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Phenolic acclimation to ultraviolet-A irradiation in *Eucalyptus nitens* seedlings raised across a nutrient environment gradient

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Abstract

We investigated the effects of long-term acclimation of *Eucalyptus nitens* seedlings to ultraviolet-A (UV-A) irradiation (320–400 nm) on phenolic compounds (gallotannins, stilbenes, and flavonols), photochemical efficiency, and chlorophyll and carotenoid contents. Seedlings were raised under four nutrient regimes, ranging from low to high application rates, in an environment that included or excluded UV-A irradiance. Our aims were: to classify phenolic compounds that absorb in the UV-A and their relative contribution to total UV-A absorption; to identify how phenolic compounds respond to UV-A exposure and exclusion, and to determine how plant nutrient status affects acclimation of photo- and pigment-chemistry to UV-A exposure and exclusion. Gallotannins contributed to only a minor fraction of total absorption within the lower range (320–360 nm) of the UV-A spectrum. Stilbene and flavonol compounds dominated absorption within the 320–360 and 360–400 nm ranges, respectively. Contents of gallotannin were generally high in UV-A-exposed seedlings. Although there was a significant effect of UV-A on contents of stilbenes, a general response (across nutrient treatment comparisons) was not evident. Contents of flavonols were not affected by UV-A exposure. Contents of gallotannin, stilbene, and flavonols decreased from low to high nutrient-application treatments. There were no effects of UV-A on photochemical efficiency or pigment-chemistry.

Additional key words: carotenoids; chlorophyll; flavonol; fluorescence induction; gallotanins; nitrogen; stilbene; xanthophyll cycle.

Introduction

Plant leaves must balance absorption of photon energy against their capacity to use and dissipate it, to prevent: (*1*) over-excitation of electron transport processes in photosynthesis, (*2*) photosystem 2 (PS2) inactivation, and (*3*) the generation of activated oxygen radicals that can oxidise leaf proteins, pigments, and membranes, and potentially cause plant death (Wise and Naylor 1987). PS2 inactivation can be induced by both visible (*e.g.* Long *et al.* 1994) and ultraviolet (UV) (*e.g.* Krause *et al.* 1999) irradiance. The likelihood of PS2 inactivation can be increased by abiotic stresses, such as nutrient deficit (Grossman and Takahashi 2001), water logging (Vartapetian and Jackson 1997), drought or salinity (Munné-Bosch *et al.* 2001), and low or high temperature (Paolacci *et al.* 1997).

Plants employ strategies of photoprotection to counter the detrimental effects of excessive visible and UV radiation absorption. Strategies of photoprotection against visible irradiance include regulating the rate of chlorophyll (Chl) degradation (Kyparissis *et al.* 1995, Close *et al.* 2001a), as energy absorption is proportional to Chl content; and by increasing the xanthophyll-cycle pool size (Adams and Demmig-Adams 1992), which increases the capacity to dissipate photon energy absorbed

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in excess. Plant responses to increased UV radiation can include increased xanthophyll-cycle pool size (Helsper *et al.* 2003, but see Kirchgessner *et al.* 2003) as well as the increase of contents of gallotannin, stilbene, and flavonol phenolic compounds (Landry *et al.* 1995, Rice-Evans *et al.* 1996, Hagerman *et al.* 1998, Cuendet *et al.* 2000, Grace and Logan 2000, Lee *et al.* 2003).

There have been many investigations of photoprotective responses of plants to abiotic stress under natural light (*i.e*. comprising visible and UV wavelengths, *e.g*. Adams and Demmig-Adams 1992, Long *et al*. 1994, Close *et al.* 2003a). Recently Krause *et al.* (2004) investigated the effects of natural irradiation on processes that photoprotect against visible and UV irradiance. Their study found significant increases in the pool size of the xanthophyll cycle pigments and in contents of ascorbate and of ultraviolet-B-absorbing phenolic compounds after leaves had been transferred from shade to full-light. However, to our knowledge no study has compared and separated the relative effects of both visible and UV components of radiation on photoprotective characteristics of leaves.

