Chapter 6: Plant responses to fluctuating UV environments

Paul W. Barnes[1*], T.M. Robson[2], M.A. Tobler[1], I.N. Bottger[1] and S.D. Flint[3]

[1]Department of Biological Sciences and Environment Program, Loyola University New Orleans, 6363 St. Charles Avenue, New Orleans, Louisiana 70448, USA;

[2]Department of Biosciences, Plant Biology Division, University of Helsinki, PO Box 65, Helsinki, Finland;

[3]Department of Forest, Rangeland and Fire Sciences, UIPO 441135, University of Idaho, Moscow, Idaho 83844-1135, USA.

*Author for correspondence: Email: pwbarnes@loyno.edu; Phone: +1 504.865.2008

6.1 Introduction

The solar ultraviolet (UV; ~280-400 nm) radiation regime experienced by plants in nature varies across multiple time scales (inter-annual, seasonal and diurnal). Long-term (year-to-year) variability in UV irradiance at the Earth's surface in modern times is driven largely by changes in stratospheric ozone, which influences the attenuation of ultraviolet-B radiation (UV-B; 280-315 nm), and climate change, which can alter both UV-B and ultraviolet-A radiation (UV-A; 315-400 nm) via changes in cloud cover, aerosols and tropospheric ozone (Bais et al., 2015, Bornman, 2017). Variation in incident UV over shorter time frames (seasonal and diurnal) results primarily from the natural rhythms in prevailing solar angles that occur over a year or day though intra-seasonal fluctuations in ozone can play a significant role in certain regions (Madronich et al., 2011, Bais et al., 2015). These natural cycles in UV irradiance are routinely disrupted by changes in atmospheric conditions and vegetative cover, which themselves can exhibit some degree of seasonal and diurnal periodicities (e.g., pronounced rainy (monsoonal) seasons and phenological patterns in canopy development; Fig. 6.1A). Rapid fluctuations in solar UV can also occur via changes in cloud cover, which can either increase or decrease UV irradiance depending on the position and type of clouds in relation to the solar disk (Fig. 6.1B, C; Thiel et al. (1997), Lopez et al. (2009)), as well as gaps in the canopy that create periodic sunflecks in the understory (Fig. 6.1D; Flint and Caldwell (1998), Heisler et al. (2003), Aphalo (2017)). Consequently, plants and individual leaves can experience considerable change in UV exposure over their life spans, from one day to the next, and within a given day that is both regular and, at times, erratic and unpredictable.

Over the past several decades, considerable attention has been given to understanding plant responses to changes in average UV conditions that occur as a result of stratospheric ozone depletion and to the interactive effects of enhanced UV-B and other climate change factors (e.g., elevated CO_2 and increased temperature; Björn (2015), Bornman et al. (2015) and references therein). By comparison, we know far less about plant responses to changes in solar UV fluxes that occur 1) naturally over the course of a given growing season or day, 2) in response to changes in cloud cover, or 3) as leaves alternate between shaded and sunlit conditions within canopies and in understory environments.

Given that plants have evolved a photosensory system to sense ambient UV-B (Rizzini et al., 2011, Jenkins, 2017) and that this photoreceptor appears to interact with other photoreceptor systems (e.g., cryptochromes and phytochromes) to provide information to a plant about its light environment (Xie and Hauser, 2012, Tilbrook et al., 2013, Mazza and Ballaré, 2015), it is relevant to ask how quickly and to what degree plants can respond to changes in their UV environment, and what these responses might mean for plant fitness. For instance, do rapid fluctuations in UV (seconds to minutes) in fact matter to plants or do they integrate and respond to UV over much longer time frames (hours, days or weeks)? Also, is the rate and magnitude of the response to changing UV conditions contingent upon developmental stage (e.g., young vs. old leaves) and environmental factors that influence resource availability and levels of physiological stress (e.g., atmospheric CO_2 concentration, temperature and moisture availability)? Finally, what is the adaptive significance of variation among plant species in their abilities to respond to temporal fluctuations in UV and what might these differences

mean for the timing of defenses against other abiotic (e.g., drought) and biotic (e.g., herbivory) factors that share commonalities in photosensory signaling (Demkura and Ballaré, 2012) and biosynthetic pathways (Izaguirre et al., 2007) with UV responses?

In this chapter, we review research to date on what is known about the prevalence and mechanisms of plant responses to short-term fluctuations in UV exposure and explore the possible adaptive and ecological significance of these responses. We address only changes in total UV irradiances and not shifts in spectral quality, which can be associated with fluctuating UV conditions particularly in understory environments (Parisi and Kimlin, 1999). Our emphasis is on physiological responses to UV, and we specifically focus on UV screening protection as this is a primary avenue of UV acclimation and one of the most frequently documented and readily observable responses to UV in plants (Searles et al., 2001).

6.2 Overview of plant responses to UV and the time course of UV acclimation

Abundant evidence from studies conducted in controlled environmental conditions indicates that exposure to UV can elicit specific photomorphogenic responses in plants as well as induce a variety of effects that are generally considered to be detrimental to plant function (Jansen and Bornman, 2012, Li et al., 2013, Robson et al., 2015). The highly energetic shorter wavelengths of UV (i.e., UV-B) are particularly efficient at producing a number of deleterious effects in plants, including disruption of the integrity and function of important macromolecules (DNA, proteins and lipids), oxidative damage, partial inhibition of photosynthesis and growth reduction (Strid and Hideg, 2017). To combat these adverse effects, plants have developed a suite of

biochemical, physiological and morphological mechanisms that collectively protect or repair sensitive targets from direct and indirect UV-induced injury (Britt, 1999, Favory et al., 2009, Hectors et al., 2009, Jacques et al., 2009, Schreiner et al., 2017). This acclimation to UV appears sufficient to largely minimize any detrimental effects of UV-B on plant growth and productivity when plants are grown under ambient or realistically enhanced UV-B in the field (Ballaré et al., 2011) such that photomorphogenic (and often beneficial) effects of UV generally predominate under these conditions (Wargent and Jordan, 2013, Ballaré and Austin, 2017).

