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Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: foliar chemistry and consequences for two lepidopteran herbivores

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Abstract White clover growing in New Zealand is experiencing increasing levels of ultraviolet-B (UV-B) radiation as a result of ozone depletion. We evaluated the effects of UV-B radiation on the foliar chemistry of two populations of white clover (*Trifolium repens* L.), 'Huia' and 'Tienshan,' and the consequences for the performance of armyworms (*Spodoptera litura*) and cutworms (*Graphania mutans*). Plants were grown in controlled environment rooms with and without supplemental UV-B radiation at a dose of 13.3 kJ m⁻² day⁻¹, corresponding to a 25% mid-summer ozone depletion above Palmerston North, New Zealand. In both white clover populations, UV-B radiation elicited changes in foliar chemistry, including slight increases in nitrogen concentrations and decreases in carbohydrate concentrations. In addition, the 'Huia' population showed decreases in fiber concentrations and marked increases in cyanogenic activity. No change in UV-absorbing compounds was detected in either population. Long- and short-term feeding trials were conducted to assess dietary effects on insect growth, consumption, and food utilization. Changes in the performance of both insect species were generally small. The most pronounced effect was a 36% reduction in weight of *S. litura* after 2 weeks of feeding on Huia grown at high UV, but larval development times were only slightly prolonged and pupal weights were unaffected. *S. litura* short-term performance was affected by differences in white clover population. The long-term performance of *G. mutans* was not affected and its short-term performance (stadium duration and consumption rate) was only marginally affected by the high-UV treatment. We conclude that the effects of elevated UV-B radiation on white clover

plant chemistry can be specific to certain plant populations. The differences in sensitivity of the two generalist insect species suggest that effects may also be specific to certain plant-herbivore associations. These results indicate that future UV-B herbivory studies should examine genotypic effects in both plants and animals.

Key words Herbivory · Phytochemistry · Plant-insect interactions · *Trifolium repens* · Ultraviolet radiation

Introduction

Recent changes in ultraviolet (UV)-B radiation due to atmospheric ozone depletion are widely recognized as having significant consequences for plant biochemistry, physiology, and productivity (Caldwell et al. 1998). UV-B-mediated changes in the quantity and quality of plant components are also likely to affect heterotrophs, via trophic interactions and cascades (Mazza et al. 1999). Very few studies, however, have addressed the impact of UV-B on such interactions (Paul et al. 1997).

The consequences of high UV-B irradiances for consumer organisms are of particular interest in New Zealand, where UV-B doses are markedly higher than at comparable latitudes in the northern hemisphere (Seckmeyer and McKenzie 1992). This is due to hemispheric differences in stratospheric and tropospheric (pollutant) ozone, differential aerosol loading, and because the distance between the sun and earth is minimal during the austral summer (Seckmeyer and McKenzie 1992). Furthermore, while studies have demonstrated UV-B sensitivity for several legumes (e.g., Teramura 1990; Krupa et al. 1998), only limited research has been conducted on the UV-B sensitivity of white clover (*Trifolium repens* L.) (Matthew et al. 1996; Rozema et al. 1997a). White clover is the dominant legume of the pasture ecosystems in New Zealand, performing a vital role for ecosystem processes as a primary source of soil nitrogen and also as a highly nutritious component of the diet for grazing ruminants (Caradus et al. 1996).

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We investigated the impact of UV-B radiation on the chemical composition of two populations of white clover, 'Huia' and 'Tienshan,' and subsequent effects on the performance of two phytophagous insects, *Spodoptera litura* (Lepidoptera: Noctuidae) and *Graphania mutans* (Lepidoptera: Noctuidae). The white clover population Huia is a commonly used New Zealand cultivar, whereas the Tienshan population is a high-altitude ecotype from northwest China. In other experiments, Tienshan demonstrated more tolerance to UV-B than did Huia (R.W. Hofmann, unpublished data). The generalist tropical armyworm, *S. litura*, became established in northern New Zealand in the mid-1970s. It has been known to cause severe, though localized, defoliation of clover (Chapman 1984). The generalist cutworm, *G. mutans*, is native to New Zealand and causes damage to pasture, vegetable, and fruit crops (McGee 1987; McGregor et al. 1987).

A small but growing number of studies have assessed the effects of UV-B-mediated changes in plant chemistry on insects. Some studies suggest that UV-B-induced effects may alter herbivory through changes in plant primary metabolism, e.g., increased nitrogen levels (Hatcher and Paul 1994) and decreased sugar concentrations (Yazawa et al. 1992). Other research points to the involvement of secondary metabolites such as flavonoids and related compounds of the shikimic acid pathway (e.g., Grant-Petersson and Renwick 1996).

