

Diurnal changes in epidermal UV transmittance of plants in naturally high UV environments

Paul W. Barnes^{a,*}, Stephan D. Flint^b, James R. Slusser^c, Wei Gao^c and Ronald J. Ryel^b

^aDepartment of Biological Sciences, Loyola University New Orleans, New Orleans, LA 70118, USA

^bDepartment of Wildland Resources and Ecology Center, Utah State University, Logan, UT 84322-5230, USA

^cNatural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499, USA

Correspondence

*Corresponding author,
e-mail: pwbarnes@loyno.edu

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Studies were conducted on three herbaceous plant species growing in naturally high solar UV environments in the subalpine of Mauna Kea, Hawaii, USA, to determine if diurnal changes in epidermal UV transmittance (T_{UV}) occur in these species, and to test whether manipulation of the solar radiation regime could alter these diurnal patterns. Additional field studies were conducted at Logan, Utah, USA, to determine if solar UV was causing diurnal T_{UV} changes and to evaluate the relationship between diurnal changes in T_{UV} and UV-absorbing pigments. Under clear skies, T_{UV} , as measured with a UV-A-pulse amplitude modulation fluorometer for leaves of *Verbascum thapsus* and *Oenothera stricta* growing in native soils and *Vicia faba* growing in pots, was highest at predawn and sunset and lowest at midday. These patterns in T_{UV} closely tracked diurnal changes in solar radiation and were the result of correlated changes in fluorescence induced by UV-A and blue radiation but not photochemical efficiency (F_v/F_m) or initial fluorescence yield (F_o). The magnitude of the midday reduction in T_{UV} was greater for young leaves than for older leaves of *Verbascum*. Imposition of artificial shade eliminated the diurnal changes in T_{UV} in *Verbascum*, but reduction in solar UV had no effect on diurnal T_{UV} changes in *Vicia*. In *Vicia*, the diurnal changes in T_{UV} occurred without detectable changes in the concentration of whole-leaf UV-absorbing compounds. Results suggest that plants actively control diurnal changes in UV shielding, and these changes occur in response to signals other than solar UV; however, the underlying mechanisms responsible for rapid changes in T_{UV} remain unclear.

Introduction

The epidermis of higher plant leaves has long been considered a selective filter, removing much of the potentially damaging UV radiation (<400 nm) while transmitting most of the PAR (400–700 nm; Caldwell

et al. 1983, Day et al. 1993). For many plants, exposure of leaves to UV-B (280–320 nm) radiation during development is known to induce the production and accumulation of UV-absorbing compounds (e.g. flavonoids and hydroxycinnamic acids) in epidermal cells (Bidel et al.

Abbreviations – F_{BL} , chlorophyll fluorescence induced by blue light; F_o , initial fluorescence yield; F_{UV} , chlorophyll fluorescence induced by UV radiation; F_v/F_m , photochemical efficiency; PAM, pulse amplitude modulation; PAR, photosynthetically active radiation; PFD, photon flux density; T_{UV} , epidermal UV transmittance; USDA, US Department of Agriculture; UV-B_{BE71}, biologically effective UV-B weighted according to the Caldwell (1971) generalized plant action spectrum; UV-B_{BE03}, biologically effective UV-B weighted according to the Flint and Caldwell (2003) plant action spectrum.

2007, Bornman et al. 1997). This, in turn, reduces the penetration of UV to sensitive targets in the underlying tissues (Day 1993, Grammatikopoulos et al. 1999, Liakoura et al. 2003, Mazza et al. 2000, Olsson et al. 1999, Robberecht and Caldwell 1983). These phenylpropanoid compounds therefore serve as UV 'sun-screens', and their biosynthesis is generally viewed as an adaptive response to UV exposure (Beggs and Wellmann 1994, Caldwell et al. 2007, Jansen et al. 1998). Indeed, mutant plants deficient in epidermal flavonoids show appreciable UV-induced injury (Fiscus et al. 1999, Landry et al. 1995), and increased accumulation of UV-absorbing compounds is one of the most frequently observed responses of plants to elevated UV-B simulating ozone depletion (Searles et al. 2001).

Epidermal UV transmittance varies considerably among species and populations (Bilger et al. 2007, Day et al. 1992, Nybakken et al. 2004a), and in certain cases, this variation is related to the solar UV environment in which the taxa evolved (Nybakken et al. 2004b, Robberecht et al. 1980). These observations, and others (Barnes et al. 1987, Caldwell et al. 1980, 1982, Sullivan et al. 1992), suggest that the prevailing solar UV climate is an important selective force in the evolution of baseline UV protective mechanisms in plants. However, solar UV irradiance, like the light environment in general, is temporally dynamic, and UV fluxes at ground level can change quickly and appreciably depending on atmospheric conditions (e.g. cloud cover and ozone levels), solar elevation (time of day) and canopy position (sun vs shade) (Flint and Caldwell 1998, Grant and Heisler 1996, Searles et al. 1999). To what degree plants can modulate or adjust their UV sunscreens and epidermal UV transmittance in response to these dynamic UV conditions has received little attention to date.