Ultraviolet radiation is categorised into several components based on different wavelength ranges (UV-A 320–400 nm, UV-B 280–320 nm, UV-C 180–280 nm). Research on the effects of increased UV-B exposure on plant photosynthesis has been driven by concerns about atmospheric ozone depletion, since this increases the levels of incident UV-B reaching the Earth's surface (Caldwell *et al.* 1989). Although UV-A is not affected by ozone depletion, it can penetrate deeper into leaves than UV-B can (Vogelmann 1993). The biological effects of UV-A radiation have only recently received attention (Turcsányi and Vass 2000, White and Jahnke 2002, Krause *et al.* 2003). UV-A causes PS2 inactivation and contributes to oxidative pressure (Turcsányi and Vass 2000, White and Jahnke 2002). Questions arise as to how plants combat the effects of UV-A.

Flavonols have been implicated to provide photoprotection against UV irradiation through a screening function (Landry *et al.* 1995). Correlations between UV exposure and epidermal flavonol concentrations (Schnitzler *et al.* 1997) suggest that at least part of their function is to directly absorb radiation (Mazza *et al.* 2000, Kolb *et al.* 2001). The absorbance of flavonol peaks within and overlapping the spectral range of UV-A suggests that flavonols play a role in screening against UV-A irradiance (Yamaguchi 1970). In contrast, gallotannins absorb across the UV-B, but not the UV-A wavelengths (Yamaguchi 1970). As a consequence they may be effective as screens against UV-B, but not UV-A.

As well as functioning as UV screens, flavonol and gallotannin compounds have strong anti-oxidant properties *in vitro* (flavonols: Rice-Evans *et al.* 1996; gallotannins: Hagerman *et al.* 1998) and may function as anti-oxidants *in vivo* (Grace and Logan 2000). Likewise stilbenes have strong anti-oxidant properties *in vitro* (Cuendet *et al.* 2000, Lee *et al.* 2003). Using *Eucalyptus nitens* seedlings as our model, we have demonstrated that the level of flavonol and gallotannin compounds in leaves increases with increased photoinhibition associated with decreased plant nutrient status (Close *et al.* 2003b). This is consistent with a potential role(s) in photoprotection. Further, it raises the question of how plants respond to conditions varying simultaneously in terms of nutrient status and UV-A. In the present study we sought to characterise and compare the dynamics of phenolics, particularly flavonols, gallotannins, and stilbenes, after long-term acclimation of *E. nitens* seedlings to two factors: UV-A exposure and nutrient status. Understanding how they respond to these two factors is fundamental to defining their photoprotective roles more completely.

In this study, our aims were to: (*1*) characterise the classes of phenolic compounds that absorb across the UV-A spectrum and their respective contribution to absorption of UV-A radiation in leaf extracts of *E. nitens* seedlings, and (*2*) investigate how phenolic compounds in leaves of *E. nitens* seedlings respond to UV-A exposure and exclusion. We simultaneously imposed variation in nutrient-status of the seedlings into the study, so that our third aim was to (*3*) determine how plant nutrient status affects the capacity of plants to respond, in terms of photo- and pigment-chemistry, to UV-A exposure and exclusion. We found that compounds within the phenolic class of stilbenes contribute significantly to absorption within the UV-A. Thus the potential effects of UV-A and nutrient treatments on stilbenes were also investigated.

Materials and methods

Plants and treatments: Seedlings of *Eucalyptus nitens* (Deane and Maiden) Maiden were raised from a single-family seed lot (Forestry Tasmania improved seed) in *Lannen 81*® 85-cm^3 plugs at Forestry Tasmania's tree nursery, Perth, Tasmania (41º34′S, 147º11′E). The potting mix was saturated with *Aquasol*® every 7 d (N : P : K 23 : 4 : 18; solution concentration 1 kg m^{-3}) for four months. In late February 2003, seedlings were moved to an outdoor growing area and 'thinned' to an equal density of 41 seedlings per tray to ensure similar radiation interception between individual seedlings.