One of the most important and widespread protective responses of plants to UV radiation involves the induction and synthesis of flavonoids, hydroxycinnamic acids (HCAs) and other related phenylpropanoid compounds that function as "UV sunscreens" and antioxidants (Caldwell et al., 1983, Agati et al., 2012, Emiliani et al., 2013). The accumulation of flavonoids and other UV-absorbing compounds in epidermal tissue decreases epidermal UV transmittance (T_{UV}) but has minimal effect on attenuating photosynthetically active radiation (PAR; 400-700 nm) needed for photosynthesis of underlying mesophyll tissue (Fig. 6.2; Day et al. (1994), Mazza et al. (2000), Bidel et al. (2007)). The epidermal screening of UV is a primary avenue by which plants acclimate to changing UV environments, including alterations resulting from stratospheric ozone depletion and climate change (Williamson et al., 2014, Bornman et al., 2015). This UV acclimation response entails a measurable energetic and fitness cost (Snell et al., 2009, Guidi et al., 2011, Hofmann and Jahufer, 2011), varies within and among species (Day et al., 1992, Qi et al., 2010, Randriamanana et al., 2015) and is linked with cross-tolerance

to other abiotic and biotic stresses (e.g., drought, herbivory and pathogen infection; Mewis et al. (2012), Bandurska et al. (2013), Zavala et al. (2015)).

Flavonoid biosynthesis is influenced by UV-B as well as UV-A, PAR (Flint et al., 2004, Siipola et al., 2015) and other environmental factors, such as temperature (Bilger et al., 2007). Orchestration of UV-B-induced flavonoid biosynthesis appears to involve the UV-B photoreceptor UV RESISTANCE LOCUS 8 [UVR8] (Jenkins, 2014) with UV-B exposure leading to the expression of UVR8-dependent gene transcripts involved in phenylpropanoid metabolism (Morales et al., 2013). While exposure to UV-B has been shown to induce rapid (within minutes) activation of UVR8 (Kaiserli and Jenkins, 2007), the production and accumulation of UV-absorbing compounds and resultant decrease in T_{UV} typically occurs over much longer time frames (i.e., days; Fig. 6.3A; Hectors et al. (2014), Bidel et al. (2015), Wargent et al. (2015)). During this period of acclimation, leaves may be susceptible to UV-induced injury (e.g., inhibition of photosynthesis; Wargent et al. (2015)). Prolonged exposure to UV-B eventually leads to reduced sensitivity to UV and may even enhance photosynthetic performance and provide some protection against photoinhibition (Wargent et al., 2011, Wargent et al., 2015).

The epidermal UV transmittance of leaves is a highly plastic trait and within a given species can vary from <5% to near 100% depending upon the radiation environment (UV and PAR) experienced during leaf development (Fig. 6.3A, B). Thus, leaves that are produced in low UV and/or low PAR environments, such as those in deep shade in canopies or understory environments, or in glasshouses that lack UV, typically display limited UV screening capabilities (Fig. 3A,B; Krause et al. (2003a), Agati et al. (2008), Pollastrini et al. (2011), Barnes et al. (2013)). However, the relationship between

UV shielding and the light (UV and PAR) regime is strongly non-linear such that leaves that develop under even rather moderate shade can still exhibit relatively high UV protection (low T_{UV}; Fig. 6.3A-inset; 6.3B). For leaves that do develop under deep shade or very low UV they can be particularly susceptible to UV-induced injury if they suddenly encounter high UV environments. This would occur for certain vegetable crops that are propagated as seedlings in glasshouses or shade structures before being transplanted to the field (Wargent, 2017), shade leaves within plant canopies that are exposed to full sunlight as a result of canopy gaps or perturbations due to herbivory, wind (Fig. 6.1D) or other factors (Kolb et al., 2001, Barnes et al., 2013), and understory plants exposed to periodic sunflecks (Krause et al., 2003b).

6.3 Responses to sun-shade transitions

As indicated above, the ability of leaves to adjust their flavonoid levels and UV shielding in response to UV exposure during leaf development is well established. What is less clear, however, is how pliable these UV optical properties are once leaves have matured and developed under one set of conditions and are then confronted with rapid changes in these conditions. Some studies suggest that mature leaves or leaf segments are generally unresponsive in their UV-sunscreen capacities when exposed to sudden changes in environmental conditions such as temperature (Nybakken et al., 2004b, Bilger et al., 2007). Other studies however have shown that the UV-screening response can be more flexible in response to changes in the light environment (Krause et al., 2007, Agati et al., 2011, Morales et al., 2011, Bidel et al., 2015). For example, field studies with fully developed sun and shade leaves of *Populus tremuloides* (aspen) and *Vicia faba* (fava

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bean) have shown that there can be considerable flexibility in UV acclimation in mature leaves, at least for those produced under low light conditions (i.e., shade leaves; Fig. 6.3C). Specifically, shade leaves were shown to decrease T_{UV} when transferred to sun environments, but sun leaves did not change their UV sunscreen protection when suddenly exposed to shade conditions (Barnes et al., 2013). Although increases in UV shielding were detected 1-2 days following the transfer of plants from shade to full sun conditions, full transition of shade leaves to the equivalent protection of sun leaves required 4-10 days depending on species and conditions. These changes in leaf optical properties were generally associated with increases in UV-absorbing compounds. Similarly Krause et al. (2004) showed that mature leaves of tropical understory plants can also respond to abrupt increases in solar UV radiation by increasing UV-absorbing compounds. In the case of *P. tremuloides* and *V. faba*, this adjustment in epidermal UV transmittance of shade leaves required both UV-B and UV-A, whereas PAR and UV were involved in the establishment of UV sunscreen protection during leaf development (Barnes et al., 2013). Similarly, Bidel et al. (2015) showed that UV-B was required to induce additional UV shielding in mature leaves of *Centella asiatica*. Thus, relatively high levels of PAR appear to provide a high foundation of UV-protection, which then is further enhanced by UV exposure (Götz et al., 2010, Wargent et al., 2015).