The earliest report of diurnal (daily) fluctuations in epidermal UV transmittance was by Lautenschlager-Fleury (1955), who observed that the UV-B transmittance in epidermal peels of *Vicia faba* (faba bean) was low during midday on a sunny day but remained relatively high on a cloudy day. Veit et al. (1996) reported substantial midday increases in flavonoid levels in an alpine fern (*Cryptogramma crispera*) and a tropical tree (*Anacardium excelsum*), and these changes were not evident when solar UV-B was filtered out of the sunlight. Mycosporine-like amino acids, the functional equivalent of flavonoids, have also been found to exhibit a diurnal course in marine algae (Taira et al. 2004). However, to what extent the changes in UV sunscreens affected epidermal or cell wall UV transmittances were not investigated in either of these two studies.

Until recently, a significant limitation in probing short-term changes in epidermal UV transmittance has been

that this information could only be obtained through destructive (i.e. epidermal peels; Flint et al. 1985, Robberecht and Caldwell 1983) or invasive (i.e. microprobe; Ålenius et al. 1995, Day et al. 1992) sampling techniques. Thus, repetitive measurements of the same leaf over time were not possible. The relatively recent development of a non-invasive technique for the quantification of epidermal UV transmittance (Bilger et al. 1997, 2001, Cerovic et al. 2002) has now provided a non-destructive approach to follow the temporal dynamics of UV shielding in individual leaves. This technique uses UV-excited chlorophyll fluorescence to indirectly assess epidermal UV transmittance, and these measurements have been found to be highly correlated with direct measurements of UV transmittance from epidermal peels as well as UV-absorbing pigment levels (Barnes et al. 2000, Markstädter et al. 2001). Several investigators have used variations of this technique to examine changes in UV shielding in response to UV-B or other environmental factors that occur over days to weeks (Barnes et al. 2000, Bidet et al. 2007, Bilger et al. 2007, Kolb et al. 2001, Mazza et al. 2000), but responses over shorter time periods (minutes, hours) have yet to be reported.

In this study, we used this chlorophyll fluorescence technique to document rapid, diurnal changes in epidermal UV transmittance for three herbaceous species grown in naturally high UV environments in tropical subalpine habitats on Mauna Kea, Hawaii. We also experimentally test whether diurnal patterns in epidermal UV transmittance are influenced by alteration in solar radiation (total and UV) and determine whether these changes in transmittance are associated with changes in whole-leaf UV-absorbing pigments.

Materials and methods

Studies were conducted on *Verbascum thapsus* L. and *Oenothera stricta* Ledeb. Ex Link, two naturalized herbaceous species growing in native volcanic soils on the southwest slope of Mauna Kea, Hawaii, USA (2800 m elevation; 19°45'N, 155°27'W). Additional studies were conducted on *Vicia faba* L. cv. Broad Windsor grown from seed at this Hawaiian site as well as at a field site in Logan, Utah, USA (1460 m elevation; 41°44'N, 111°49'W). Established plants of *V. thapsus* and *O. stricta* were growing in fully sunlit clearings of sparsely vegetated shrublands and received ambient solar radiation and rainfall. *V. faba* plants were grown in pots (0.15 l volume) containing organic potting soil and placed in sunlit locations. In Hawaii, *Vicia* plants were grown under UV transparent film (Aclar type 22 A, 0.038 mm thick; Honeywell, Pottsville, PA) and moved into the open for diurnal transmittance measurements. In Utah,

premium cellulose triacetate (0.13 mm thick; Liard Plastics, Salt Lake City, UT) was used as the UV-transmitting filter, while clear Llummar (0.13 mm thick, part number UVCLSRPS, cutoff near 390 nm; CPFilms Inc., Martinsville, VA) was used as a UV-absorbing film. These plants were kept well watered throughout the study. Nomenclature follows Wagner et al. (1999); hereafter, species are referred to by genus.

Non-invasive measurements of epidermal UV transmittance were made on intact leaves with a field-portable pulse amplitude modulation (PAM) chlorophyll fluorometer (UV-A-PAM; Gademann Instruments, Würzburg, Germany). This instrument provides indirect estimates of epidermal UV-A transmittance by measuring the fluorescence yield of chlorophyll (F_o , $\lambda > 650$ nm) induced by UV-A (UV, 375 nm) and blue (BL, 470 nm) radiation, as outlined by Kolb et al. (2005). The technique is based on the premise that both UV and BL can induce chlorophyll fluorescence and that reductions in the penetration of UV to the mesophyll (e.g. from UV-absorbing pigments in the epidermis) will reduce UV-induced chlorophyll fluorescence (F_{UV}). Fluorescence induced by BL (F_{BL}), which is not absorbed by UV pigments, serves as a reference to account for variation in chlorophyll content. Ideally, true values of epidermal UV transmittance are obtained by electronically adjusting the output from the BL source (light-emitting diodes) such that F_{UV}/F_{BL} is unity for an epidermis-free leaf of the species being measured. As it is usually not possible to readily remove the epidermis for most species, F_{UV}/F_{BL} values are normally expressed relative to a blue plastic standard (Heinz Walz GmbH, Effeltrich, Germany), which has emission properties similar to those of an epidermis-free green leaf. Such was the case in this study. The epidermal UV transmittances (hereafter referred to as T_{UV}) reported here should therefore be considered as approximations of the true transmittances for these species; however, they are still reflective of diurnal changes in UV transmittance.

