

RESEARCH PAPER

Can dual chlorophyll fluorescence excitation be used to assess the variation in the content of UV-absorbing phenolic compounds in leaves of temperate tree species along a light gradient?

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Abstract

The present study assesses light-induced variations in phenolic compounds in leaves of saplings of two co-occurring temperate species (*Acer platanoides* L., and *Fraxinus excelsior* L.) along a light gradient using a new non-invasive optical method (DualEx). The DualEx-derived UV absorbance of leaf epidermis (the sum of the adaxial and abaxial faces, A_{UV}) increased significantly with increasing light in both species. A_{UV} values were correlated with absorbance of the leaf extract at 305 nm and 375 nm (A_{305} and A_{375}) in both species with similar slopes for both species. However, a large difference in intercept was observed between the two species when A_{305} was regressed against A_{UV} . Similarly, A_{UV} values were well correlated with the amount of phenolics in the leaf extracts assessed by the Folin–Ciocalteu method, but slopes were significantly different for the two species. Thus, the UV-A epidermal transmittance, despite being a reliable indicator of the UV-screening capacity of the leaf epidermis, cannot be used for any quantitative estimate of UV-B screening capacity or of energetic requirement for leaf construction without a species-specific calibration.

Key words: *Acer platanoides* L., epidermis, fluorescence, *Fraxinus excelsior* L., phenolic compounds, UV absorbance, shade.

Introduction

Plants produce a diverse array of phenolics, ranging from simple molecules such as phenolic acids to large highly polymerized compounds such as tannins and other polyphenolics. They play several important roles in plants and any change in plant chemistry may have a large influence on ecosystem functions; like trophic interaction in food webs, outcome of competition between plant species, or nutrient cycling (Baas *et al.*, 1989; Harborne and Williams, 2000; Ballaré *et al.*, 2001; Rousseaux *et al.*, 2004). Some phenolics serve as defence against herbivory, pathogens, or ultraviolet (UV) radiation, while others are important attractants for pollinators and seed dispersers or exhibit allelopathic activity (Harborne, 1989; Bormann *et al.*, 1997; Cockell and Knowland, 1999; Ballaré *et al.*, 2001). Flavonoids and hydroxycinnamic acid conjugates are the most efficient UV-screening compounds because of their strong absorbance in this wavelength region (Caldwell *et al.*, 1995; Cockell and Knowland, 1999). Their concentrations in epidermal layers are significantly increased by UV radiation (Burchard *et al.*, 2000; Kolb *et al.*, 2001).

The synthesis of phenolics uses a lot of energy (on average 2.6 g glucose g^{-1} phenolics; Poorter and Villar, 1997). Thus, a large investment in phenolic compounds may affect the plant's growth and vitality despite the advantages they provide. Phenotypic plasticity will enable plants to match their phenolic contents with the

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environmental conditions. This is particularly important in a light gradient where the photoprotective involvement of phenolics decreases with increasing shade while the energetic cost of their synthesis becomes more disadvantageous for the plant carbon balance.

Commonly used techniques for quantifying the amount of UV-absorbing compounds include quantification of phenolic compounds in leaf extracts using spectrophotometry, chromatography, and other techniques (Veit *et al.*, 1995; Appel *et al.*, 2001), measurements of the spectral transmittance of epidermal peels using an integrating sphere (Flint *et al.*, 1985), or direct measurements of the UV levels inside the leaf using fibre optic microprobes (Day *et al.*, 1994). All of these methods have distinct advantages and limitations. Two specific problems with methods using leaf extracts are that compounds of interest may be insoluble and that compounds located within the mesophyll do not protect the leaf against UV radiations. Another problem that is common to all methods is that they are time-consuming and, therefore, have limited applications in field studies that require multiple and rapid comparisons among genotypes or among plants subjected to contrasting environments.

Recently, methods using chlorophyll fluorescence have been developed to assess the UV-absorbing properties of leaves. The ratio of chlorophyll fluorescence induced by UV radiation and by visible radiation is well related to the transmittance of the epidermis in the UV region (Bilger *et al.*, 1997, 2001; Barnes *et al.*, 2000; Burchard *et al.*, 2000; Markstädter *et al.*, 2001; Cerovic *et al.*, 2002; Krause *et al.*, 2003). A large part of the UV radiation is absorbed in the epidermis, depending on the relative amount of UV-absorbing substances, while visible radiation reaches the chloroplasts largely undiminished. Accumulation of UV-absorbing compounds in the epidermis will reduce the fluorescence excited by the UV beam, without affecting the fluorescence excited by the visible beam. These non-invasive methods have the great advantage of estimating UV penetration without introducing any perturbation in the optical properties of the leaves, and using natural UV target (chlorophyll) as a reporter of the UV climate within the mesophyll. Dualex (dual excitation of chlorophyll fluorescence) is a portable leaf-clip device that allows non-destructive estimation of the absorbance by the leaf epidermis using two excitation wavelengths, one in the UV part of the spectrum at 375 nm (epidermal UV-A shielding), and one red reference wavelength at 650 nm that crosses the epidermis without being absorbed before reaching the chlorophyll in the mesophyll (Ounis *et al.*, 2001; Goulas *et al.*, 2004).