The experiment was set up as a randomised block design with two factors: (*1*) nutrients, 4 levels, and (*2*) UV-A, 2 levels. In late March, seedling trays were randomly allocated to one of the four nutrient treatments: *A*, non-fertilized; *B*, fertilized every 14 d; *C*, fertilized every 7 d; *D*, fertilized every 2 d. Each seedling received approximately 7.8 mg *Peters Excel*® (N : P : K 20.0 : 2.2 : 6.6; solution concentration 1 kg^{-3}) when fertilized. Nutrient treatments were each randomly

allocated (one tray per plot) twice within four blocks orientated east-west. In early April, one week after nutrient treatments started, each of the nutrient treatments within blocks were then randomly allocated to UV-A treatments. Glass screens, supported by metal frames, conferred either (*1*) UV-A exposure (+UV) (6 mm *Pilkington Optifloat™ Clear*, *88/65* Visible/UV transmission specification; *Pilkington (Australia) Ltd.*, Dandenong, Victoria, Australia) or (*2*) UV-A exclusion (–UV) (6.38 mm *Pilkington Optilam™ Clear* [0.38 mm PVB interlayer], *89/<1* Visible/UV transmission specification). Both glass types transmitted approximately the same amount of visible radiation and both glass types did not transmit UV-B radiation (Fig. 1). The glass was tilted at 2º towards north, to cut low angle radiation, to prevent rainwater pooling on the glass, and to facilitate the escape of warm air to avoid any glasshouse effect. Seedlings were at least 10 cm in from the perimeter of the screen to avoid absorption of unscreened radiation early and late in the day and screens were positioned 10–15 cm above the tops of the seedlings. The experiment ran until early October, 6 months after UV treatments were first imposed. At the conclusion, seedlings were sampled and analysed as described below.

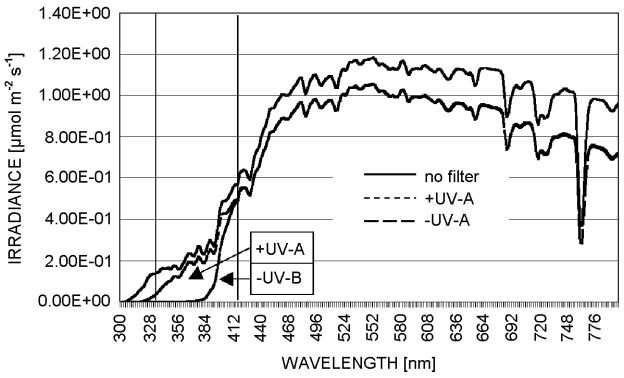

Fig. 1. Irradiation (measured using a *LiCor 1800* spectroradiometer) incident under clear-sky (no filter) and incident on seedlings under UV-A exposure (+UV-A) and UV-A excluded (–UV-A) glass screen treatments. UV-A encompasses the 320–400 nm spectrum as indicated by the vertical lines.

Photo- and leaf-chemistry: Pre-dawn photochemical efficiency (F_v/F_m) of 16 seedlings from each treatment (4 seedlings from each of 4 plots) was measured using a *PAM-2000* fluorometer and *2030-B* leaf-clip holder (*H. Walz*, Effeltrich, Germany). On the same day, the two most recently fully expanded leaf pairs from 16 seedlings were randomly sampled from each plot (n = 4 plots per treatment). The leaf pairs of eight seedlings per plot were placed immediately into porous cloth bags and submerged in liquid nitrogen. These were then transferred to a freezer at –20 ºC until extraction [not longer than 24 h for Chl and carotenoid (Car) extraction and not longer than 72 h for phenolic extraction]. Leaf pairs from the other eight seedlings were placed into paper bags and put in an oven at 65 ºC until they were dried to a constant mass, for percentage dry matter determination and nitrogen (N) analysis.

N, Chl, and Car extraction and analysis: For N extraction and analysis, leaves were cooled over desiccation salts before being finely ground in a hammer mill. Material was then digested in concentrated sulphuric acid as described by Lowther (1980). The digest was diluted and colourimetrically analysed for N (*QuikChem* method *10-107-06-2E*, *Lachat Instruments*, Wisconsin, USA) on a continuous flow injection analyser (*QuikChem 800*, *Lachat Instruments*). Standard samples of known N concentration and blank samples were included to validate the efficiency of digestion and elemental analysis.

For pigment extraction and analysis, the cloth bags containing the sampled leaves were crushed so that the frozen leaves were broken into a coarse powder. The leaf powder was mixed and sub-samples (approximately 0.5 g, but carefully weighed individually to allow expression of concentrations on a dry matter basis) were extracted in 100 % acetone to yield Chls and Cars, and analysed using high-performance liquid chromatography (HPLC) (Close *et al.* 2001a).