6.4 Seasonal and diurnal changes in UV-sunscreen protection

6.4.1 Occurrence and patterns among species and environments

The above studies indicate that mature leaves can, in some cases, respond, albeit relatively slowly, to sudden changes in their UV environment. Additionally, there is now

growing evidence that fully developed leaves can modulate their levels of UV sunscreen protection on more rapid time scales such as one day to the next or over the course of an individual day. The earliest report suggesting that plants may be capable of rapid adjustment in UV-screening came from observations by Lautenschlager-Fleury (1955) who found that the UV-B transmittance of epidermal peels from *Vicia faba* leaves was low during midday on a sunny day but remained relatively high on a cloudy day. Subsequently, Sullivan et al. (2007) detected significant day-to-day changes in UV-absorbing compounds in field-grown *Hordeum vulgare* (barley) and *Glycine max* (soybean) and this variation was correlated with variation in ambient UV-B in both species and UV-A in soybean. This variability was dampened in plants grown under reduced (near 90% reduction) levels of UV-B and no significant relationship was found between variation in solar UV and UV-screening compounds in these plants. By comparison, Kotilainen et al. (2010) found no clear relationships between UV doses and seasonal variation in flavonoids and other phenolics in *Alnus incana* (alder) and *Betula pubescens* (birch) leaves. Other investigators have also shown that flavonoids and epidermal UV screening can vary over the course of a season (Fischbach et al., 1999, Liakoura et al., 2001, Louis et al., 2009, Nenadis et al., 2015) though it is sometimes difficult to assess whether this variation is the result of UV acclimation during leaf development as opposed to rapid adjustment in fully developed leaves, especially in species that produce long-lived leaves and multiple leaf cohorts/season.

The first evidence that plants could adjust levels of UV photoprotection on a diurnal basis came from field studies conducted by Veit et al. (1996) who found that the concentration of flavonoids from whole-leaf extracts of *Anacardium excelsum*, a tropical

tree, and *Cryptogramma crispa*, an alpine fern, increased progressively during the

morning and then declined in a similar fashion in the afternoon in both species. More

recently, Barnes et al. (2008) used chlorophyll fluorescence (UVA-PAM; Kolb et al.

(2005) to non-invasively measure epidermal UV-A shielding and reported small (ca. 1-

2% change in absolute T_{UV}; 13-16% change in relative T_{UV}), but statistically significant

diurnal changes in epidermal UV transmittance in three plant species (*Vicia faba*,

Oenothera stricta and *Verbascum thapsus*) growing in a high-UV tropical alpine

environment in Hawaii. Following this study, Barnes et al. (2016a) surveyed 37 species

(63 taxa of wild and cultivated species with multiple cultivars/species for several

cultivated species) growing in different locations (Hawaii, Utah, Idaho and Louisiana,

USA) and found that diurnal change in T_{UV} occurred in nearly half of the species

examined. Diurnal changes in T_{UV} were found in plants at all locations, in monocots as

well as dicots and in both herbaceous and woody growth forms. Species that did not

exhibit diurnal change in T_{UV} included grasses and dicots of cultivated, wild and exotic

species (e.g., *Symphoricarpus albus*, *Zea mays*, *Phalaris arundinacea* and *Malva*

parviflora), and in some cultivated species (*Brassica rapa*, *Triticum aestivum, and*

Citrullus lanatus) there was significant intraspecific variation in the prevalence of this

phenomenon. Diurnal changes in flavonoid levels have also been observed in *Centaurea*

uniflora and *Geum montanum*, two herbaceous alpine species growing in the French

Alps, using similar non-invasive techniques (i.e., Dualex; Goulas et al. (2004); T. Robson

and S. Hartikainen, unpubl. data; Fig. 6.4). Thus, diurnal adjustment in UV sunscreen

protection appears not limited to species in extreme UV environments but is in fact

widespread among higher plants. However, at present there appears to be no apparent

functional or ecological distinction between diurnally 'responsive' vs. 'unresponsive'
species.

Results from the above survey study further revealed that significant interspecific
variation existed in the magnitude of these diurnal changes in epidermal UV
transmittance. Certain species, such *Abelmoschus esculentus* (okra) displayed large (62%
relative change) decreases in T_{UV} from dawn to midday (absolute $\Delta T_{UV} = 16.1\%$; Fig.
4A) whereas others, such as *Typha latifolia*, showed minimal diurnal changes in T_{UV} of
over the day ($\Delta T_{UV} < 1\%$). Across taxa and locations, the largest diurnal changes in T_{UV}
were found for plants growing in locations with warm nights (i.e., Louisiana). Low
temperatures are known to induce the production of flavonoids (Neugart et al., 2013) and
decrease T_{UV} (Bilger et al., 2007), such that many plants growing in cold, high-elevation
or high-latitude sites often exhibit high constitutive levels of UV sunscreen protection
(Barnes et al., 2000, Nybakken et al., 2004a, Albert et al., 2009). The small diurnal
changes in T_{UV} observed in plants from cooler locations (temperate latitudes and high
elevations) may thus be a consequence of low temperature effects on UV-absorbing
compounds, which then restricts the range of diurnal transmittances possible in these
species. Interestingly, across all taxa and locations the magnitude of ΔT_{UV} and the daily
minimum value of T_{UV} (midday values) were not correlated with daily UV fluxes or the
amplitude of diurnal changes in solar UV-B irradiance. The magnitude of the diurnal
changes in epidermal UV transmittance therefore appears to be governed more by
nighttime values of T_{UV} than those at midday. Collectively, these findings suggest that
the largest diurnal changes in T_{UV} will likely occur in plants inhabiting warm subtropical
and tropical environments.

6.4.2 Mechanisms and drivers

Although diurnal adjustment in UV shielding appears common among plant species, the underlying mechanisms and drivers of these rapid responses remain unclear. Direct transmittance measurements made on epidermal peels of *V. faba* have confirmed that the rapid changes in T_{UV} measured using chlorophyll fluorescence are indeed the result of changes in the optical properties of the epidermis (Barnes et al., 2016b). These diurnal alterations in T_{UV} are further associated with changes in the levels of whole-leaf UV-absorbing compounds in responsive species (e.g., *A. esculentus* and *Solanum lycopersicum*) but this is not the case for unresponsive species (e.g, *Zea mays*; Barnes et al. (2015), Barnes et al. (2016b)).