The objectives of the present study were (i) to assess the light-induced variations in phenolic compounds in leaves of saplings of two temperate species (*Acer platanoides* L., and *Fraxinus excelsior* L.) along a light gradient using this new non-invasive optical method, (ii) to check whether

the epidermal UV absorbance of leaves obtained using the Dualex were related to the UV absorbance of the total leaf methanolic extract and to the soluble phenolic contents obtained by a colorimetric method based on their reducing properties (Folin–Ciocalteu), and (iii) to test whether these relations were species-specific or more generic.

Materials and methods

Site description

The study was carried out in a naturally regenerated temperate forest (Graouilly Forest, Moselle, France, 49°05' N, 6°02' E, 300 m elevation (Barthod and Epron, 2005). The overstorey is dominated by *Acer pseudoplatanus* L. and *Fagus sylvatica* L. In addition to these two species, understorey vegetation is composed of saplings of *Sorbus torminalis* L.; *Sorbus aria* L.; *Acer campestre* L.; *Acer platanoides* L.; *Ulmus glabra*; *Fraxinus excelsior* L. and *Tilia cordata*. Monthly average air temperature ranged between 1.6 °C in January and 18.7 °C in July and total annual rainfall was 745 mm (data from Météo France, Metz-Augny 1946–2001 periods).

Estimation of irradiance conditions

Hemispherical photographs were taken above each sapling with a digital camera (Coolpix 4500, Nikon, Japan) equipped with a fisheye converter (FC-E8, Nikon) as already described in Barthod and Epron (2005). The hemispheric photographs (3.9 millions pixels) were analysed using Gap Light Analyser software (GLA V2.0, Institute of Ecosystem Studies, New York, USA; Canham *et al.*, 1990; Pacala *et al.*, 1994; Fraser *et al.*, 2001). The threshold for calculating canopy openness was manually fixed using pixel histograms (lowest frequency value) and visually checked. Potential diffuse (standard overcast) and direct photosynthetic active radiation transmitted through the canopy above each sapling was calculated from canopy openness and expressed relative to incoming radiation above the canopy (T_{dif} and T_{dir} , respectively). Sky regions were defined from eight azimuth classes and 20 zenith classes and the solar time step was set to 2 min. The Global Site Factor ($GSF = (T_{dir} + T_{dif})/2$) was calculated assuming an equal proportion of diffuse and direct radiation above the canopy (Anderson, 1964; Canham *et al.*, 1990). GSF values were averaged over a period starting on 1 May and ending on 31 August.

Sampling and analysis

Twenty-four saplings (0.5–1.0 m high) of *Acer platanoides* L. and of *Fraxinus excelsior* L. were sampled in the stand in a large range of light environments in August 2003.

The aerial part of each plant was harvested, stored in an icebox, and transferred to a fridge (4 °C). At the earliest opportunity (within 24 h) 10–20 leaves from each sapling were sampled and their Dualex absorbance was immediately recorded (see following paragraph). Leaf area was measured with a leaf area portable meter (LI-3000A, Li-Cor, Nebraska, USA). Leaf dry mass was estimated after freeze-drying and specific leaf area of leaves was calculated as the ratio between leaf area and leaf dry mass. All samples were ground to a fine powder and stored dry until analysis.

Optical measurements of epidermal UV absorbance

A dual excitation fluorimeter (Dualex® Dual Excitation, prototype CNRS-LURE, France) was used for the non-destructive assessment of phenolics present in leaf epidermis (Goulas *et al.*, 2004; Cartelat *et al.*, 2005). Briefly, the measurement of leaf epidermal absorbance