Diurnal measurements of T_{UV} were made on adaxial (upper) surfaces of mature, healthy leaves from 5–12 individual plants (two to four leaves per plant) five times during the day, including approximately 1 h before sunrise (predawn) and 1–2 h after sunset. For these measurements, the placement of the sampling cuvette (surface area = 0.5 cm²) on the leaf was denoted with a permanent-ink marker, such that repeat measurements during the day were obtained from the same location on each leaf. Measurements were made during June to July under clear skies. Studies examining leaf surface and age effects on diurnal changes in T_{UV} were conducted on established, vegetative plants of *Verbascum* (10 plants, five leaves/plant). Leaves were classified as relatively

‘young’ and ‘old’ based on position within the basal rosette and leaf angle (i.e. young leaves = inner near vertical leaves, old leaves = outer more horizontal leaves). While we do not know the precise age of these leaves, both ‘young’ and ‘old’ leaves appeared to be mature and fully expanded at the time of measurement (i.e. we avoided sampling the youngest, emerging leaves at the very center of the rosette). Maximal diurnal change in T_{UV} was determined from measurements conducted over a cloudless 2-day period in June.

To test whether diurnal patterns in T_{UV} could be disrupted by changes in solar radiation, we conducted several experiments. In the first study, we shaded established plants of *Verbascum* with structures consisting of rectangular plywood tops (1.3 × 0.6 m) and neutral density shade cloth on the sides. The plywood sheet was placed on a metal frame approximately 0.5 m above the plants to allow for ventilation, and the side shade cloth was temporarily lifted during measurements to allow access to the experimental plants, but care was taken to ensure that plants were not exposed to direct beam solar radiation. Measurements of photon flux density (PFD, 400–700 nm) made at midday with a quantum sensor (LI-185; Li-COR Inc., Lincoln, NE) at plant height indicated that these shade structures reduced light to approximately 1% of full sun. Three plots (one unshaded control and two shade treatments) were established with five experimental plants in each plot. Measurements of T_{UV} (UV-A-PAM) were made on two to three leaves per plant (adaxial surface only), with the initial measurements made on unshaded plants at predawn. After these measurements, the shade structures were placed over two of the three plots, and these remained in place for varying time periods over the next 2 days. Both shade structures were removed from the plots during the intervening night. At each sampling time and immediately following measurements of T_{UV} , initial fluorescence yield (F_o) and photochemical efficiency (F_v/F_m) were measured on dark-adapted (10 min) experimental leaves using a portable continuous excitation fluorescence system (Handy PEA; Hansatech Instruments Ltd., Norfolk, UK; saturating PFD approximately 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

In a separate study, we tested whether exposure to solar UV was required for expression of diurnal changes in T_{UV} and assessed whether diurnal changes in T_{UV} were associated with increases in UV-absorbing pigments. Logistical and facility constraints prevented us from conducting this study on the species growing at our Hawaii field site. Therefore, for this study, we grew potted plants of *Vicia* under either UV transparent (premium cellulose triacetate) or UV-absorbing (Llummar) film during August to September in Logan, Utah. Plants were grown

in 'filter tents' (base dimensions = 2.1 × 0.6 m) and treatment films were suspended over the plants (from base to base) using arched plastic tubing with a maximum height of 0.6 m. The ends of the tent were plywood with 20 × 40 cm openings covered with shade cloth (10% PAR transmittance) to provide ventilation; a portable fan (HT-380; Kax Inc., Southborough, MA) was placed in one end to vent air to the outside and maintain ambient temperatures within the tents. After 15 days of post-emergence growth, diurnal measurements of T_{UV} were made on 10 plants (one pair of healthy, mature, upper canopy leaflets per plant) in each treatment (day 1). Prior to sunrise of the next day (day 2), plants from each growth treatment were transferred to the alternate treatment (i.e. +UV plants were moved to the -UV treatment, and -UV plants were moved to the +UV treatment) and diurnal measurements of T_{UV} were repeated on the same leaves. Skies were clear on all sampling days. Prior to sunrise of day 3, the +UV plants were placed in the +UV treatment; plants originally from -UV were left in the +UV treatment. Leaf samples (1 cm²) from the plants currently in the +UV treatment (10 plants per treatment) were collected at predawn (from one of the marked leaflet pairs used for T_{UV} measurements) and midday (the second leaflet from the same leaf and plant) on day 3 to quantify UV-absorbing pigments. Care was taken to collect tissue samples from within the part of the leaflet used for T_{UV} measurements, which were taken just prior to collection of the pigment samples. These samples were oven dried (60°C for 24 h) and extracted at room temperature (approximately 20°C) in the dark for 24 h in an acidified methanol solution (5 ml of 70% methanol, 29% H₂O and 1% HCl). Extract absorbances were measured with a scanning UV/Vis spectrophotometer (Model DU640; Beckman Coulter Inc., Fullerton, CA). The concentration of UV-B- and UV-A-absorbing pigments is here expressed as the absorbance/5 ml at 305 and 360 nm, respectively.