Here we analyzed plant chemistry linked to primary metabolism and of relevance to insect feeding. We also examined aspects of plant secondary metabolism, including levels of UV-absorbing compounds and cyanogenesis, a mechanism present in several populations of white clover and more than 2000 other angiosperm species (Kakes 1990). Hydrogen cyanide (HCN) is produced when cyanide-containing glucosides (linamarin and lotaustralin) and the hydrolytic β -glucosidase linamarase, normally separated by compartmentalization, are brought together during lesion of plant tissues. Increased cyanogenesis in white clover is a common response to several forms of stress, including herbivory, frost, and drought (Williams 1987; Caradus et al. 1990). UV-B could possibly alter this potential defense pathway by a general stimulatory effect on secondary metabolism (Rozema et al. 1997b).

In this study, we sought to determine whether UV-B would affect key attributes of white clover chemical composition, and whether the two white clover populations would be differentially affected. We also predicted that performance of *S. litura* and *G. mutans* would decline when reared on foliage of white clover grown under high levels of UV-B, and that the magnitude of this effect would vary between white clover populations.

Materials and methods

Experimental design

The overall design was a 2×2 factorial, with two levels of UV-B radiation and two populations of white clover. The UV treatments

were applied in two separate growth rooms and thus our experimental design was, of necessity, pseudoreplicated. The plants and UV treatments were rotated several times between our two experimental rooms in order to equalize potential room effects. In a separate room maintained with no UV supplementation, feeding experiments were first conducted with *S. litura*, then repeated with *G. mutans*.

Plant cultivation and UV irradiation

Stolon cuttings of Huia and Tienshan white clover populations were planted into separate 3-l pots containing 3 kg of soil (Karapoti brown sandy loam). The soil was supplemented with 2 g of lime and 10 g of slow-release fertilizer (15-4-10 N-P-K plus micronutrients). Plants were watered daily by an automatic drip irrigation system. Relative humidity in the growth rooms was 70% and day/night temperatures were 24°C and 18°C, respectively. Daylength was 14 h and mean photosynthetic photon flux (PPF) was 420 $\mu\text{mol m}^{-2} \text{s}^{-1}$, supplied by four 1-kW Sylvania 'metal-arc' high-pressure discharge lamps, together with four 1-kW Phillips tungsten iodide lamps. PPF was measured using a Li-Cor 185 meter with an LI 190S quantum sensor. Trolleys containing the plant material were rotated regularly to equalize variation in light distribution.

Elevated UV-B radiation was supplied by Phillips TL 40W/12 RS fluorescent UV tubes, enclosed in cellulose diacetate filters. Biologically effective UV-B (UV-B_{BE}) levels were 13.3 kJ m⁻² day⁻¹, calculated from the generalized plant action spectrum (Caldwell 1971) and normalized to 300 nm. This dose corresponds to a 25% depletion of summer ozone levels above Palmerston North, New Zealand (latitude 40.2 S, longitude 175.4 E). The UV treatment started 1 h after onset of the light period and ended 1 h before darkness. A feedback control system continuously monitored and adjusted the output of the UV lamps in response to degradation of the filters and aging of the tubes in order to maintain the set UV-B level. The system incorporated a Solar Light Co. UV-B biometer to measure incident radiation. This sensor was connected to a personal computer via an analog-to-digital interface to monitor and control UV levels. To achieve the required UV-B output level from the lamps, the computer varied an analog voltage supplied to Osram high-frequency dimming ballasts, thus directly controlling fluorescent-lamp output. The sensor was regularly calibrated against the generalized plant action spectrum using a Bentham DM150 UV spectroradiometer. In the control room, Mylar filter sheets were used to screen out UV-B but maintain UV-A levels similar to those in the UV-B treatment. Filters were changed regularly. The plants were subjected to the UV treatments for 29 days prior to initiation of insect bioassays.

Our purpose in using these two contrasting UV-B treatments was to clearly expose underlying genetic differences among plants, with attendant changes in insect performance, in response to UV-B. Moreover, the zero UV-B treatment (control) could be used to discern UV-B effects per se, which would not have been possible with two levels of UV-B irradiation but no control.

Foliar chemistry related to primary metabolism

Leaf samples were collected from five pairs of pots within each treatment. Fully unfolded trifoliate laminae were frozen in liquid nitrogen and freeze-dried. Samples were then finely ground in a coffee grinder and stored at -20°C until chemically analyzed. Nitrogen was measured using a Carlo Erba Instruments NA 1500 Series II Nitrogen Analyzer, which detects N₂ by thermal conductivity after flash combustion of the plant material (Carlo Erba 1988). Available carbohydrates (largely soluble carbohydrates and starch) were solubilized with dimethyl sulfoxide, digested with amylase and amyloglucosidase, acid hydrolyzed, and subsequently measured colorimetrically at 420 nm as reducing sugars using *p*-hydroxybenzoic acid hydrazide (Blakeney and Mutton 1980; Southgate 1991). Fiber content was measured as neutral-detergent fiber

(NDF), yielding mainly hemicellulose, cellulose, and lignin after solution in neutral detergent, digestion with amylase, and several washing steps using ethanol and acetone (van Soest et al. 1991).