of UV light is based on the screening effect of the epidermis that absorbs part of the incident UV light and therefore decreases the amount of light available for chlorophyll fluorescence excitation. An incident red light is used as a reference for chlorophyll fluorescence as the leaf epidermis is almost transparent in this spectral region. The leaf is alternately illuminated by UV (375 nm) and red (655 nm) light-emitting diodes (LED) at a modulation frequency of 1 kHz. For a constant UV-LED intensity, epidermal transmittance in the UV is equal to the reference red LED intensity when it is adjusted to induce the same chlorophyll fluorescence as the UV LED, thanks to an electronic feed-back loop. Absorbance was defined as the base-10 logarithm of the transmittance reciprocal (Goulas *et al.*, 2004). Absorbance values displayed on the Dualex after 1 s were recorded for the lamina of 10–20 fully-developed leaves of each plant which had no noticeable insect or mechanical damage. Depending on the leaf area, one or two measurements were taken first on the adaxial (upper) and then on the abaxial (lower) side of each leaf. Values for the adaxial and abaxial leaf side were averaged for each sapling. Dualex readings of the adaxial and abaxial were summed to provide an area-based estimate of total UV absorbance (A_{UV}) of the leaf epidermis (Goulas *et al.*, 2004; Cartelat *et al.*, 2005; Meyer *et al.*, 2006).

Extraction of phenolic compounds

A 100 mg aliquot of the homogenized leaf sample was extracted twice with 9 ml of a mixture containing methanol and chloroform (2/2, v/v) according to Bligh and Dyer (1959). Samples were vortexed and kept at room temperature for 30 min to allow for complete solvent extraction. The addition of water into the methanol/chloroform mixture (1/2/2: by vol.) produces a chloroform phase which contains chlorophylls and carotenoids, and a methanol/water phase on the top devoid of chlorophyll and carotenoids, which contains the soluble phenolic compounds. The methanol/water phase was collected with a Pasteur pipette and filtered through syringe filters (Interchrom, PVDF: polyvinylidene difluoride, pores size 0.45 μm) and kept at $-20\text{ }^{\circ}\text{C}$ until analysis. Extractions were repeated for each sapling to duplicate the reading.

Quantification of phenolic compounds

Total soluble phenolics content was measured using the Folin–Ciocalteu assay (Singleton and Rossi, 1965). Briefly, 50 μl of sample and 475 μl of 0.25 N Folin–Ciocalteu reagent were mixed and kept at room temperature for 5 min. Next, 475 μl of a 7% sodium carbonate solution were added and the mixture was kept at $40\text{ }^{\circ}\text{C}$ for 15 min. Detection of phenolic compounds was performed at 724 nm, using a Beckman DU 640B spectrophotometer. Phenolic content was standardized against coumaric acid. The standard curve was linear from 0 to 250 g l^{-1} giving an absorbance range of 0.05–0.80 ($R^2=0.998$). Total soluble phenolics content (*PHE*) was expressed on a leaf area basis knowing the specific leaf area of leaves.

Absorbance spectra (250–400 nm)

Absorbance of 10-fold-diluted leaf extracts were scanned from 250 nm to 400 nm (Beckman DU 640B spectrophotometer). All absorbance values of extracts were recalculated taking into account the dilution and the specific leaf area of leaves to make them equivalent to Dualex surface-based measurements. UV-absorbance values at 305 nm and 375 nm (A_{305} and A_{375}) were further used as relative measures of soluble phenolic compounds absorbing in the UV-B and UV-A spectral region, respectively.

Statistical analyses

Linear regression analyses were performed using JMP® version 3.2 (SAS Institute Inc, SAS campus Drive Cary, IN 27513) to examine

the relationship between *GSF*, A_{UV} , A_{305} , A_{375} , and *PHE*. Regression coefficients were calculated and tested for significant deviation from zero ($P < 0.05$). Differences between slope values or between intercept values were not significant when 95% confidence intervals overlapped. Intercept values and slopes values were also tested for significant deviation from, respectively, zero and 1 ($P < 0.05$).

Results

Changes in UV absorbance and composition of phenolics in a light gradient

Saplings of both species were sampled within the same light gradient, with global site factor values (*GSF*) ranging between 6% and 52%. Unfortunately, intermediate values of *GSF* (20–40%) were underrepresented for both species.

Dualex values were first compared between the adaxial side and the abaxial side for each leaf (Fig. 1). For the two temperate species, Dualex values of the two sides of leaves are highly correlated ($R^2=0.89$ for *F. excelsior* and $R^2=0.76$ for *A. platanoides*). Adaxial values were three time higher than those in abaxial epidermis for both species. The Dualex-derived UV absorbance of leaf epidermis (the sum of the adaxial and abaxial faces, A_{UV}) increased significantly with increasing light in both species ($R^2=0.81$ and $R^2=0.66$, for *F. excelsior* and *A. platanoides*, respectively; Fig. 2A). The slope of *F. excelsior* is significantly steeper than that of the other species.

Absorbance of the leaf extract (A_{305} and A_{375}) increased significantly with increasing light in both species (Fig. 2B, C) but the correlation with *GSF* was much better for *F. excelsior* ($R^2=0.78$ and 0.79) than for *A. platanoides* ($R^2=0.43$ and 0.62). The slopes were significantly different between species for A_{375} in agreement with the Dualex data, but not for A_{305} .

