Responses of pasture plants to UV-B radiation and interaction with drought

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Abstract. Our studies investigated UV-B radiation effects and their interaction with drought stress in the important New Zealand pasture legume white clover (*Trifolium repens* L.). Population studies showed intraspecific differences in UV-B responsiveness. Slow-growing white clover ecotypes adapted to other stress factors were tolerant to UV-B, whereas UV-B sensitivity was observed in productive white clover cultivars. Plants exposed to drought showed decreased UV-B sensitivity, resulting in beneficial interaction effects between these stress factors. Levels of protective flavonoid pigments were highest in the stress-tolerant white clover ecotypes and in the combination of UV-B and drought. The results emphasise roles for flavonols in UV-B tolerance, both on a population level and in conferring cross-tolerance via other stress factors.

Introduction

New Zealand's pasture-based industries depend on clover and other legume species, providing essential nitrogen to the associated grasses in the swards and representing excellent feed value to grazing animals. Clover production alone adds an estimated $3 billion per annum to the economy. White clover (*Trifolium repens* L.) is the main legume species in New Zealand pastures, grown in close association with perennial ryegrass (*Lolium perenne* L.).

During the New Zealand summer, high levels of UV-B radiation often coincide with periods of drought. White clover is sensitive to drought (Wang *et al.* 1996), but also to New Zealand UV-B radiation levels (Matthew *et al.* 1996). Studies are needed to examine how the combination of these stress factors impacts on this important pasture species. Detrimental effects of UV-B on plants include DNA damage, altered phytohormone production and decreases in plant growth parameters (e.g. leaf size) (Jordan & Hofmann 2004). These effects can vary among species and within populations of the same species. Drought can limit plant productivity even further, e.g. due to strong decreases in cell expansion and reduced photosynthesis (Reddy *et al.* 2004).

However, plants have also developed a number of strategies to guard themselves against such effects. These strategies include mechanisms of stress avoidance (e.g. accumulation of UV-B-screens or stomatal responses) and of stress tolerance (e.g. DNA repair or synthesis of antioxidants). The accumulation of phenolic compounds is a key protective response of plants against UV-B radiation. Flavonoids are important plant phenolics which act as UV-B-screens, antioxidants and energy-dissipating agents (Smith & Markham 1998). Flavonoids are frequently found in or on epidermal layers where they can increase markedly following UV-B treatment. Studies with mutants further highlight the importance of flavonoids for UV-B protection (Ryan *et al.* 2001).

Our studies examined whether UV-B effects on white clover are modified by drought and whether accumulation of flavonoids can be linked to UV-B protection in this species.

Experimental

The investigations included controlled environment studies as well as long-term research under New Zealand field conditions (Campbell *et al.* 1999; Hofmann *et al.* 2003a; Hofmann *et al.* 2003b). Main features are outlined in Table 1, exemplified here by the controlled environment studies. Plants were grown in large growth chambers of the National Climate Laboratories in Palmerston North, New Zealand. Nine white clover populations were investigated, including important New Zealand cultivars as well as ecotypes collected in the wild.

In the controlled environment study, drought was applied during the last four weeks of a 12 week UV-B-supplementation period. Plants were harvested at the end of the drought phase. Above-ground plant biomass was dried at 80 °C for 48 h to provide measures of plant productivity (yield). Biochemical investigations examined flavonoid accumulation in white clover using high-performance liquid chromatography (HPLC) (Hofmann *et al.* 2000). Statistical analyses included ANOVA of main and interaction effects and regression analyses.

Table 1. Methodology and key results from controlled environment (CE) experiments of UV-B and drought effects on white clover.

Features	CE studies
Duration of UV-B treatment	12 weeks
Level of supplemental UV-B[a]	13.3 kJ m^{-2} d^{-1}
Equivalent to % ozone depletion above Palmerston North (mid-summer value)	25%
Plant material	9 populations
UV-B sensitivity (growth reduction)	✓
Differences in UV-B sensitivity among white clover populations	✓
Positive UV-B × drought interaction for plant growth	✓
Highest levels of flavonol accumulation under UV-B × drought and in UV-B-tolerant ecotypes	✓

[a]Plant-weighted, biologically effective UV-B (Caldwell 1971), normalised to 300 nm

Results and Discussion

Results showed an overall UV-B-induced decrease in plant productivity by 24% across white clover populations (Figure 1). However, this effect differed among the white clover populations (Table 1), with no UV-B sensitivity in ecotypes collected in the wild and pronounced UV-B-induced growth reductions of 40% in cultivars bred for agronomic performance. Compared to the UV-B effect, the drought-induced reduction in plant productivity was much more pronounced, showing average growth reductions by about 70% across white clover populations in the controlled environment study (Figure 1).

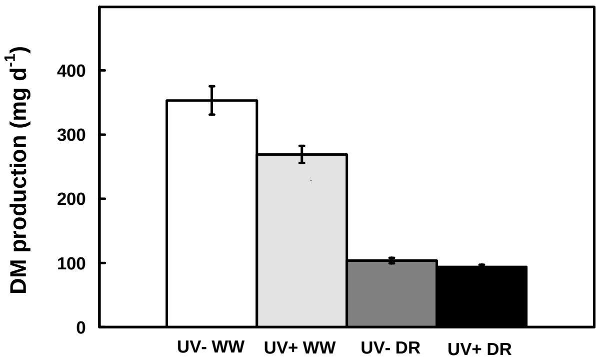

Figure 1. Above-ground dry matter (DM) production across nine white clover populations grown with (UV+) and without (UV-) supplementation of 13.3 kJ m^{-2} d^{-1} UV-B. Error bars are ± SE. WW, well watered plants; DR, plants that were exposed to four weeks of drought.

When investigating the UV-B × drought interaction, we found beneficial effects of the stress combination on plant performance: the UV-B effect on biomass production in droughted plants was much less pronounced than under well-watered conditions (Figure 1).

Beneficial stress interaction effects were also observed in our field studies (data not shown) and this 'cross-tolerance' can be explained by a number of physiological changes that are of relevance for protection against either stress, including growth delay and radical scavenging (Beggs *et al.* 1986). HPLC analysis demonstrated that the major flavonoids enhanced by both stress factors were derivatives of the flavonols quercetin and kaempferol. This increase was more pronounced for quercetin than for kaempferol glycosides and levels were highest in the UV-B-tolerant white clover ecotypes and in the UV-B × drought combination.

Conclusions

The results help characterise the role of key metabolites involved in stress protection. Quercetin is not only an efficient UV-B-screening compound but also high in antioxidant activity and these attributes may confer UV-B tolerance to slow-growing white clover ecotypes and general cross-tolerance to plants exposed to UV-B and drought stress.

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