For all species, diurnal T_{UV} data were analyzed using repeated measures analysis of variance (ANOVA) where the experimental unit was the individual leaf with individual plant used as a fixed factor. Mean comparisons were made using preplanned least square difference (LSD) tests or paired Student's *t*-tests at $P < 0.05$. Analysis of covariance was used to compare predawn and midday concentrations of UV-B- and UV-A-absorbing pigments with predawn UV-A absorbance used as the covariate and individual plant used as a fixed factor. Pearson's correlation coefficients (*r*) were calculated and tested for significance by Fisher's *r* to *z* transformation.

Solar UV irradiance data for the relative UV portrayed in the figures were obtained from an on-site broadband UV sensor (Skye UV-B; Skye Instruments Ltd., Powys, UK) or from the US Department of Agriculture (USDA) UV-B

monitoring station radiometer located at the Mauna Loa Observatory, Hawaii, USA (3397 m elevation; approximately 30 km from our sampling site on Mauna Kea). For the UV irradiance doses presented in the figure legends, data were obtained from a USDA UV-B monitoring station near Logan, Utah, USA, and the above-mentioned Mauna Loa station. Biologically effective UV-B irradiances were derived by weighting the raw UV spectral irradiance data, obtained from synthetic spectra on the USDA UV monitoring Web site (http://uvb.nrel.colostate.edu/UVB/home_page.html), with the Caldwell (1971) generalized plant action spectrum and a more recent plant action spectrum (Flint and Caldwell 2003), both of which were normalized to unity at 300 nm. Here, these weighted UV-B irradiances are denoted as UV-B_{BE71} and UV-B_{BE03}, respectively. We adjusted the UV-B_{BE} data for the differences in elevation between the Mauna Loa station and the Mauna Kea study site using a regression equation generated from data by Nullet and Juvik (1997). The UV-A irradiances are reported as unweighted irradiances integrated over 321–400 nm and were not adjusted for elevation differences, which were minor.

Results

Under clear skies, significant diurnal changes in epidermal UV transmittance (T_{UV}), as measured using chlorophyll fluorescence (F_{UV}/F_{BL}), were detected in the upper (adaxial) leaf surfaces of *V. thapsus* (Fig. 1A), *O. stricta* (Fig. 1B) and *V. faba* (Fig. 1C) growing at high elevations in Hawaii ($P < 0.05$ for time effect, repeated measures ANOVA). For all species, T_{UV} declined from predawn to midday and then increased to predawn values soon after sunset. The midday minimum in T_{UV} occurred at, or shortly after, the daily peaks in ambient solar UV (Fig. 1) and PAR (not shown). Diurnal changes in T_{UV} occurred in the context of highly correlated F_{UV} and F_{BL} for all species ($r > 0.7$, $P < 0.01$), with the slope of the linear regressions between F_{UV} and F_{BL} changing over the course of the day as T_{UV} changed (data not shown).

While the absolute changes in T_{UV} were small (1–2%), these midday changes represented substantial (13–16%) increases in UV shielding potential relative to predawn values. For horizontal leaves of *Verbascum*, the daily dose of UV-A (375 nm) reaching the mesophyll without these diurnal changes (i.e. a constant epidermal transmittance at predawn values) was calculated to be 11.6% of incident solar UV. With these diurnal changes in T_{UV} , the midday UV dose to the mesophyll decreased to 9.9% (a 14.7% decrease) and the daily UV dose decreased to 10.2% of incident (a 12.1% decrease). By comparison, if midday values of T_{UV} were maintained over the day, the calculated UV dose to the mesophyll would be 9.9% of incident.