Cyanogenesis

HCN production of fully unfolded young trifoliolate laminae was examined in a two-step procedure using a modified picrate paper test (Williams et al. 1998). The picrate paper technique is a semi-quantitative method involving scoring of color intensity by eye, which gives satisfactory correlation with quantitative procedures (Melville et al. 1940). In the first assay, a leaflet from each pot was tested for HCN production with picric acid as indicator. After addition of 10 μ l toluene and overnight incubation at 37°C, the color change in picric acid was examined by comparison with a standard color scale, ranging from 0 (unchanged, yellow) to 5 (dark orange) (Corkill 1940). Cyanogenesis intensity per leaf was expressed on a dry weight basis. Plants that did not prove cyanogenic in this first test were then examined in a second assay for the presence of substrates (linamarin and lotaustralin). The same picric acid procedure was used, but included addition of 20 μ l of 0.5% linamarase (a glucosidase enzyme) to liberate HCN (Williams et al. 1998). We used this amount to ensure that enzyme availability did not limit the reaction.

UV-absorbing compounds

Methanol-extractable UV-absorbing compounds were estimated by standard procedures (Mirecki and Teramura 1984). In other controlled-environment studies on white clover, phenolic UV-absorbing compounds mainly consisted of flavonoids and phenolic (e.g., caffeic) acids (R.W. Hofmann, unpublished data). Fully unfolded young trifoliolate laminae were oven-dried and 15 mg of ground material was weighed into Eppendorf tubes. The extraction was performed with 1.2 ml of 79:20:1 (volume) MeOH/H₂O/HCl, followed by spectrophotometric analysis. Absorbance readings at 300 nm were calculated on the basis of leaf dry weight. This procedure was repeated on seven replicates for each white clover population under each UV treatment.

S. litura bioassays

S. litura egg masses were provided by Biodiscovery of Auckland, New Zealand. All rearing was conducted in a controlled-environment room similar to those used for the UV treatments at 24:18°C, with a 14 h:10 h light:dark cycle.

We used a long-term feeding trial to assess the effects of UV-B-mediated changes in white clover composition on insect growth, development, and pupal weight. We apportioned groups of 12–14 neonate larvae to 9-cm plastic petri dishes, one dish for each of seven pots per white clover population per UV treatment. Trifoliolate leaves were clipped from potted plants and petioles inserted into 1.5-ml microcentrifuge tubes containing water. Leaves were placed into the petri dishes with larvae and replaced every 1–3 days. At 1 week of age, larvae showed signs of cannibalism, so we randomly selected seven surviving larvae from each petri dish and placed them individually into dishes (subsamples). Larval weights were recorded weekly until the onset of pupation. Upon pupation, we recorded pupal weight, sex, and development time (egg hatch to pupation).

We conducted a short-term feeding trial with penultimate (fourth) instars to determine the effects of experimental treatments on larval feeding, growth rates, and food-processing efficiencies. Penultimate instars were used because the insects were large enough to measure consumption rates accurately, and because the beginning and end of the penultimate stadium is demarcated by clearly defined molts. Insects were reared on white clover population 'Kopu' (grown in the low-UV room) from egg hatch through the third stadium. Upon molting into the fourth stadium, larvae

were weighed and placed individually into 9-cm plastic petri dishes containing a weighed white clover trifoliolate. Leaves were kept hydrated and replaced as described previously. We assayed two insects for each of seven pots per white clover population and UV treatment. Upon molting into the fifth stadium, larvae were frozen, then larvae, frass, and uneaten leaf tissue were freeze-dried and weighed. We calculated average growth rate (AGR), average consumption rate (ACR), approximate digestibility (AD), and efficiency of conversion of ingested food (ECI) according to standard formulas (Waldbauer 1968). Initial dry weights of larvae were estimated from a wet:dry conversion factor derived from a sample of 15 newly molted fourth instars. Similar conversion factors for leaves were obtained from leaf samples collected for chemical analyses.

G. mutans bioassays

G. mutans egg masses were obtained from gravid females light-trapped in the vicinity of Palmerston North, New Zealand. Bioassays were conducted following completion of the *S. litura* feeding assays, using the same procedures except as follows. For the long-term feeding trial, we apportioned groups of 12–15 larvae to 9-cm petri dishes. Larvae were transferred to 15-cm petri dishes as third instars. When larvae reached the fifth stadium, we reduced the number to six per container and transferred them to 1-l ventilated plastic boxes. For the short-term feeding assay, penultimate-instar larvae were in the fifth stadium. We assayed one insect for each of nine pots per white clover population and UV treatment.

Statistics

We used two-way analysis of variance as the primary statistical method for assessing UV-B and white clover population effects on white clover chemistry and insect performance. Data from the foliar chemical determinations and long-term feeding trials were analyzed using the general-linear-model procedure in SigmaStat (1995). For short-term feeding trials such as those we employed, differences in insect performance variables may result from differences in initial larval weight. Analysis of variance revealed no significant differences in initial larval weight for insects in various treatments. Moreover, analysis of covariance (SAS 1988) revealed that initial larval weight was not a significant covariate for the insect performance variables reported here as showing statistically significant effects.

