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# UV radiation promotes flavonoid biosynthesis, while negatively affecting the biosynthesis and the de-epoxidation of xanthophylls: Consequence for photoprotection?

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ABSTRACT

There is evidence that UV radiation may detrimentally affect the biosynthesis of carotenoids, particularly de-epoxided xanthophylls, while strongly promoting phenylpropanoid, particularly flavonoid biosynthesis in a range of taxa. Here we tested the hypothesis that mesophyll flavonoids might protect chloroplasts from UV-induced photo-oxidative damage, by partially compensating for the UV-induced depression of xanthophyll biosynthesis. To test this hypothesis we grew two members of the Oleaceae family, *Ligustrum vulgare* L. and *Phillyrea latifolia* L., under either partial shading or fully exposed to sunlight, in the presence or in the absence of UV radiation. The examined species, which display very similar flavonoid composition, largely differ in their ability to limit the transmission of UV and visible light through the leaf and, hence, in the accumulation of flavonoids in mesophyll cells. We conducted measurements of photosynthesis, chlorophyll *a* fluorescence kinetics, the concentrations of individual carotenoids and phenylpropanoids at the level of whole-leaf, as well as the content of epidermal flavonoids. We also performed multispectral fluorescence micro-imaging to unveil the intra-cellular distribution of flavonoids in mesophyll cells. UV radiation decreased the concentration of carotenoids, particularly of xanthophylls, while greatly promoting the accumulation of flavonoids in palisade parenchyma cells. These effects were much greater in *L. vulgare* than in *P. latifolia*. UV radiation significantly inhibited the de-epoxidation of xanthophyll cycle pigments, while enhancing the concentration of luteolin, and particularly of quercetin glycosides. Flavonoids accumulated in the vacuole and the chloroplasts in palisade cells proximal to the adaxial epidermis. We hypothesize that flavonoids might complement the photo-protective functions of xanthophylls in the chloroplasts of mesophyll cells exposed to the greatest doses of UV radiation. However, UV radiation might result in adaxial mesophyll cells being less effective in dissipating the excess of radiant energy, e.g., by decreasing their capacity of thermal dissipation of excess visible light in the chloroplast.



## 1. Introduction

The effects of UV, particularly UV-B radiation on plant physiology and biochemistry have received increasing interest from scientists over the last three decades, in view of the depletion of the stratospheric ozone layer, which is particularly severe in some regions of the Earth (for review articles, see Ballaré et al., 2011; Williamson et al., 2014; Bornman et al., 2015). High doses of UV radiation have the potential to damage Photosystem II (PSII) reaction centers (Vass, 2012) as well as DNA integrity (Frohnmeyer and Staiger, 2003; Biever and Gardner, 2016). Nonetheless, photosynthesis and biomass production decrease little in plants exposed to UV radiation under natural sunlight (Bassman et al., 2002; Wargent and Jordan, 2013; Kataria et al., 2014; Bornman et al., 2015; Siipola et al., 2015; Wargent et al., 2015). Blue light-activated photolyase, which repairs UV photoproducts in DNA (Biever and Gardner, 2016), effectively limits the damage driven by

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short-wave solar radiation (Aphalo et al., 2012, 2015; Hideg et al., 2013; Bornman et al., 2015; Klem et al., 2015).

During extended periods of exposure to UV and blue light radiation, the stimulation of phenylpropanoid biosynthesis (Agati and Tattini, 2010; Agati et al., 2013; Kaling et al., 2015; Siipola et al., 2015; Wargent et al., 2015; Huchè-Thélier et al., 2016) offers further photoprotection to the photosynthetic apparatus, despite an initial decline in photosynthetic performance (Kolb et al., 2001; Tsormpatsidis et al., 2008). UV-absorbing hydroxycinnamates (HCA) and flavonoids serve a multiplicity of functions in photoprotection: they efficiently absorb short-wave solar radiation, thus decreasing the risk of photo-oxidative stress, as well as countering photo-oxidative damage by scavenging free radicals and reactive oxygen species, such as singlet oxygen ($^1O_2$) and hydrogen peroxide (Agati et al., 2007, 2012). The potential of HCA and flavonoids to serve as antioxidants in photoprotection stems from the observation that these compounds accumulate in mesophyll, not only in epidermal cells, in response to high solar irradiance (Semerdjieva et al., 2003; Polster et al., 2006; Tattini et al., 2004, 2005; Ferreres et al., 2011). Flavonoids accumulate in the chloroplasts, other than in the vacuolar compartment in some species (Saunders and McClure, 1976), apparently associated to the chloroplast outer envelope membrane (Agati et al., 2007). High sunlight almost exclusively activates the biosynthesis of flavonoids with the greatest antioxidant capacity, in the presence or in the absence of UV-irradiance (Agati et al., 2009, 2011a; Siipola et al., 2015). This adds further support to the idea that flavonoids may serve antioxidant functions in photoprotection (Ryan et al., 1998; Agati et al., 2007, 2012; Ferreres et al., 2011).