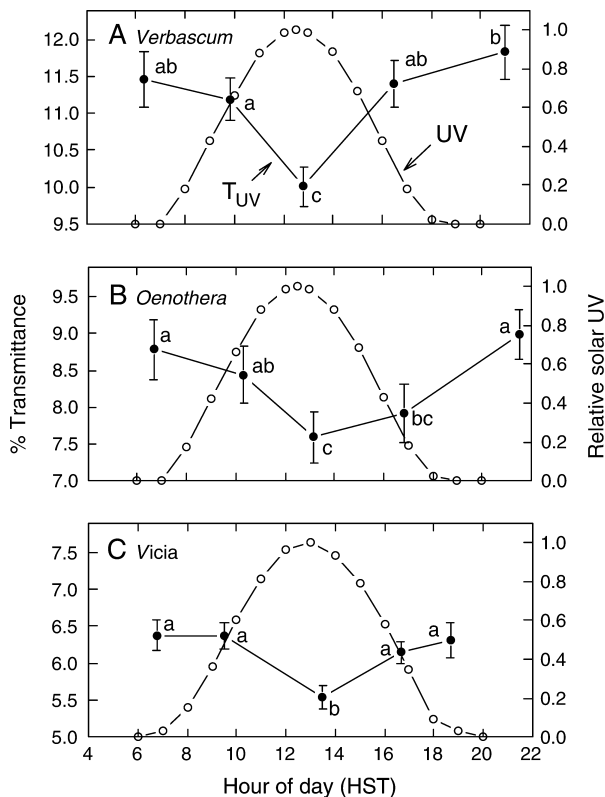


Fig. 1. Diurnal patterns of adaxial epidermal UV transmittance (T_{UV} , solid symbols) for *Verbascum thapsus* (A), *Oenothera stricta* (B) and *Vicia faba* (C) growing under clear skies at 2800 m elevation on Mauna Kea, Hawaii (mean \pm SE, $n = 5$ –12 plants, two to four leaves per plant). Within a species, means with different letters are significantly different at $P < 0.05$ as determined by Fisher's LSD test. Relative solar UV data (open symbols) are from the 368 nm channel of the UV multifilter rotating shadowband radiometer at the USDA UV monitoring station on Mauna Loa, Hawaii. Maximum daily UV-B_{BE71}, UV-B_{BE03} and UV-A at the Mauna Kea site were 0.60, 1.75 and 68.2 W m⁻², respectively. HST = Hawaiian Standard Time.

The magnitude of the diurnal changes in T_{UV} tended to be greater ($P < 0.10$) for younger than for older basal rosette leaves of *Verbascum* (Fig. 2). Diurnal changes in T_{UV} were statistically comparable between lower (abaxial) and upper (adaxial) surfaces in this species, and there was no indication of a significant interaction between leaf age and surface.

Both the addition and the removal of artificial shade altered the diurnal patterns of T_{UV} in established, sun-grown plants of *Verbascum* (Fig. 3). Specifically, we found that the imposition of deep shade (1% of incoming PAR) prior to sunrise eliminated the normal midday reductions in T_{UV} (Fig. 3C, D). Indeed, the shade treatment caused T_{UV} to increase rather than to decrease during the morning. Shading the leaves at midday caused T_{UV} to increase (Fig. 3D), whereas removal of the shade

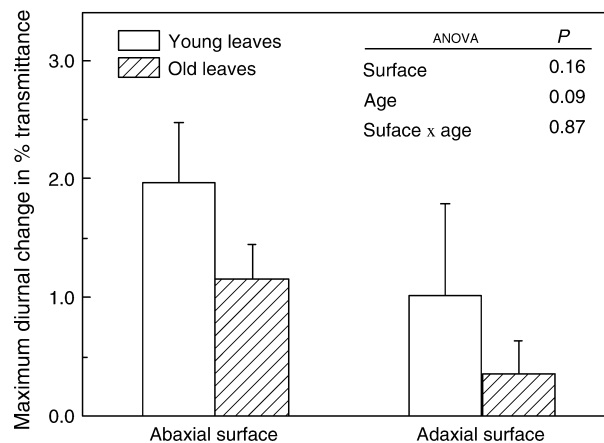


Fig. 2. Effect of leaf surface and age on the magnitude of diurnal changes in epidermal UV transmittance (T_{UV}) in *Verbascum thapsus* growing at 2800 m elevation on Mauna Kea, Hawaii (mean \pm SE, $n = 10$ plants, five leaves per plant). Measurements were conducted under clear skies where the maximum daily UV-B_{BE71}, UV-B_{BE03} and UV-A were 0.61, 2.00 and 85.4 W m⁻², respectively.

treatment, either in the morning (Fig. 3C) or at midday (Fig. 3D), caused T_{UV} to decrease. These changes in T_{UV} in response to experimental shade or full sun were relatively rapid (1–2 h) such that the temporal pattern of T_{UV} in the treatment leaves following shade removal quickly resembled that of the unshaded controls (Fig. 3A, B).

PSII quantum efficiency (F_v/F_m) of *Verbascum* in this shade study varied significantly over the day ($P < 0.01$ for time effect, repeated measures ANOVA) and was also affected by the shading treatments (Fig. 3). However, the diurnal variation in F_v/F_m was not significantly correlated with diurnal variation in T_{UV} in any of the treatments, including ambient controls (Table 1). Similar results were found for initial fluorescence yield (F_o , Table 1).

Diurnal changes in T_{UV} in potted plants of *V. faba* were evident in plants grown and measured under both ambient (+UV \rightarrow +UV) and reduced (-UV \rightarrow -UV) solar UV in Logan, Utah (Fig. 4A, $P < 0.05$ for time effect, repeated measures ANOVA). Diurnal changes in T_{UV} in the +UV plants occurred without detectable changes in whole-leaf UV-absorbing pigments (Fig. 4B). Transferring the +UV plants to the -UV treatment (+UV \rightarrow -UV) did not significantly alter diurnal T_{UV} patterns ($P = 0.41$ for day effect, repeated measures ANOVA, Fig. 4A). There was also no significant difference in maximum diurnal change in T_{UV} for the +UV plants measured in the presence or absence of solar UV ($P = 0.97$, Student's paired t -test). By comparison, transferring the -UV plants to the +UV treatment (-UV \rightarrow +UV) caused a gradual decrease in T_{UV} (Fig. 4A), and this change was associated with an increased accumulation of UV-absorbing pigments (Fig. 4B).