Phenolic extraction and analysis: Additional subsamples (approximately 0.5 g) from the coarse leaf powder material that was used for Chl and Car subsampling were homogenized with a *Polytron* homogenizer in 2 % (v/v) H_2SO_4 in methanol (Close *et al.* 2001b). Samples were extracted overnight at 4 °C, before HPLC analysis for phenolic compounds. Extracts were analysed on a *Waters Alliance 2690* HPLC connected to *Waters 996* Photodiode Array (PDA) detector using an *Agilent Zorbax 300SB-C3* column (2.1×150.0 mm). Mobile phases were: solvent *A* – 2 % acetic acid in methanol, solvent *B* – 2 % acetic acid in water, solvent *C* – hexane. A gradient was run from 5 : 95 *A : B* to 15 : 85 *A : B* at 4 min, then to 40 : 60 *A : B* at 60 min, then to 100 % *A* at 90 min, then to 80 : 20 *A : C* at 91 and this was held until 95 min. Re-equilibration was back through 100 % *A*, then to starting conditions. Flow rate was 0.25 cm^3 per min.

Where photodiode array data were not adequate to categorise compounds to class, they were analysed for molecular mass by negative ion electro-spray mass spectrometry (MS), by coupling the effluent from the PDA detector to a *Finnigan LCQ* ion trap mass spectrometer. Experimental conditions were: sheath gas 5.624 kg cm^{-2}, auxillary gas 1.055 kg cm^{-2}, needle voltage 5.2 kV, capillary voltage 45 V, capillary temperature 250 ºC.

The complexity of leaf phenolic extracts precluded complete HPLC separation of all components, and this was also beyond the scope of the experiment. Instead, UV wavelengths from 230 to 410 nm were monitored at

a resolution of 2.4 nm, and chromatograms of 'average absorbance' within defined 20 nm wide UV windows were generated using *Waters Millenium* software. Identifications of specific compounds or the compound class of major contributors to individual chromatographic peaks within the averaged 20 nm wide UV windows were based on previous work with *Eucalyptus* extracts using HPLC, UV, MS, and MS/MS characteristics (Barry *et al.* 2001, Close *et al.* 2001b, Eyles *et al.* 2003). Absorbance, as indicated by peak area, was integrated and calculated using the *Waters Millenium* software. The relative area under identified peaks, as indicated in Fig. 2, was then summed from the generated peak area tables for the major compound classes of gallotannins, stilbenes, and flavonols.

Statistical analyses: We used the general linear model procedure (*PROC GLM*, SAS Institute 1989) to test for effects of nutrient and UV-A treatments on seedling N content, pre-dawn F_v/F_m, F_0, and F_m, and contents of Chl, Car, and phenolic compounds. Data were log transformed for analysis to satisfy assumptions of normality where required (Zar 1996). A Tukey-Kramer adjustment was made for pair-wise comparisons. Least-squares' means and standard errors of untransformed data are presented.

Results

There was a significant nutrient treatment effect on foliar N contents, with a gradual increase in foliar N from treatments *A* to *D* (Table 1). There was no effect of UV-A, nor of the nutrient×UV-A interaction on foliar N (Table 1). Likewise there were significant nutrient effects on F_v/F_m, F_0, F_m, contents of Chl and total xanthophylls (V+A+Z; violoaxanthin, antheraxanthin, zeaxanthin) per unit Chl, and xanthophyll cycle conversion ratio (A+Z)/(V+A+Z) (Table 1). F_v/F_m and Chl (*a*+*b*) content were lower in treatment *A* than in the other three ones, whereas (V+A+Z) per Chl and (A+Z)/(V+A+Z) were higher in treatment *A* than in the other three treatments (Table 1). There were no effects of UV-A nor nutrient×UV-A treatment interactions on F_v/F_m, F_0, F_m, Chl (*a*+*b*), and (A+Z)/(V+A+Z) (Table 1). However, the effect of UV-A treatment of (V+A+Z) per unit Chl was significant (comparisons in order –UV-A then +UV-A: 91.8 and 68.9 for treatment *A*; 63.7 and 55.6 for treatment *B*; 55.6 and 53.9 for treatment *C*; and 56.7 and 54.3 for treatment *D*; SE±5.8).