How plants actually achieve these rapid and reversible changes in UV-screening and flavonoids remains is not known. As indicated above, the induction and accumulation of UV-absorbing compounds and resultant decreases in T_{UV} typically occur over time frames (days) considerably longer than these rapid changes (Hectors et al. 2014; Wargent et al. 2015). The relocation of flavonoids among different pools in leaf tissues (Schnitzler et al., 1996, Burchard et al., 2000), the rapid alterations in absorptive properties of individual compounds (e.g., Dean et al. (2014)) and/or the UV-induced conversion of phenylpropanoid structures (e.g., Boulton, 2001) would seem more likely mechanisms to account for the rapid changes in these UV-sunscreens than *de novo* synthesis and degradation of these compounds. Indeed, relatively rapid changes (<1 day) in the flavonoid composition of leaves have been observed in several plant species. For example, after one day of moderate UV-B exposure, Neugart et al. (2012) found that

leaves in juvenile plants of *Brassica oleracea* showed a number of structurally-dependant changes in flavonol (quercetin and kaempferol) glycosides with some compounds increasing and others declining. Similarly, Barnes et al. (2016b) found significant diurnal shifts in the foliar composition of quercetin glycosides in *A. esculentus* with low molecular weight compounds changing to a greater degree than higher weight compounds. Because of the nature of these studies there was no way to ascertain the precise cellular location of these compounds and thus it is unclear whether these compounds function primarily as UV sunscreens or as antioxidants.

It is conceivable that this diurnal change in UV-shielding represents some aspect of an endogenous circadian rhythm in plants (McClung, 2001). Indeed, diurnal rhythms in gene expression, metabolites and the activities of key enzymes involved in phenylpropanoid biosynthesis (e.g., chalcone synthase) are known to occur (Peter et al., 1991, Kim et al., 2011), and the circadian clock in *Arabidopsis thaliana* appears to interact with UVR8-controlled UV-B signaling (Feher et al., 2011). Whether these rapid UV-shielding responses are mediated all or in part by the UV-B photoreceptor UVR8 is unknown at present. Several studies have, however, demonstrated that manipulation of the light regime can significantly influence these diurnal changes. For example, Barnes et al. (2008) showed that the imposition of dense shade at different times of the day could effectively eliminate diurnal changes in T_{UV} in *Verbascum thapsus*, and conversely, the removal of shade caused T_{UV} to revert to "normal" conditions within a matter of minutes. Subsequent studies with *A. esculentus* showed that the diurnal adjustment in T_{UV} in this species could be reduced by ca. 50% when plants were placed under plastic film that attenuated much of the ambient solar UV (Barnes et al. 2016). Also, the diurnal changes

in UV-shielding in this species and others are usually less pronounced on cloudy than sunny days and for *A. esculentus* there is a strong negative correlation between T_{UV} during the day and incident UV-B and PAR when data from clear and cloudy days are combined (Barnes et al., 2016b). Field filter experiments by Robson and Hartikainen (unpubl.) have also shown that diurnal change in epidermal flavonoids in *Centaurea uniflora* was not detectable when plants were kept in continuous darkness or when UV-B or UV-B + UV-A was filtered out of sunlight (Fig. 6.4). Removing blue and UV radiation produced a diurnal pattern somewhat different than that of plants experiencing full sunlight. These findings are consistent with those of Veit et al. (1996) who reported that removal of solar UV-B eliminated the diurnal changes in flavonoids in their study species and the studies of Sullivan et al. (2007) who found a significant positive correlation between day-to-day variation in UV-absorbing compounds and ambient solar UV-B. Thus, it appears that these rapid changes in leaf optical properties represent a specific response to sunlight (UV and perhaps visible) though some involvement with the circadian clock has not been ruled out (e.g., Atamian et al. (2016)).

6.4.3 Costs, benefits and ecological significance

Irrespective of mechanisms, these diurnal changes in UV shielding likely provide clear benefits to plants in UV protection, at least when compared to hypothetical situations where low predawn levels of UV shielding remain unchanged throughout the day (Fig. 6.5). However, the benefits of diurnal adjustment in UV protection relative to plants that maintain consistently high (i.e., midday equivalent) levels of UV protection over the day are less clear. In comparison to these kinds of plants (e.g., *Z. mays*), model

calculations suggest that plants that exhibit diurnal adjustment in UV shielding (e.g., *A. esculentus*) may experience increased penetration of UV to the underlying mesophyll both in the morning and afternoon but not at midday. This is likely due to the time lag between solar UV change and that of T_{UV}. It is conceivable that increased penetration of UV to photosynthetic tissue at these times of day may protect leaves from photoinhibition (Wargent et al., 2015) that can occur under high irradiances in the middle of the day. However, studies on *V. thapsus* have shown no correlation between T_{UV} and photochemical efficiency (F_v/F_m) when leaves encounter sudden changes in light regime (Barnes et al., 2008). There is evidence that UV-A can drive photosynthesis (Turnbull et al., 2013) and increased penetration of UV-A might thereby increase photosynthesis at times of the day when leaves are light-limited (mornings and afternoons). If this is the case, elimination or reduction in diurnal change in T_{UV} could potentially reduce plant carbon gain and growth. Finally, there is the possibility that maintaining constant high levels of flavonoids might interfere with plant growth during the night. Several of the flavonoids induced by UV (e.g., quercetin and kaempferol) are known to interfere with auxin metabolism and transport (Ringli et al., 2008, Kuhn et al., 2011), which in turn could influence plant growth and morphology (Hectors et al., 2012). If this is the case, the benefits of diurnal cycling in UV shielding may lie not in UV protection but rather in other functional roles of flavonoids.