Results

Foliar chemistry

White clover chemical composition varied in response to UV treatment and between populations. Concentrations of available carbohydrates declined an average of 22% in high-UV clover, but did not differ between populations (Fig. 1). Levels of NDF declined 14% in high-UV Huia, but were unaffected in Tianshan (significant UV \times population interaction) (Fig. 1). Levels of nitrogen tended to increase in UV-B-treated plants and the amount of nitrogen was slightly, but significantly higher in Huia than in Tianshan (Fig. 1). While UV-B did not change the proportion of acyanogenic to cyanogenic Huia plants (15 plants each), cyanogenic activity showed a strong 50% increase in response to UV-B treatment in cyanogenic Huia (Table 1). The first cyanogenesis assay indicated that the Tianshan population was acyanogenic. Applica-

tion of linamarase to the acyanogenic plant tissues indicated that acyanogenesis could be due to deficiencies in the enzymatic steps of cyanide production, rather than to absence of substrates. The low levels of linamarase-catalyzed cyanide production in Tienshan increased under high UV, but this effect was only half as pronounced as that exhibited by acyanogenic Huia plants supplied with

linamarase (Table 1). The UV-B-induced cyanide levels in Tienshan were the same as the constitutive, linamarase-catalyzed cyanide levels in Huia. Levels of UV-absorbing methanol-extractable compounds did not change in response to UV treatment (Table 1).

S. litura performance

The long-term bioassays showed that growth of larvae reared on high-UV-treated foliage was reduced for insects fed the white clover population Huia (Fig. 2). At 2 weeks of age, body weights of larvae in the high-UV Huia treatment were only 64% of those of larvae in the

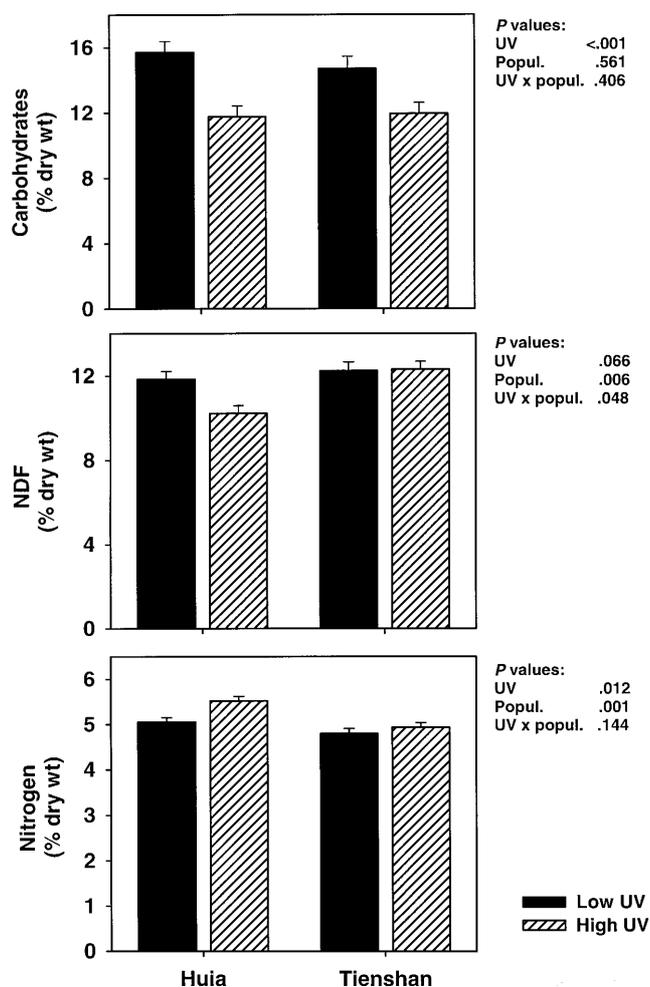


Fig. 1a–c Foliar chemical composition (mean±1 SE) of two populations of white clover grown under low and high UV radiation (NDF neutral-detergent fiber)

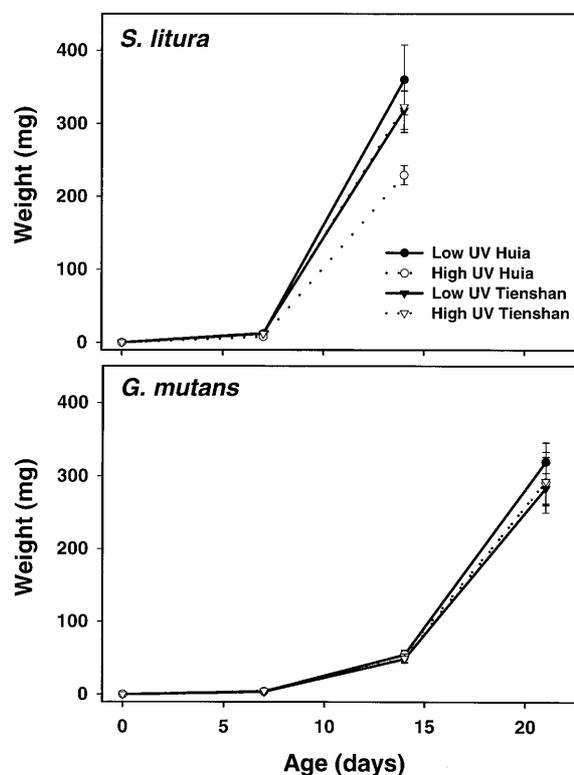


Fig. 2 Growth of *Spodoptera litura* (a) and *Graphania mutans* (b) larvae between egg hatch and pupation. Data collection at weekly intervals was terminated when the first larva in a treatment pupated. Vertical lines indicate ±1 SE