Table 1. Nitrogen (N) [%(DM)] and chlorophyll (Chl) [mol kg^{-1}(FM)] contents, Chl *a* fluorescence (pre-dawn photochemical efficiency; F_v/F_m, baseline fluorescence; F_0, maximum fluorescence; F_m), total xanthophylls per unit chlorophyll (VAZ per Chl) [mmol mol^{-1}(Chl)], and xanthophyll cycle conversion ratio [A+Z]/[V+A+Z] of seedling nutrient treatments *A*, *B*, *C*, and *D* (not fertilized and fertilized every 14, 7, and 2 d, respectively, for 6 months). Results are presented with respect to nutrient treatment only because neither the main effect of UV-A (+/–) nor the interactions of UV-A and nutrient treatment were significant except in the case of VAZ per Chl, see text for details. DM, dry matter; FM, fresh matter.

	Nutrients					Nutrient effect	UV-A effect	Interaction effect
	A	*B*	*C*	*D*	SE	F value, P value	F value, P value	F value, P value
N	0.64 a	0.96 b	1.24 b	1.73 c	0.05	$F_{3,28}$ = 13.09, 0.0001	$F_{1,28}$ = 13.09, 0.809	$F_{3,28}$ = 13.09, 0.5830
F_v/F_m	0.66 a	0.72 b	0.73 b	0.73 b	0.01	$F_{3,110}$ = 13.09 0.0001	$F_{1,110}$ = 0.03, 0.8526	$F_{3,110}$ = 1.54, 0.2094
F_0	0.19 a	0.23 b	0.23 b	0.24 b	0.03	$F_{3,110}$ = 11.31, 0.0001	$F_{1,110}$ = 0.77, 0.3807	$F_{3,110}$ = 0.59, 0.6218
F_m	0.58 a	0.88 b	0.96 b	0.90 b	0.05	$F_{3,110}$ = 29.61, 0.0001	$F_{1,110}$ = 0.52, 0.4723	$F_{3,110}$ = 0.60, 0.6142
Chl (*a*+*b*)	327 a	520 a,b	568 b	912 c	104	$F_{3,29}$ = 14.81, 0.0001	$F_{1,29}$ = 0.14, 0.7150	$F_{3,29}$ = 1.55, 0.2306
VAZ per Chl	80.4 a	59.6 a	54.7 a	55.5 a	5.80	$F_{3,29}$ = 6.68, 0.0022	$F_{1,29}$ = 4.55, 0.0444	$F_{3,29}$ = 1.33, 0.2892
(A+Z)/(V+A+Z)	0.52 a	0.32 a,b	0.21 b	0.16 b	0.05	$F_{3,29}$ = 17.88, 0.0001	$F_{1,29}$ = 0.37, 0.5501	$F_{3,29}$ = 0.05, 0.9830

Absorption within the UV-B range (between 300–320 nm) was dominated by gallotannin and stilbene compounds (59 and 35 % of total absorbance; Fig. 2). Absorption by gallotannins was minor within the 320–340 nm (13 %) and 340–360 nm (5 %) UV-A wavelengths and there was no absorption by gallotannins in the UV-A range windows of 360–380 nm and 380–400 nm (Fig. 2). Stilbene compounds dominated absorption in the 320–340 nm (62 %) window; stilbenes and flavonols in the 340–360 nm (48 and 47 %, respectively) windows, and flavonols dominated in the 360–380 nm (92 %) and 380–400 nm (100 %) windows. Total absorption by extracted phenolic compounds declined with successively increasing windows (Fig. 2).