As an initial test to assess the costs and benefits of diurnal changes in UV sunscreen protection we compared the effects of attenuating ambient solar UV on the growth and morphology of two species (*A. esculentus* and *Capsicum annuum*) that differed in their UV protection "strategies". Although statistically significant diurnal

adjustments in T_{UV} were evident in both species, this change was much greater in *A. esculentus* than in *C. annuum* (Fig. 6.6A). In addition, overall levels of UV screening protection were higher and less affected by UV exclusion in *C. annuum* than *A. esculentus* (Fig. 6.6B). When comparing shoot growth and morphology, it appeared that *C. annuum* was influenced by UV exclusion to a greater degree than *A. esculentus* (Table 6.1). Thus, the species that was more flexible in its UV screening (*A. esculentus* in this case) was less affected in its growth by UV. Whether these differences in morphological sensitivity to UV are due to these differences in UV screening is difficult to assess, as these species likely differ in other aspects of UV protection (e.g., DNA damage repair, canopy architecture and others), which were not examined in this study. Nonetheless, these findings do suggest that, at the very least, there are no clear negative consequences in terms of UV effects on growth and production for species exhibiting diurnal changes in UV shielding. Additional study is needed to determine if there is any general association between flexibility in UV screening protection and sensitivity to UV-induced alterations in growth and morphology.

Diurnal fluctuations in UV sunscreen protection may also have consequences for the timing of plant responses to other abiotic and biotic stresses (e.g., drought and herbivory) that can vary in severity over the course of a day (e.g., Goodspeed et al. (2012)) and which, in some cases, employ similar suites of secondary compounds for both defense and UV protection (Kuhlmann and Müller, 2010, König et al., 2014). For example, are plants that exhibit diurnal changes in flavonoids more susceptible to attack by herbivores or pathogens early and late in the day than in the middle of the day? No studies to date have explored this possibility but understanding how UV protection

interacts with these, and other physiological and ecological functions, may be required to fully evaluate the costs and benefits associated with "static" vs. "dynamic" UV protection strategies in plants.

Table 6.1. Effects of ambient solar UV on the growth, morphology and midday epidermal UV transmittance (T_{UV}) in *Capsicum annuum* (pepper) and *Ablemoschus esculentus* (okra). Data are expressed as % difference of mean attenuated – mean ambient UV with ambient UV values as the base. Plants in the ambient UV treatment were grown under UV-transparent film (aclar) whereas plants in the attenuated UV treatment were grown under clear film that did not transmit UV-B or UV-A radiation (llumar). Significant treatment differences at $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$(***) as determined by ANOVA; ns = not significant at $P>0.05$. N = 24 per species and treatment (I. Bottger, M. Tobler and P. Barnes, unpubl. data).

Parameter	*Capsicum annuum* % difference	*P*	*Abelmoschus esculentus* % difference	*P*
Shoot height	-10.2	*	-4.4	ns
Internode length	-38.3	**	-16.8	*
Leaf area	-22.6	*	2.4	ns
Leaf dry mass	-17.4	ns	2.8	ns
Leaf mass/area	5.7	ns	1.3	ns
Shoot dry mass	-11.2	ns	5.0	ns
Midday T_{UV}	19.2	***	71.9	***

6.5 Practical considerations

   The existence of a temporally dynamic UV sunscreen protection system in plants has a number of important practical implications for how plant UV research is conducted and culturing plants in controlled environments when UV-B (and UV-A) is employed to enhance food plant quality and vigor (Schreiner et al., 2012, Wargent, 2017). For research aimed at quantifying the effects of UV radiation on flavonoids and UV protection it is important that the time of day (and time of year) when samples are collected or leaves are measured be standardized and recorded. For field-grown plants, determination of the seasonality of flavonoid levels should be assessed and sample collections and measurements should then be done at midday during the seasonal peak to provide the best measure of maximal levels of UV protection. As noted by Kotilainen et al. (2010) this seasonal maximum may well differ for different species that are sympatric. Care should also be taken to collect samples/data under similar sky conditions. When plants are grown in controlled environments (growth chambers or greenhouses) artificial sources of UV are typically employed (e.g., UV fluorescent bulbs or LEDs). While it is not yet clear whether plants grown in these environments exhibit "typical" diurnal patterns in UV shielding, it is well known that plants are more susceptible to UV injury in these environments than those grown in the field (Caldwell and Flint, 1994). It is conceivable that this heightened sensitivity to UV, at least for some species, may well be the result of diminished UV sunscreen protection when natural diurnal cycles in UV radiation are muted or absent in controlled environments. In these environments it may be necessary to develop artificial lighting and UV exposure systems that promote natural

diurnal (and seasonal) adjustments in UV screening so as to promote the beneficial

effects of UV-B while avoiding excessive UV injury.

6.6 Summary and conclusions

The epidermis of leaves is a selective filter of solar radiation—absorbing much of

the potentially detrimental solar UV while transmitting visible wavelengths (PAR) that

drive photosynthesis in the underlying mesophyll. The epidermis is also a variable UV

filter and the increase in the concentration of UV-absorbing compounds (flavonoids and

related phenolics) and resultant decrease in epidermal UV transmittance represents a

primary mechanism by which plants acclimate to changing UV environments.

Understanding the nature and limitations of this acclimation response is fundamental to

evaluating the ecological and agronomic significance of variation in solar UV that occurs

over multiple temporal scales (inter-annual, seasonal and diurnal).

Results from a number of relatively recent studies conducted on diverse cultivated

and wild plant species reveal that this UV protective mechanism is much more dynamic

and flexible than previously thought. While the mechanistic underpinnings of this

temporal variation is relatively clear in some cases (e.g., the adjustments of UV shielding

and flavonoid levels over ontogenetic time) the basis of other responses (e.g., diurnal

changes in UV shielding) are less understood. To date, most studies on rapid responses

to UV have focused on herbaceous plants that are adapted to high light environments

(i.e., heliophytes); less attention has been given to exploring the temporal dynamics of

UV protection in forest understory species (i.e., sciophytes) that are adapted to survive in

low light environments but which routinely encounter brief periods of intense UV and

PAR in sunflecks. Additional studies are also needed to evaluate the adaptive significance of rapid modulation in UV shielding in plants and the potential implications of a dynamic UV protection system for other abiotic (drought, nutrient limitation and temperature extremes) and biotic factors (herbivore and pathogen protection) that are cross-linked to this UV protection response.