Table 1 Effects of UV treatment on cyanogenesis (HCN score mg⁻¹ leaf dry matter) and UV-absorbing compounds (A₃₀₀ mg⁻¹ leaf dry matter) in white clover (mean±1 SE). For the HCN score, a *t*-test revealed a significant effect of UV treatment on Huia foliage (*P*=0.017). ANOVA revealed effects of treatment and popu-

lation (both *P*<0.001) and of treatment × population (*P*=0.043) on the linamarase-catalyzed HCN score, but revealed no significant effects of UV or population on UV-absorbing compounds (nd not detectable)

UV treatment	Clover population	HCN score (mg ⁻¹)	Linamarase-catalyzed HCN score (mg ⁻¹)	A ₃₀₀ mg ⁻¹
Low	Huia	0.565±0.053	0.350±0.050	1.088±0.037
Low	Tienshan	nd	0.256±0.030	1.089±0.042
High	Huia	0.850±0.097	0.619±0.047	1.090±0.051
High	Tienshan	nd	0.358±0.031	1.089±0.042

Fig. 3 Development times and pupal weights (mean+1 SE) of female (a) and male (b) *S. litura* reared on two populations of white clover grown under low and high UV radiation

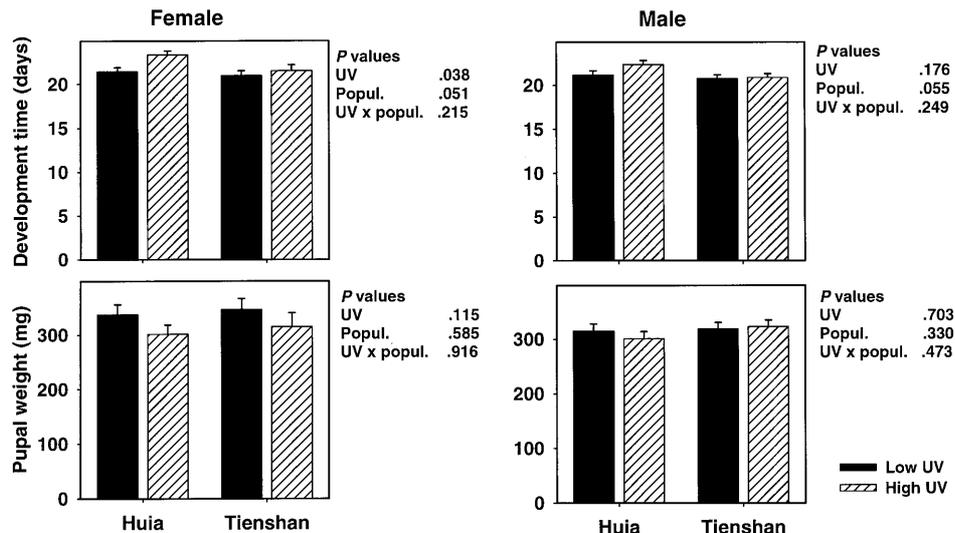
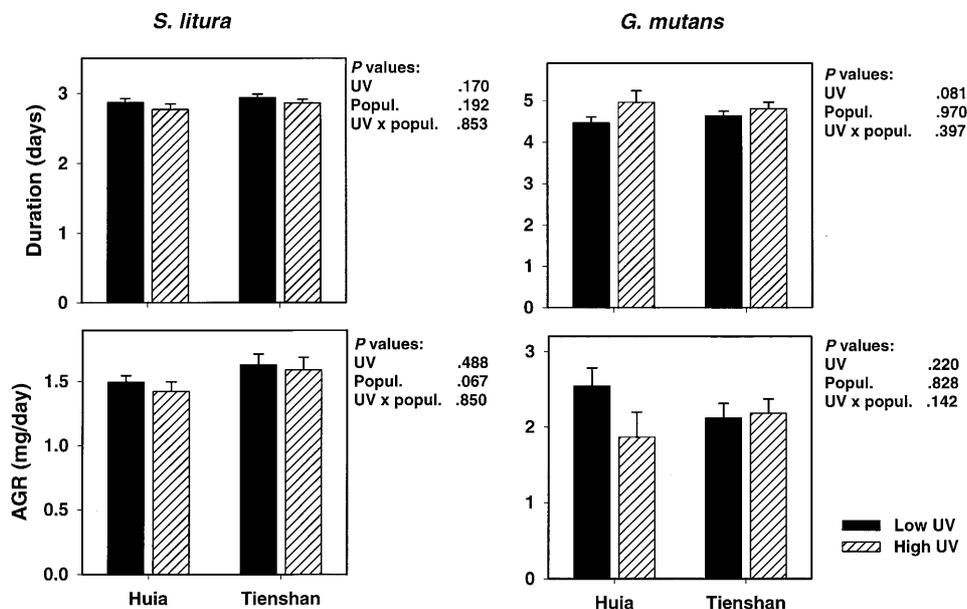