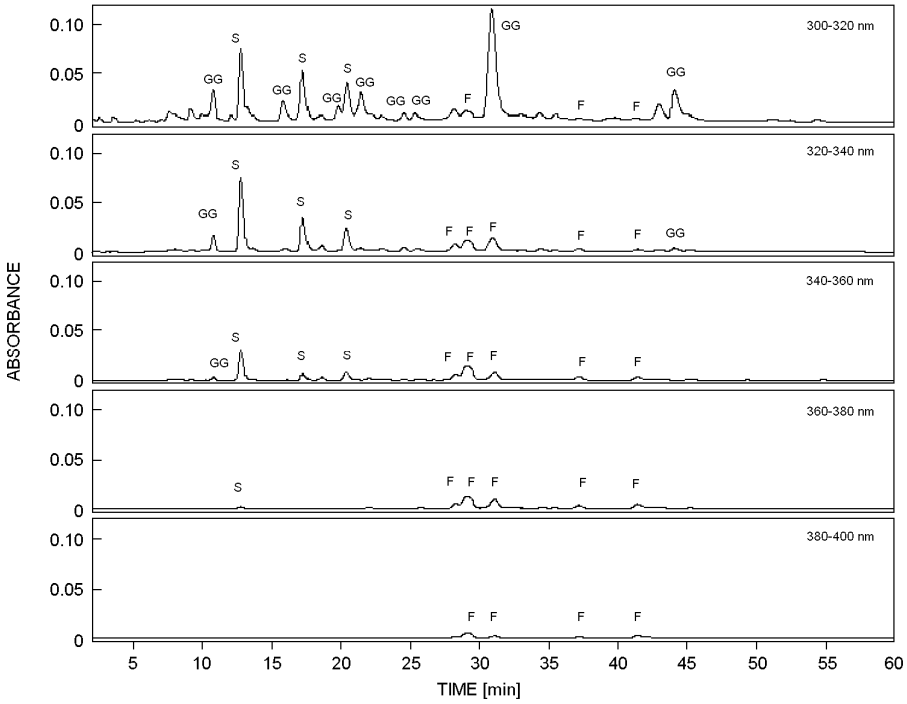

Fig. 2. Chromatograms showing the absorbance, averaged within 20 nm windows, for a UV-A exposed sample from a non-fertilised *E. nitens* seedling. Some significant peaks with each chromatogram have been labelled (G = gallotannins, S = stilbene glycosides, F = flavonols) to the level of compound class to illustrate their contribution to absorption within each window.

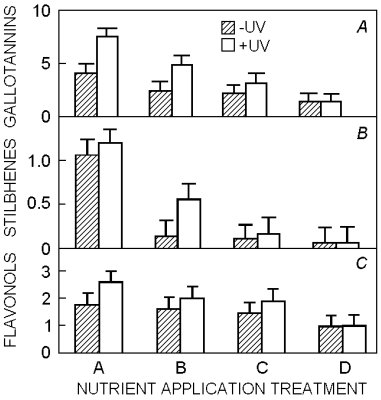

Fig. 3. Average absorbance [relative peak areas from HPLC analysis] of gallotannins between 300 and 320 nm (*A*), stilbenes, between 340 and 360 nm (*B*), and flavonols, between 360 and 380 nm (*C*) from UV-A exposed and UV-A excluded seedlings of fertiliser treatments *A*, *B*, *C*, and *D* (not fertilised and fertilised every 14, 7, and 2 d, respectively, for 6 months). Least square means within one SE bar.

There were significant nutrient ($F_{3,20}$ = 19.04, p<0.0001) and UV-A ($F_{1,20}$ = 13.57, p=0.0015) effects on gallotannins (Fig. 3*A*) but no significant nutrient×UV-A interaction effect ($F_{3,20}$ = 0.46, p=0.7152). Gallotannin content decreased with increasing nutrients, and was higher in +UV-A than –UV-A treatments in the low nutrient treatments. There was a significant nutrient ($F_{3,18}$ = 39.46, p<0.0001) and UV-A ($F_{1,18}$ = 5.16, p=0.0357) effect on contents of stilbenes, with a weak interaction ($F_{3,18}$ = 2.84, p=0.0670; Fig. 3*B*). On average, stilbene contents were 60 % higher in the lowest nutrient treatment (*A*; 1.15±0.12 total relative peak area) than the highest nutrient treatment (*D*; 0.70±0.12 total relative peak area), and were 45 % higher in +UV-A then –UV-A (0.51±0.09, 0.35±0.09 total relative peak area averaged across fertiliser treatments, respectively). There was a significant nutrient effect on contents of flavonols ($F_{3,27}$ = 6.04, p=0.0042) where contents gradually decreased from treatment *A* to treatment *D*, but there was no UV-A treatment ($F_{1,27}$ = 2.72, p=0.1517) nor interaction ($F_{3,27}$ = 0.07, p=0.9765) effect (Fig. 3*C*).