6.7 Acknowledgements

6.8 References

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Figure 6.1. Variability in ultraviolet-B irradiance at multiple time scales. Panel A shows integrated daily plant effective UV-B over three years at a Sonoran Desert location in southern Arizona, USA, with the annual summer monsoon period noted. Panel B shows daily plant effective UV-B at Pullman, Washington, USA over a period in early spring (April-early May 2014) when new leaves are emerging in many native plant species. Panel C show diurnal patterns of plant effective UV-B (30 min means) under clear and cloudy sky conditions in the Arizona Sonoran Desert in July 2015. Panel D shows instantaneous UV-B at the same location (to the nearest cm) in the understory of a 20-year old *Betula pendula* stand in a provenance trial at Little Wittenham, Oxfordshire, UK, on a windy and relatively calm day. All irradiance data are weighted according to the generalize plant action spectrum (Caldwell, 1971) and normalized to unity at 300 nm. Data in A and C were obtained from an on-site broad-band UV-B sensor (YES UVB-1) calibrated against a double-monochromator spectroradiometer (Optronics 756). Data in B were obtained from the USDA UV monitoring network (http://uvb.nrel.colostate.edu/UVB/index.jsf) using data from narrow waveband spectrometers to produce synthetic spectra. Data in D were obtained from a diode array spectroradiometer (OceanOptics Maya Pro2000+) measuring spectral irradiance every 0.1 seconds for a 10 second period.

Figure 6.2. Illustration of a typical dicot leaf showing the penetration of PAR (400-700 nm) and UV (280-400 nm) radiation through the epidermal tissue. The majority (ca. 90% of the incident UV is absorbed by flavonoids and other UV-absorbing compounds in the epidermis while most of the PAR is transmitted to the underlying mesophyll where

photosynthesis occurs. The epidermal UV transmittance (T_{UV}) of the epidermis varies over time in relation to the stage of leaf development, UV and PAR exposure and, in some species, time of season, day and cloud cover.

Figure 6.3. The response of adaxial epidermal UV transmittance (T_{UV}; measured with a UVA-PAM) to different light environments in pot-grown *Arabidopsis thaliana* (Panel A) and field-grown *Populus tremuloides* (aspen; Panels B, C). Panel A shows the time course of T_{UV} in *A. thaliana* grown in a growth chamber with no UV-B and then transferred to different conditions (indoor and outdoors) that differed in levels of UV-B (as measured with a calibrated broad band UV-B sensor). Inset shows the relationship between T_{UV} measured 5 days after transfer and relative UV irradiances. Panel B shows the relationship between adaxial T_{UV} and midday photon flux density (PFD) of PAR (400-700 nm) in *P. tremuloides* leaves occupying different canopy environments. Panel C shows the response of T_{UV} in sun and shade leaves of *P. tremuloides* subjected to different solar radiation treatments in the field. Data are means ±SE. Panel A is from an unpublished study by T. Atunes, M. Tobler, and P. Barnes; Panels B and C are reproduced from Barnes et al. (2013).

Figure 6.4. The absorbance of UV at 375 nm by leaf upper epidermal flavonoids (AU) in *Centaurea uniflora* as measured using chlorophyll fluorescence (Dualex Scientific+, Force-A, Paris). Measurements were taken on plants growing in a subalpine meadow (2130 m asl) in the French Alps during July 2015. Cylindrical filters (220 mm diameter × 300 mm height) made from special plastic films that completely attenuated either UV-

B radiation (polyester, 0.125 mm thick, Autostat CT5; Thermoplast, Helsinki, Finland); UV-A and UV-B radiation (Rosco #226 supergel, Westlighting, Helsinki, Finland); or blue, UV-A and UV-B radiation (Rosco 31~~2~~3 Canary Yellow) provided the filtration treatments, and were compared to a full-spectrum control (polyethene, 0.05 mm thick, 04 PE-LD; Etola, Jyväskylä, Finland) and a control in complete darkness (polyethene, solid white outside, solid black inside). Data are means ±SE change in absorbance of each leaf from its initial value (n=5 per filter type). * = means within a filter treatment significantly different at $P<0.05$ based on ANOVA (T.M. Robson and S.M. Hartikainen, unpubl. data).

Figure 6.5. Diurnal change in adaxial epidermal UV transmittance (T_{UV}; measured with a UVA-PAM) in *Abelmoschus esculentus* (okra; Panel A) and calculated plant effective UV-B irradiance directly beneath the epidermis (Panel B) under near-clear summer sky conditions in New Orleans, Louisiana, USA. T_{UV} data in Panel A are means ±SE. Panel B shows the diurnal course of calculated UV-B irradiance just beneath the adaxial (upper) epidermis for three scenarios: measured diurnal change in T_{UV} (circles), assuming constant dawn epidermal T_{UV} (squares) and assuming constant midday T_{UV} (triangles). Calculations assumed a horizontal leaf and no qualitative change in the UV-absorbance spectrum (Reproduced from Barnes et al. 2015).

Figure 6.6. Diurnal change (predawn to midday) in adaxial epidermal UV transmittance (T_{UV}; measured with a UVA-PAM) in pot-grown *Capsicum annuum* (pepper) and *Abelmoschus esculentus* (okra; Panel A) and midday T_{UV} in these species grown under