Fig. 4 Stadium duration and growth rates (mean+1 SE) of penultimate-instar *S. litura* (a) and *G. mutans* (b) reared on two populations of white clover grown under low and high UV radiation (AGR average growth rate)



low-UV Huia treatment. In contrast, growth of larvae fed population Tienshan over the same period was unaffected by UV treatment. We observed no significant UV-B effect on pupal weights, and only a slight prolongation of developmental time, especially in females (Fig. 3). White clover population only slightly affected larval development times, with insects fed Huia requiring an additional day (relative to insects fed Tienshan) before the onset of pupation. Final pupal weights of males and females did not differ between white clover populations.

Short-term (fourth instar) bioassays revealed few if any effects of UV treatment on *S. litura* performance (Figs. 4, 5). AD tended to improve in insects fed high-UV foliage, but the difference was slight and only marginally significant. Effects of white clover population were more pronounced. Insects fed Tienshan had 20% higher AGRs but 10% lower ECIs compared with insects

fed Huia. Consequently, these insects exhibited a marginally significant 10% increase in growth rates.

G. mutans performance

Over the course of the 3-week period spanning egg hatch to onset of pupation, growth of *G. mutans* larvae was nearly identical on the four experimental diets (Fig. 2). Moreover, neither UV treatment nor white clover population influenced larval developmental periods or final pupal weights (Fig. 6). Relative to *S. litura*, *G. mutans* required an additional week for larval development and attained smaller pupal weights.

The short-term (fifth-instar) feeding trials provided results similar to those of the long-term trials. Neither UV treatment nor white clover population affected insect growth rates or food utilization efficiencies (Figs. 4, 5).

Fig. 5 Consumption rates and food utilization efficiencies (mean+1 SE) of penultimate-instar *S. litura* (a) and *G. mutans* (b) reared on two populations of white clover grown under low and high UV radiation (ACR average consumption rate, AD approximate digestibility, ECI efficiency of conversion of ingested food)

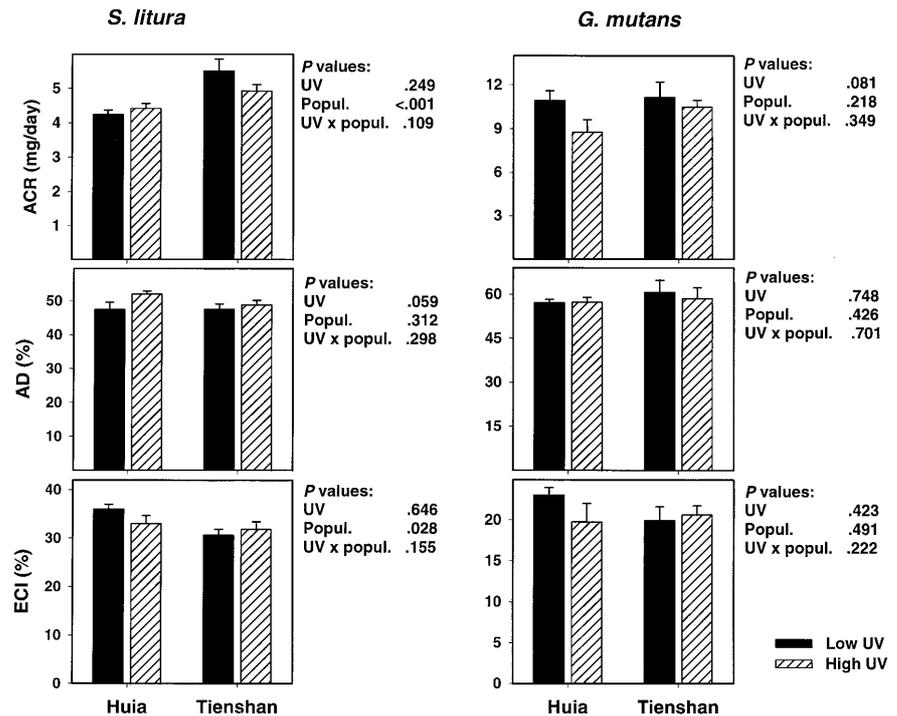
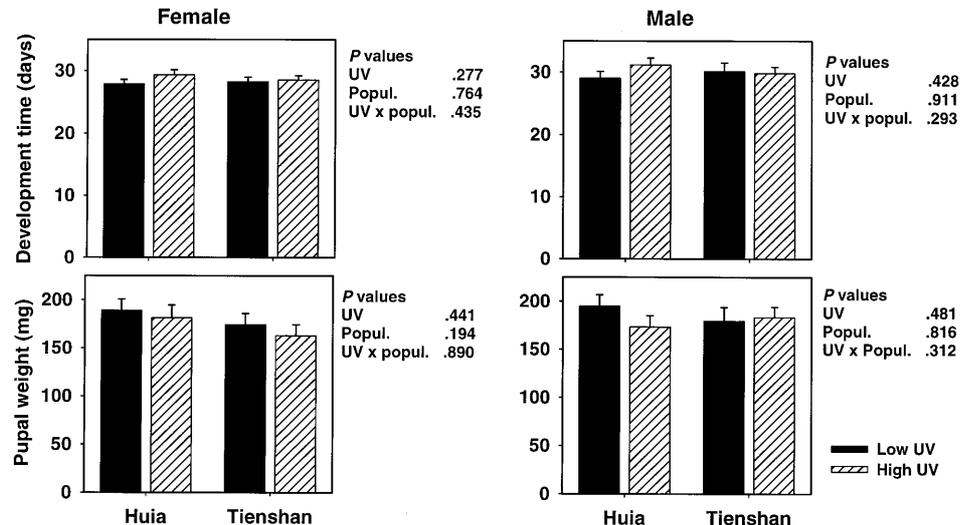