Discussion

The majority of compounds that contributed to absorption across the UV-A spectrum was identified to the class level by a combination of UV spectra and negative ion electrospray MS/MS. The major classes detected were gallotannins, stilbenes, and flavonols. UV-A absorption by gallotannins, however, was minor and occurred only within the shorter wavelength range of 320–360 nm. In contrast, stilbenes contributed the majority of UV-A absorption between 320–340 nm, both stilbenes and flavonols between 340–360 nm, and flavonols provided virtually all absorption between 360–400 nm. These results confirm the potential for stilbenes and flavonols to act as UV-A screens in the leaves of *E. nitens* although we caution that the leaf extracts were from whole leaves and not specifically from epidermal tissues.

Gallotannin and stilbene contents were significantly augmented in seedlings of low nutrient status when exposed to UV-A, but flavonol contents did not appear to

increase. These results are intriguing because, combined with our knowledge of the differing UV spectral ranges in which these three phenolic classes absorb, they suggest some specificity in function and response to UV-A between the three phenolic classes. Gallotannins could only play an extremely limited role as UV-A screens (given their limited absorption of UV-A), yet their active increase in seedlings acclimated to UV-A suggests a function. Given their demonstrated anti-oxidant capacity, at least *in vitro* (Hagerman *et al.* 1998), we suggest that it is enhanced anti-oxidant capacity that is being realised through the elevated contents of gallotannins observed under UV-A exposure, particularly in seedlings of low nutrient status.

We found that stilbenes, like gallotannins, are an active part of the UV-A response. Stilbenes have absorbance properties consistent with a function as UV-A screens (our results). Antioxidant activity has also been reported for stilbenes (Cuendet *et al.* 2000, Lee *et al.* 2003), consistent with induction of stilbenes by exposure to ozone and UV-B radiation (Schöppner and Kindl 1979, Thalmair *et al.* 1996). The relative importance of UV-A screening and antioxidant functions in UV-A-exposed plants cannot be determined from our experiment. Further, it is unclear why the greatest response in levels of stilbenes to UV-A occurred in the nutrient treatment *B*. It is possible that the relatively low nutrient level was sufficient to induce stilbenes only with the additional presence of UV-A. The two-fold increase in stilbenes once the nutrient level was lowered further (treatment *A*) may subsequently have been sufficient to cope with ambient UV-A.

Similar to stilbenes, flavonols could function as UV-A screens (our results) and/or anti-oxidants (Rice-Evans *et al.* 1996). We did not detect a significant increase in flavonols with UV-A exposure, although average flavonol contents were greater in +UV-A than –UV-A treatments. It therefore appears that flavonols are not induced by UV-A even if they play some role in protecting leaves against its damaging effects. This contrasts to their responsiveness to nutrient conditions.

No differences were found in PS2 efficiency, Chl or Car contents in UV-A exposed *versus* excluded treatments, within a given nutrient-application treatment. We suggest that long-term (six-month) acclimation to UV-A exposure (resulting in increased contents of gallotannins, and possibly stilbenes and flavonols) provided an adequate photoprotective response and enabled avoidance both of PS2 damage (indicated by Chl fluorescence) and damage caused by oxidative pressure (indicated by similar Chl contents and xanthophyll-cycle dynamics). The response to UV-A contrasts with long-term acclimation of leaves to the nutrient conditions imposed in this study and in a previous study (Close *et al.* 2003b) where nutrient deficit induced a reduction in Chl content and an increase in Car content.

We have identified three classes of phenolic compounds that show similar, though not identical, responses to UV-A radiation. The increased contents, in particular of gallotannins and stilbenes, reflect the type of increases also seen when nutrient levels are reduced in seedlings. Both responses point to a function in photo-protection, *via* two possible mechanisms: UV-A screening and as anti-oxidants. However, the implications of these findings are broader than simply the interaction between plants and abiotic conditions. Phenolic compounds have negative effects on many natural enemies of plants, including fungi and insect and mammalian herbivores (Derks and Creasy 1989, McArthur and Sanson 1993, Dudt and Shurer 1994). Just as natural light and nutrients affect herbivory through effects on plant secondary chemistry such as phenolics (Bryant *et al.* 1983), we found now that UV-A can specifically alter the contents of these compounds. Variation in the interaction between plant-available nutrients and incident UV-A is therefore likely to affect ecosystems at multiple trophic levels *via* modifications to foliar chemistry and subsequent plant-herbivore interactions.

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