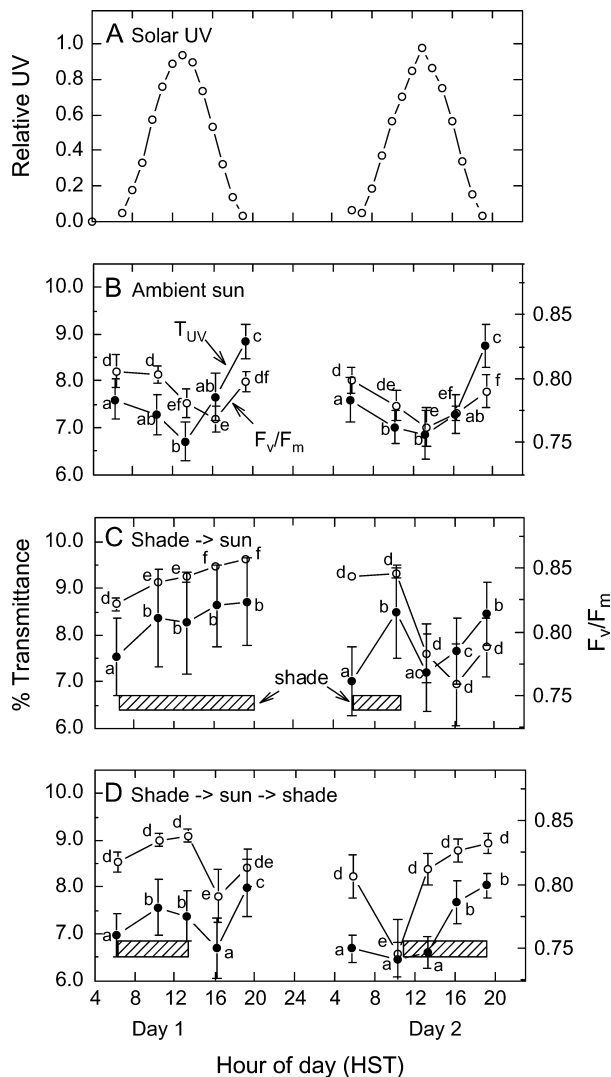


Fig. 3. Influence of artificial shade on adaxial diurnal epidermal UV transmittance (T_{UV} , solid circles) and photochemical efficiency (F_v/F_m , open circles) in *Verbascum thapsus* growing at 2800 m elevation on Mauna Kea, Hawaii (mean \pm SE, $n = 5$ plants/treatment, two to three leaves per plant). At different times within a given day and treatment, means with contrasting letters (a–c for T_{UV} and d–f for F_v/F_m) are significantly different at $P < 0.05$ as determined by Fisher's LSD test. Relative solar UV data are from on-site measurements made with a broadband UV sensor (280–315 nm). Maximum daily UV-B_{BE71}, UV-B_{BE03} and UV-A were 0.61, 1.82 and 71.7 W m⁻², respectively. Shaded bars denote times when the shade structure was placed over the experimental plants. HST = Hawaiian Standard Time.

Discussion

Results from our study indicate that measurable diurnal changes in epidermal UV transmittance (T_{UV}) occurred in plants growing in relatively high solar UV environments of the Hawaiian subalpine. For all three species examined (*V. thapsus*, *O. stricta* and *V. faba*), daily minima in T_{UV}

occurred at midday, when solar UV flux was maximal, and T_{UV} was highest at predawn and after sunset. To the best of our knowledge, this is the first report of changes in T_{UV} within the same leaves of higher plants over a diurnal cycle. Previous studies by Lautenschlager-Fleury (1955) examined detached epidermal peels of *V. faba* and found that epidermal UV transmittance changed from 1 day to the next depending on cloud cover.

Our study was made possible through the use of a non-destructive, non-invasive technique that measures T_{UV} based on chlorophyll fluorescence. Since its introduction, this technique has been found to provide a robust and reliable measure of T_{UV} in a variety of plant species and environments [see Bilger et al. (2007), Kolb et al. (2005) and references therein]. A key premise of this technique is that UV-absorbing pigments in the epidermis will reduce the penetration of UV to the mesophyll, which then reduces F_{UV} . It is assumed that fluorescence induced by longer wavelengths (F_{BL}) is not influenced by these UV-absorbing compounds such that F_{BL} can serve as a reference to account for variation in photosynthetic tissue. Misleading results can occur if F_{BL} is altered by non-UV-absorbing epidermal pigments (e.g. anthocyanins), which may change in response to UV or other environmental factors (e.g. cold temperatures; Barnes et al. 2000). Although we did not quantify anthocyanins in the present study, we did find that the observed changes in T_{UV} were the result of correlated changes in both F_{UV} and F_{BL} and not simply changes in F_{BL} . Also, while T_{UV} , F_v/F_m and F_o appeared to covary at certain times of day, we found no statistically significant correlations between T_{UV} and F_v/F_m or F_o . Thus, we have no evidence to suggest that our results are artifacts linked to this particular technique or to diurnal changes in photochemistry.