Fig. 6 Development times and pupal weights (mean+1 SE) of female (a) and male (b) *G. mutans* reared on two populations of white clover grown under low and high UV radiation



Trends toward longer development times and reduced consumption rates for insects on high-UV white clover were only marginally significant.

Discussion

Foliar chemistry related to primary metabolism

In this experiment, UV-B radiation elicited alterations in white clover composition that could be expected to affect the performance of herbivores. The largest effect was a greater than 20% decrease in available carbohydrate levels in both white clover populations. This

change was accompanied by decreased fiber levels in Huia. To date, few studies have evaluated the effects of UV-B radiation on plant carbohydrate or fiber content. Decreased available carbohydrate concentrations at elevated UV-B levels have been reported for glucose in pea (Mackerness et al. 1997), sucrose in mulberry (Yazawa et al. 1992), and soluble carbohydrates in *Pinus* (Katzel et al. 1996). Gehrke et al. (1995), working with leaf litter from *Vaccinium uliginosum*, reported no effect of UV-B dose on soluble carbohydrates, but observed a decrease in cellulose concentrations. Rousseaux et al. (1998), however, observed no effect of UV-B radiation on hemicellulose content of *Gunnera magellanica*.

Overall levels of nitrogen in both white clover populations were quite high, well above the limiting threshold for most insects (Mattson 1980; Slansky and Scriber 1985). The slight increase in nitrogen concentrations in Huia could therefore be regarded as less important for herbivory than the aforementioned chemical changes, and the effect in Tienshan was negligible. Shifts of comparable magnitude have been reported for other legume species, such as soybean (Murali and Teramura 1985) and pea (Hatcher and Paul 1994).

Foliar chemistry related to secondary metabolism

To our knowledge, this is the first study to assess the impact of UV-B radiation on cyanogenesis. We found a clear UV-B-induced increase in cyanogenesis levels in cyanogenic plants. Our findings from linamarase supplementation indicate that UV-B-induced increases in cyanogenesis could primarily be due to increased substrate biosynthesis and are less dependent on increases in β -glucosidase concentration/activity. The possibility of UV-induced increases in white clover cyanogenic glycosides is notable given that these compounds are not derived from aromatic amino acids. The latter are products of the shikimic acid pathway, well known to be induced by UV radiation (Rozema et al. 1997b). Linamarin and lotaustralin, however, are derived from the aliphatic amino acids valine and isoleucine (Seigler 1998). Thus, their accumulation is mediated by the effects of UV-B on biochemical mechanisms other than those of the shikimic acid pathway. Interestingly, our findings suggest that in acyanogenic white clover genotypes, cyanoglucoside levels may rise under enhanced UV-B as well.

The cyanogenesis levels in Huia can be classified as moderate-high (Crush and Caradus 1995; Caradus and Woodfield 1997). The apparent acyanogenic nature of Tienshan is consistent with its origin in a cold habitat, where frequent frost-related lesions of the tissue would put cyanogenic plants at a disadvantage (Williams 1987). The low linamarase-catalyzed cyanide levels in Tienshan could suggest lower constitutive levels of available cyanogenic substrates, which would be consistent with findings from other cyanogenesis studies on white clover populations originating in colder climates (Daday 1965; Caradus and Eerens 1992).

Foliar concentrations of flavonoids and related UV-absorbing compounds typically increase in response to UV-B radiation (Bornman et al. 1997; Rozema et al. 1997b). In contrast, we found no change in the UV-absorbing capacity of white clover grown under elevated UV-B radiation. This result is not without precedent, as several other recent studies (e.g., Rousseaux et al. 1998; Salt et al. 1998) have also failed to detect such a response. Moreover, we cannot rule out the possibility that concentrations of some individual flavonoids or other phenolics changed, while overall UV-absorbing capacity remained unchanged.

