar *et al.* 1993). This mitigation of UV-B sensitivity by drought was of particular advantage for the high-yielding white clover cultivars that were UV-B sensitive under well-watered conditions: when drought-stressed, these cultivars showed similar levels of UV-B tolerance as the less productive ecotypes (Hofmann *et al.* 2003b).

It was of further interest to examine links between possible biochemical means of UV-B protection and plant productivity that could help explain the intraspecific differences in UV-B responsiveness. Our results revealed higher levels of key protective flavonoid compounds in the less productive white clover ecotypes. For example, the comparison of the cultivar 'Grasslands Kopu' with the ecotype 'Tienshan' showed higher intrinsic and stress-induced levels of flavonoids in the latter (Figure 2). The findings suggest that a metabolic cost of UV-B protection may be lower carbon allocation towards intrinsic plant productivity.

Figure 1 Aboveground biomass dry matter (DM) production in the white clover populations 'Grasslands Kopu' and 'Tienshan' grown for 12 weeks with (+UV) and without (-UV) supplementation of UV-B, concomitant with and without exposure to 4 weeks of drought (DR). Error bars are ± SE. WW = well watered.

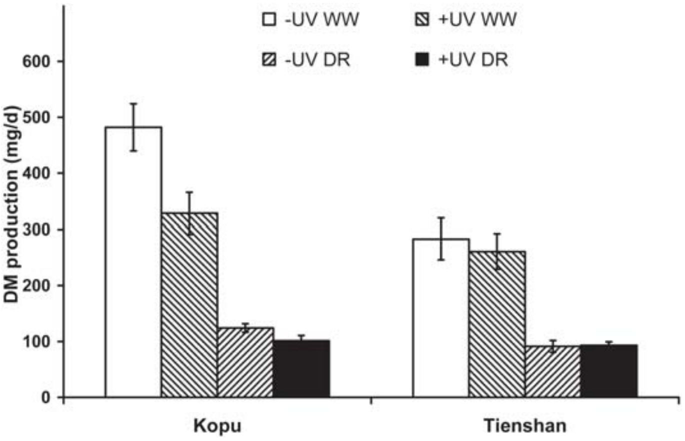
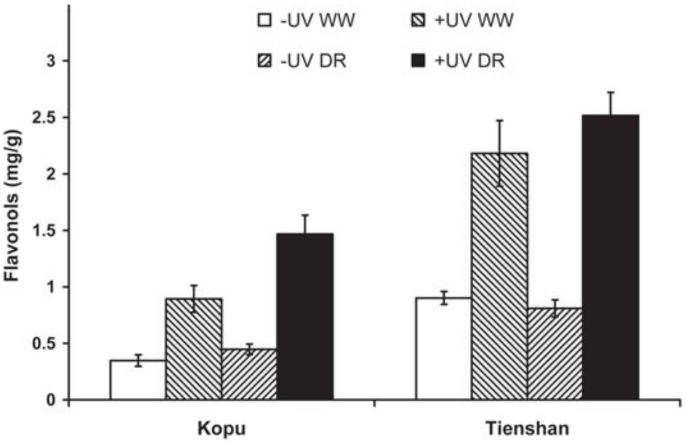


Figure 2 Flavonol glycoside levels in the white clover populations 'Grasslands Kopu' and 'Tienshan' grown for 12 weeks with (+UV) and without (-UV) supplementation of UV-B, concomitant with and without exposure to 4 weeks of drought (DR). Error bars are ± SE. WW = well watered.



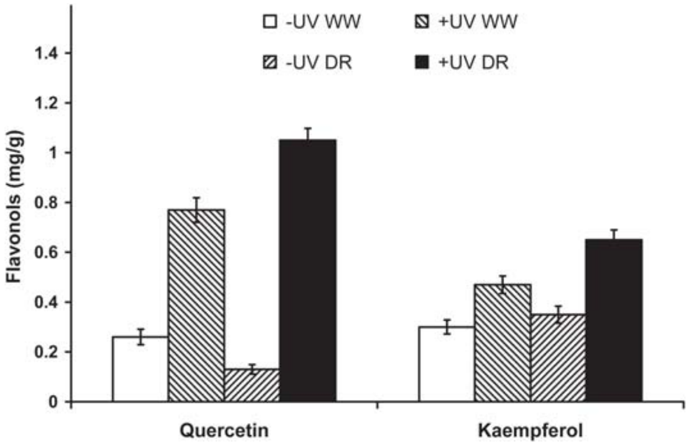
The two main flavonoid compounds enhanced by UV-B in the white clover populations were glycosides of the flavonols quercetin and kaempferol (Figure 3). In well-watered plants, the UV-B-induced increases of quercetin glycosides across white clover populations were significantly higher (200%) than those of their kaempferol counterparts (60%) ($P < 0.001$). Increases of these flavonols were even more pronounced in the UV-B × drought combination and again much higher for quercetin (300%), compared to kaempferol compounds (120%) (Figure 3). Our studies further showed that UV-B tolerance of the white clover populations was related to higher UV-B-induced accumulation of the dihydroxylated quercetin, but not of the monohydroxylated kaempferol glycosides (Hofmann *et al.* 2003a; Hofmann *et al.* 2000).

These findings are in agreement with research across a number of different plant species, environments and UV-B levels, showing that highly specific differential UV-B responses between closely related flavonoids are well conserved in the plant kingdom. Such differential UV-B responses have been demonstrated in liverworts (Markham *et al.* 1998a), gymnosperms (Fischbach *et al.* 1999), monocotyledons (Markham *et al.* 1998b) and dicotyledons, both herbaceous (Olsson *et al.* 1998) and trees (Lavola 1998). This could be a reflection of higher antioxidant activity or energy dissipation in dihydroxylated flavonoids (Kostina *et al.* 2001; Smith & Markham 1998).

Conclusions

UV-B sensitivity in white clover was reduced (i) in less productive populations adapted in their natural environment to stress (e.g. drought) and (ii) across populations when UV-B-treated white clover plants acclimatised to simultaneously applied drought. The biochemical studies suggest a key role for quercetin glycosides in white clover tolerance of UV-B stress. In future studies we plan to examine a range of productive white clover cultivars for levels of quercetin compounds and of other key metabolites providing the biochemical basis for the development of productive *and* stress-tolerant pasture plants.

Figure 3 Levels of quercetin and kaempferol glycosides averaged across nine white clover populations grown for 12 weeks with (+UV) and without (-UV) supplementation of UV-B, concomitant with and without exposure to 4 weeks of drought (DR). Error bars are ± SE. WW = well watered.



ACKNOWLEDGEMENTS

We thank Chris Hunt, Derryn Hunt, Yvonne Gray, Christine van Meer and Margaret Greig for technical assistance. We are grateful for advice on white clover populations to John Caradus, Derek Woodfield and John Ford. We thank David Baird, John Brock, Dennis Greer, Brian Jordan, Geoff Lane, Rick Lindroth, Chris Mercer, Fred Potter, Ken Ryan and Rod Thomas for valuable discussions. RWH was supported by an AGMARDT Fellowship. The research was funded by the New Zealand Foundation for Research, Science and Technology, Contract Number C10632.

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