The effect of UV irradiance on carotenoid biosynthesis is less clear, possibly due to different experimental set-ups (UV supplementation vs. UV exclusion experiments), intensity of UV 'stress' (irradiance × time of exposure), plant species (woody vs herbaceous), and even genotype (Musil et al., 2002; Láposi et al., 2009; Newsham and Robinson, 2009; Li et al., 2010; Aphalo et al., 2012, 2015; Vodović et al., 2015). Nonetheless, the overall emerging picture describes a negative effect of UV radiation on the concentration of carotenoids (Hideg et al., 2006; Hui et al., 2015; Bernal et al., 2015), particularly in UV-exclusion experiments (Bischof et al., 2002; Liu et al., 2005; Newsham and Robinson, 2009; Albert et al., 2011), with few exceptions (Láposi et al., 2009; Klem et al., 2015). UV-B irradiance was additionally shown to partially inhibit the high light-induced down-regulation of xanthophyll epoxidation (Mewes and Richter, 2002; Moon et al., 2011), and the consequential nonphotochemical quenching (NPQ) of excess light in the chloroplast, by reducing the pH gradient across thylakoid membranes (Pfündel et al., 1992; Pfündel and Dilley, 1993).

This offers the intriguingly possibility that during UV acclimation plants might enhance their capacity to effectively counter the detrimental effects of the most energetic solar wavelengths, while partially decreasing their ability to cope with an excess of photosynthetic active radiation (PAR). This might have ecological significance, since an excess of visible light may translate into a severe stressful condition plants face on seasonal and daily basis (Li et al., 2009), further exacerbated by the concurrent impact of heat and drought stresses, particularly in a Mediterranean climate (Matesanz and Valladares, 2014; Tattini and Loreto, 2014).

In our study, we investigated the potential relationship between flavonoid and carotenoid biosynthesis in photoprotection mechanisms of plants growing in the presence or in the absence of UV radiation. We hypothesize that flavonoids might serve photoprotective functions of increasing significance in leaves growing in the presence of solar UV wavelengths, because of the decreased biosynthesis of carotenoids. To test this hypothesis we grew plants under either partial shading (40% of natural sunlight) or fully exposed to solar irradiance (100%) in the absence or in the presence of UV-radiation, in an UV-exclusion experiment. We analyzed the responses to different light treatments of two members of the Oleaceae family, *Ligustrum vulgare* L. and *Phillyrea latifolia* L., which inhabit sunny or partially shaded areas, respectively, in the Mediterranean basin, and display a very similar flavonoid pool (Tattini et al., 2005; Fini et al., 2016). In *P. latifolia*, a constitutively higher frequency of secretory trichomes coupled with thicker cuticles and epidermises offer greater capacity in limiting the transmission of solar irradiance through the leaf, thus offering greater protection to the photosynthetic apparatus as compared to *L. vulgare* (Tattini et al., 2005). This hypothesis was consistent with the much higher accumulation of 'antioxidant' flavonoids in mesophyll cells of *L. vulgare* than of *P. latifolia* when plants grew in full sunlight. Therefore, in our study we tested the hypothesis that UV radiation, while promoting the biosynthesis of flavonoids might depress the biosynthesis of xanthophylls to greater extent in *L. vulgare* than in *P. latifolia*, with important consequences on photoprotection mechanisms.

## 2. Material and methods

### 2.1. Plant material and growth conditions

Self-rooted *Ligustrum vulgare* L. and *Phillyrea latifolia* L. potted plants were grown in screen houses (2 m × 2 m × 2 m, length × width × height) constructed with roof and walls using plastic foils with specific transmittances, over a six-week experimental period. Plants were exposed to 40% or 100% solar irradiance in the absence (referred as PAR plants/leaves throughout the paper) or in the presence of UV irradiance (referred as to UV plants/leaves). Solar UV radiation was excluded by LEE #226 UV foils (LEE Filters, Andover, UK), which fully excluded solar wavelengths in the range 280–380 nm, and transmitted just 3% of radiation in the 380–390 nm range. Plants grew under a 100-μm ETFE fluoropolymer transparent film (NOWOFLON® ET-6235, NOWOFOL® Kunststoffprodukte GmbH & Co. KG, Siegsdorf, Germany) in the UV treatment. Attenuation of solar irradiance was achieved by adding a proper black polyethylene frame to the LEE #226 or NOWOFOL ET-6325 foils. UV irradiance (280–400 nm) and photosynthetic active radiation (PAR, over the 400–700 nm spectral region) inside the screen houses were measured by a SR9910-PC double-monochromator spectroradiometer (Macam Photometric Ltd., Livingstone, UK), and a calibrated Li-190 quantum sensor (Li-Cor Inc., Lincoln, NE, USA), respectively. UV-A was 798 or 314, and UV-B 43.1 or 17.3 kJ $m^{-2}$ $d^{-1}$ in the UV treatment under 100 or 40% solar irradiance, respectively, on a clear day. Biologically effective UV-B radiation, $UV\text{-}B_{BE}$ (as weighed by the generalized plant action spectrum proposed by Caldwell (1971)), was 3.54 or 1.39 kJ $m^{-2}$ $d^{-1}$, at 100% or 40% solar irradiance. UV-A irradiance was 33.2 or 13.9 kJ $m^{-2}$ $d^{-1}$ in plants at 100 or 40% solar irradiance in the absence of UV radiation, respectively, on a clear day. Temperature maxima/minima were measured daily with Tinytag Ultra2 data loggers (Gemini Dataloggers, UK) and averaged 30.8/17.7 °C or 32.6/16.9 °C in plants growing at 40% or 100% sunlight, over the whole experimental period. We sampled six-week-old leaves, i.e., newly developed under the different light treatments, for measurements at midday hours (from 12:00 to 14:00 hrs), when photosynthetic and non-photosynthetic pigments play major photoprotective functions.

### 2.2. Photosynthesis and chlorophyll a fluorescence

Measurements of net $CO_2$ assimilation rate ($P_n$) were performed using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA), at PPFD of 1000 μmol photons $m^{-2}$ $s^{-1}$,