In summary, the results from our chemical analyses lend support to the pattern observed by others (e.g., Paul et al. 1997; Lavola et al. 1998), that phytochemical responses to UV-B radiation are compound-, species-, and population-specific. Such variability can be expected to influence the interactions of plants with higher trophic levels.

Insect responses

Results from this research demonstrate that insect responses to UV-B-mediated changes in white clover chemical composition are specific to the particular plant-herbivore interaction investigated. The most pronounced effect observed was for growth of *S. litura* during the first 2 weeks of the larval developmental period. Ultimately, however, UV-B-treated white clover had only a slight effect on the development time of female *S. litura* and no effect on pupal weights of either insect species. Because the fecundity of female Lepidoptera is generally correlated with pupal weight, our results suggest that consumption of UV-B-irradiated white clover is unlikely to affect reproductive success in *S. litura* or *G. mutans*. However, our experiments focused on the first generation and thus possible cumulative effects over several generations cannot be ruled out.

The cause of decreased growth in young *S. litura* larvae reared on high-UV Huia is unclear, but may be related to high cyanogenic activity. Cyanogenesis was elevated in high-UV Huia and only for this treatment was larval performance reduced. Although cyanide is a broadly toxic constituent, some insects exhibit metabolic adaptations (e.g., β -cyanoalanine synthase activity) against it (Lindroth 1991). Brattsten et al. (1983) found that cyanide stimulated larval feeding and growth in a related noctuid species, *S. eridania*. Other findings suggest that cyanogenic glycosides in white clover can act as feeding deterrents to insect pests (Ellsbury et al. 1992).

In contrast to insects feeding on cyanogenic Huia plants, a cyanogenic effect for insects feeding on Tienshan would only be possible in the presence of both appropriate amounts of cyanogenic plant substrates and of suitable β -glucosidases in the insect gut. While the presence of the latter has been reported for several lepidopteran species (Ferreira et al. 1998), the low levels of linamarase-catalyzed cyanide production in Tienshan under high UV could suggest that the lack of insect response was due to insufficient amounts of available cyanogenic substrates. For the population Huia we propose that *S. litura* required several weeks to habituate to higher levels of cyanogenic activity in high UV, after which growth accelerated and compensated for earlier growth suppression. *G. mutans* may not have exhibited reduced growth because it is better adapted than *S. litura* to dietary cyanogenic compounds. *G. mutans* larvae are noted apple pests in New Zealand (Burnip et al. 1995) and apple (*Malus pumilus*) produces cyanogenic glycosides such as amygdalin (Conn 1979).

The lack of pronounced effects on insect performance in our studies could also be attributed to the overall excellent nutritional quality of the white clover diet, which exhibited relatively high nutrient concentrations and low fiber levels across all treatments, compared with levels for this species under field conditions (Rattray and Joyce 1974; Wanjaiya et al. 1993). Whether significant UV-B-induced changes persist in the field, where compounds of relevance to nutrition are likely to occur at more limiting levels for herbivore growth remains to be investigated. In addition, several white clover populations used in pasture ecosystems have higher constitutive levels of cyanogenesis compared to Huia (Crush and Caradus 1995) and a further UV-B-induced increase could be of relevance for insect feeding as well as mammalian herbivory. Consideration of ruminant sensitivity to cyanogenesis (e.g., Lehmann et al. 1991) indicates a need for field experimentation under grazing conditions and concomitant inclusion of other stress forms (e.g., drought) which are likely to influence cyanogenesis and other aspects of white clover nutritive composition, and in turn nutrient utilization by animals (Wanjaiya et al. 1993).

Relatively few experiments have investigated the plant-mediated effects of UV radiation on insects, and results have varied among species and studies. With respect to consumption, we found no effect on feeding rates of *S. litura*, and only a slight trend toward reduced feeding in *G. mutans*. Other studies have documented the complete range of potential feeding responses to high-UV radiation, including decreases (Hatcher and Paul 1994; Grant-Petersson and Renwick 1996; Rousseaux et al. 1998), no change (Grant-Petersson and Renwick 1996), and increases (Lavola et al. 1998). In terms of growth performance, we found little if any effect of high-UV radiation. Other researchers have reported reduced growth (McCloud and Berenbaum 1994; Grant-Petersson and Renwick 1996), no change (Grant-Petersson and Renwick 1996; Stout et al. 1998), and increased growth (Hatcher and Paul 1994).

We conclude that generalizations about the implications of higher UV-B radiation levels for plant biochemistry and herbivory must take account of genotypic differences in both plant and animal populations. We have shown that important differences may occur between populations of the same plant species in the biochemical response to UV-B radiation and in the subsequent effects on herbivore performance. Further research is required to understand the underlying functional basis for differential responses in both plants and animals given that UV-B effects on plant-insect interactions are species- and population-specific. Our data suggest that a scheme for classifying the functional bases of plant-herbivore interactions should, amongst other attributes, take into account cyanogenic capacity, differential climatic adaptation, and stress tolerance of plants.

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