At present, the underlying mechanism for the relatively rapid changes in T_{UV} is unknown. Our finding that T_{UV} was responsive to artificial shade indicates that leaves can respond quickly to changes in the solar radiation regime

Table 1. Correlations between adaxial epidermal UV transmittance (T_{UV}) and photosynthetic fluorescence parameters (F_v/F_m , F_o) in *Verbascum thapsus* growing at 2800 m elevation on Mauna Kea, Hawaii, and exposed to different solar radiation treatments (see Fig. 3). Pearson's correlation coefficients (r) are for all data within a treatment collected over a 2-day period ($n = 5$ plants/treatment, two to three leaves per plant).

Comparison	Treatment	r	P
T_{UV} vs F_v/F_m	Ambient	0.14	0.34
	Shade–sun	0.07	0.64
	Shade–sun–shade	0.13	0.38
T_{UV} vs F_o	Ambient	–0.25	0.08
	Shade–sun	0.07	0.63
	Shade–sun–shade	0.07	0.63

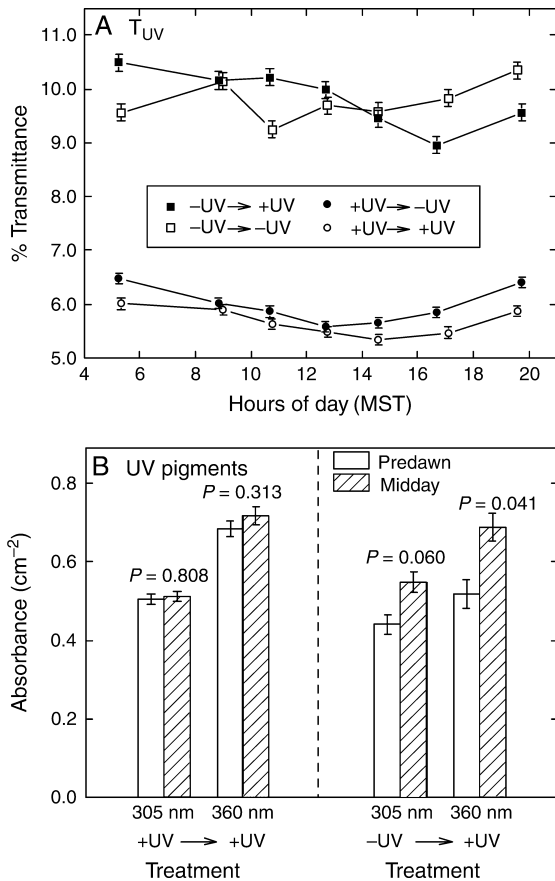


Fig. 4. Diurnal patterns of adaxial epidermal UV transmittance (T_{UV}) (A) and UV-absorbing pigments (B) in *Vicia faba* grown under different solar UV conditions at Logan, Utah. In A, data are from plants grown and measured under ambient solar UV (+UV → +UV, open circle), grown under ambient solar UV and then transferred to the reduced UV treatment for measurement (+UV → -UV, closed circle), grown under reduced solar UV and measured under reduced UV (-UV → -UV, open square), then transferred to the ambient UV treatment for measurement (-UV → +UV, closed square). In B, data are from plants grown and measured under ambient solar UV (+UV → +UV), and plants grown under reduced solar UV and then transferred to the ambient UV treatment for 2 days (-UV → +UV). Data shown are means \pm SE [$n = 5$ plants/treatment, two leaves per plant (T_{UV}) or one leaf per plant (pigments)]. P values are from analysis of covariances between predawn (open bars) and midday (shaded bars) means for UV-B-absorbing (305 nm) and UV-A-absorbing (360 nm) pigments. Significant differences were found between predawn and midday for UV-A transmittance for both treatments (+UV, $P < 0.001$; -UV, $P = 0.001$). Measurements were conducted under clear skies where the maximum daily UV-B_{BE71}, UV-B_{BE03} and UV-A were 0.28, 1.14 and 51.5 $W m^{-2}$, respectively. MST = Mountain Standard Time.

and that these diurnal changes in T_{UV} are not the result of some endogenous or circadian rhythm. The fact that diurnal changes in T_{UV} were greater for young vs old leaves further suggests a physiological basis for this response. However, our finding that the diurnal change in

T_{UV} was not affected by exclusion of solar UV indicates that this response is driven more by longer wavelength radiation and/or other environmental or physiological changes correlated with daily changes in solar radiation (e.g. temperature, water stress). Indeed, the imposition of artificial deep shade actually caused an initial increase in T_{UV} . Interestingly, these findings appear to contrast with those of Veit et al. (1996) who found that diurnal changes in flavonoid levels in *C. crispera* were not apparent in a solar UV-exclusion treatment but were evident in plants growing in the shade. However, the level of shade in our study was much greater than in the study by Veit et al. (1996), and this may limit comparisons between these two studies.