ambient (+UV) and attenuated (-UV) solar UV (280-400 nm) in New Orleans, Louisiana, USA (Panel B). Data are means ±SE (n=24). ** = means within a species significantly different at $P<0.01$ based on ANOVA (I. Bottger, M. Tobler and P. Barnes, unpublished data).
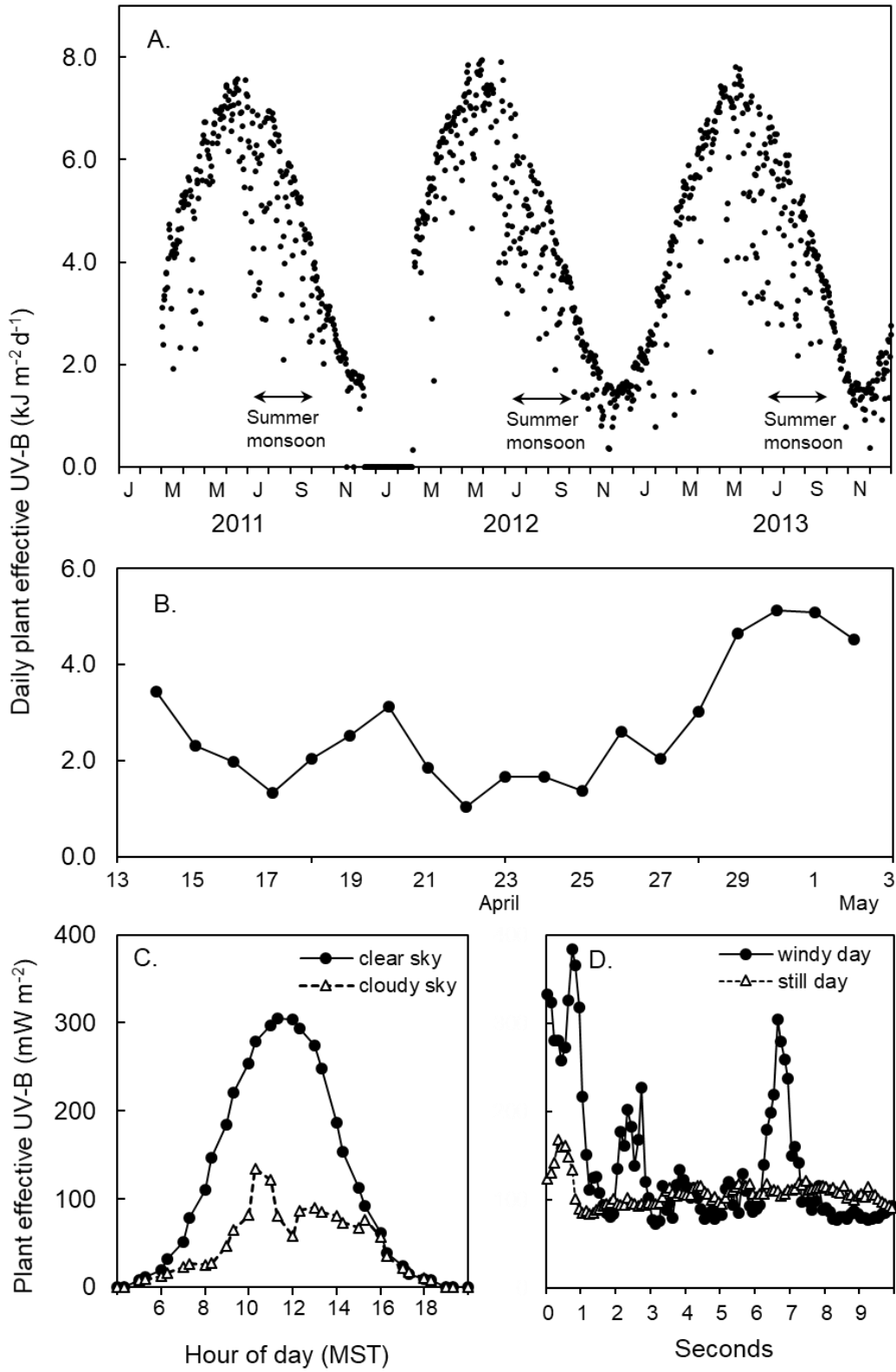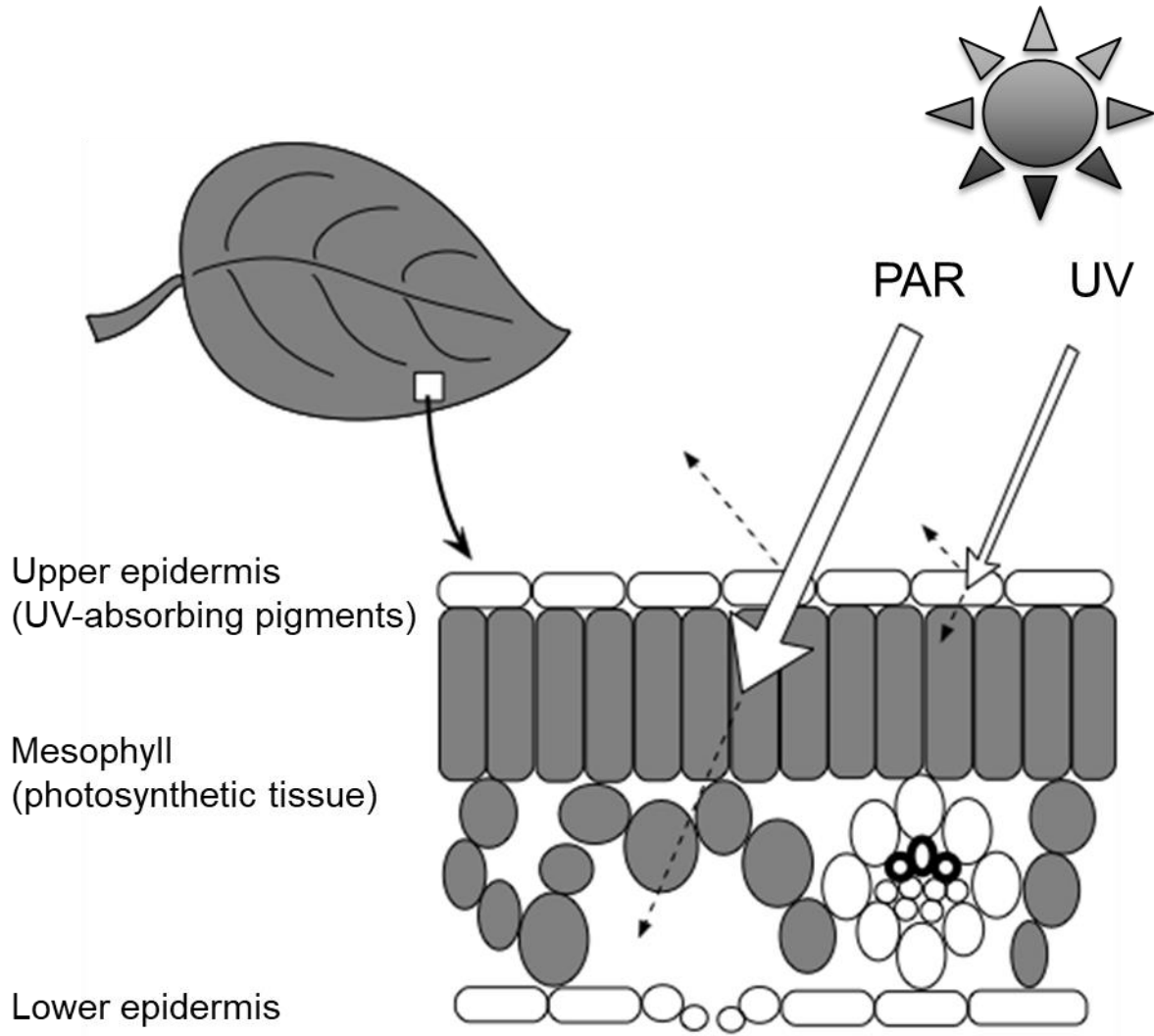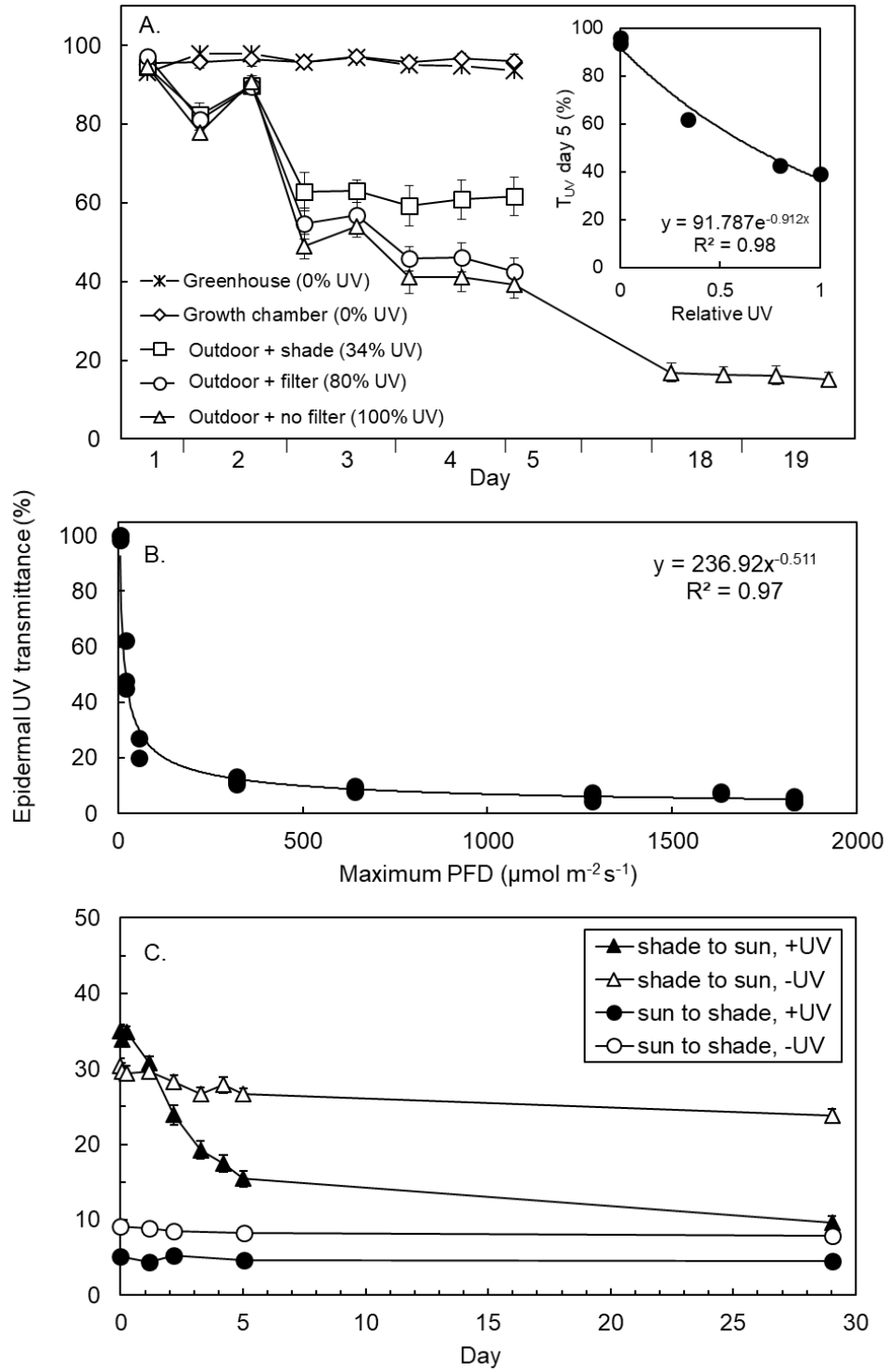
Fig 6.1

Fig 6.2

Upper epidermis
(UV-absorbing pigments)

Mesophyll
(photosynthetic tissue)

Lower epidermis

Fig. 6.3

A. (inset) $y = 91.787e^{-0.912x}$, $R^2 = 0.98$

- Greenhouse (0% UV)
- Growth chamber (0% UV)
- Outdoor + shade (34% UV)
- Outdoor + filter (80% UV)
- Outdoor + no filter (100% UV)

Inset axes: T_{UV} day 5 (%) vs Relative UV

B. $y = 236.92x^{-0.511}$, $R^2 = 0.97$

Epidermal UV transmittance (%) vs Maximum PFD ($\mu mol\ m^{-2}\ s^{-1}$)

C.
- shade to sun, +UV
- shade to sun, -UV
- sun to shade, +UV
- sun to shade, -UV

Day

Fig 6.4

Centaurea uniflora

Change in Dualex Flavonoids Abs.

Time of Day of Measurement

Fig. 6.5

Fig 6.6