Whereas Veit et al. (1996) reported relatively large diurnal changes in flavonoids in *C. crispera*, a fern growing at high elevations in the southern Alps, and *A. excelsum*, a tropical tree growing in Panama, our findings suggest that the diurnal changes in T_{UV} were not because of concurrent changes in the levels of UV-absorbing compounds. However, logistical and facility constraints prevented us from collecting and processing pigment samples from the study species in Hawaii. Thus, our analysis of the relationship between diurnal changes in flavonoids and epidermal transmittance is limited to the field studies conducted in Utah with a single species, *V. faba*. For this study, pigment samples were collected twice during the day (predawn and midday) on leaflet pairs from a total of 10 plants. It is possible that this sampling protocol was insufficient to detect subtle diurnal changes in flavonoids in the plants grown and measured under solar UV (+UV plants). Moreover, our pigment data from *Vicia* were derived from whole-leaf extracts, and these results may not be indicative of changes occurring within the epidermis. While flavonoids and other related UV-absorbing compounds are generally concentrated in epidermal tissue, they do also occur in mesophyll and vascular tissues (Grammatikopoulos et al. 1999, Reuber et al. 1996). Nevertheless, previous studies have shown that T_{UV} is generally strongly correlated with changes in whole-leaf UV-absorbing pigments, at least when plants are grown under a variety of UV exposures (Barnes et al. 2000). In addition, the studies by Veit et al. (1996) documenting diurnal changes in flavonoid levels were based on whole-leaf extracts. It is known that chalcone synthase, a key enzyme in the flavonoid biosynthetic pathway, exhibits diurnal changes in activity, at least under artificial light conditions in laboratory-grown plants (Peter et al. 1991). The fact that we observed increases in UV-absorbing pigments in plants of *V. faba* grown in the absence of solar UV and then transferred to a UV treatment indicates that these plants were physiologically capable of rapidly increasing their UV pigmentation

levels. It is conceivable that the midday decrease in T_{UV} of the +UV plants was the result of interconversions between different pools of UV-absorbing compounds (e.g. vacuolar vs membrane bound; Schnitzler et al. 1996) and not necessarily changes in flavonoid synthesis and degradation. Clearly, further studies are needed to fully assess what linkages exist between diurnal variation in leaf chemistry and epidermal UV transmittance.

Regardless of the underlying mechanism responsible for these changes in T_{UV} , it is likely that even modest increases in UV shielding could significantly reduce UV-induced damage to DNA or other chromophores that might occur on a daily basis (Rousseaux et al. 1999, Stapleton et al. 1997). Even if insufficient to entirely eliminate UV damage, these diurnal responses may be adequate to enable repair mechanisms to effectively mitigate against the transient effects of high UV exposure (Britt 1996). In addition to the changes in T_{UV} /flavonoids, other related cellular processes and metabolites have also been reported to show diurnal changes in higher plants. For example, a variety of antioxidant compounds, including flavonoids, can provide some degree of protection from free radicals formed by UV-B (Jordan 2002). In spruce (*Picea abies* L.), the antioxidant glutathione undergoes a substantial light-dependent diurnal rhythm, which peaks during midday (Schupp and Rennenberg 1988). Several antioxidants displayed a similar diurnal pattern in two alpine Asteraceae species (Wildi and Lütz 1996). There were also indications of increased midday concentrations of chlorophylls and carotenoids in these species (Wildi and Lütz 1996). In addition, the photo-repair of UV-B-induced DNA damage by cyclobutane pyrimidine dimer DNA photolyase (CPD photolyase) has been shown to increase during simulated day time periods in *Cucumis sativus* (Takahashi et al. 2002), and diurnal movements of chloroplasts appear to be a protective response to reduce the potential of photoinhibition in *Arabidopsis thaliana* (Kasahara et al. 2002). Thus, the diurnal changes in T_{UV} that we describe here may constitute one component of a larger integrated response system in plants to protect themselves against the detrimental effects of short-term exposures to high solar UV and visible radiation.

Finally, although our studies were conducted on exotic species in the Hawaiian subalpine, we have no reason to believe that similar diurnal responses in T_{UV} do not occur in native species. In the present study, we chose study species that were common, were relatively easy to measure (i.e. possessed large green leaves) and were easy to grow in this cool, high-elevation environment (e.g. *V. faba*). A number of the native alpine/subalpine plant species on Mauna Kea possess small leaves or leaves that are highly reflective or reddish in coloration. Thus, some

of these species are unsuitable for measurements of epidermal UV transmittance using chlorophyll fluorescence (see above). In the native Hawaiian alpine/subalpine species in which we can readily measure T_{UV} using this technique, we have found no indication that midday (minimum) epidermal UV transmittances differ between native and exotic species (Ryel and Barnes, unpublished data). However, we have found species (both native and exotic) that do exhibit lower overall values of adaxial T_{UV} (<5%) than the plants used in this study (T_{UV} = 5–10%). In these species with high baseline (predawn) levels of UV shielding, it may be more difficult to detect diurnal changes in epidermal transmittance. Also, while the majority of our studies were conducted in the high solar UV environments in Hawaii, we also found diurnal changes in T_{UV} for *V. faba* growing in Logan, Utah, where ambient effective solar UV-B irradiances were 40–50% less than at our field site in Hawaii. Thus, there is no indication that diurnal changes in epidermal UV transmittance are restricted to plants growing in extreme solar UV environments, as occurs in the tropical subalpine/alpine. Nonetheless, we do not yet know how widespread this phenomenon is among terrestrial plants and to what degree species may vary in their ability to modulate their UV shielding capacities.

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