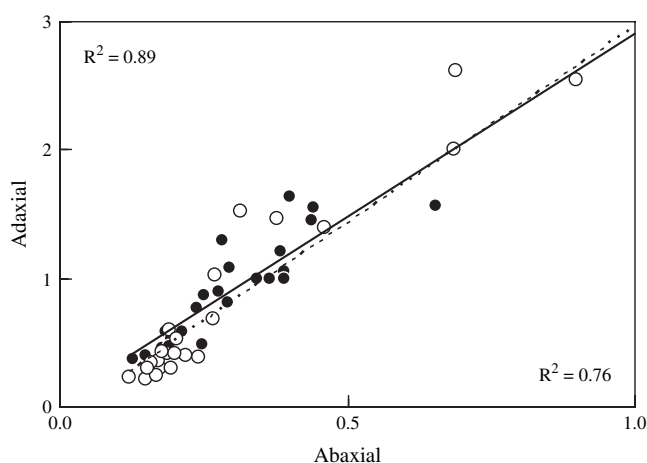


Fig. 1. Relationship between Dualex-derived UV absorbance of the abaxial and adaxial sides of leaves of *A. platanoides* (closed symbols) and of *F. excelsior* (open symbols). Determination coefficients (R^2) and linear regression lines (solid line for *A. platanoides* and dotted line for *F. excelsior*) are given when significant ($P < 0.05$).

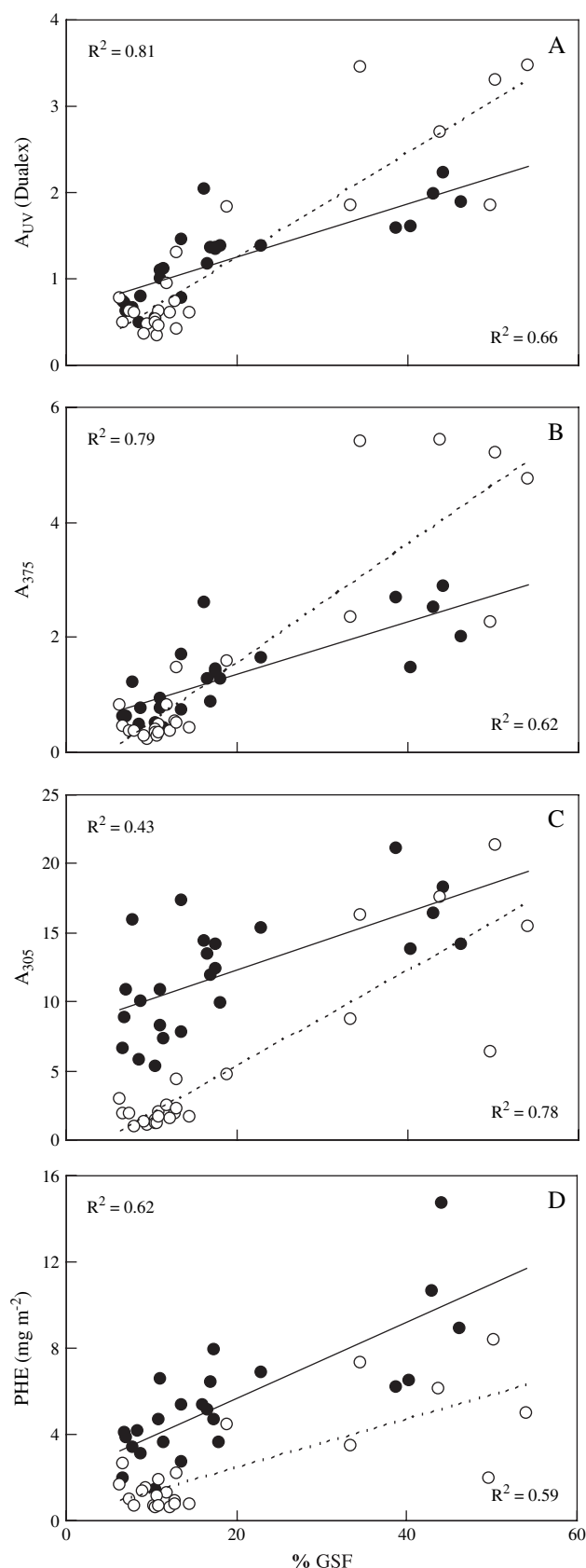


Fig. 2. Relationships between irradiance (global site factor, *GSF*) and absorbance of leaf extract at 375 nm (A_{375} , B) and 305 nm (A_{305} , C),

Content of soluble phenolic compounds (measured with the Folin–Ciocalteu method) also increased significantly with increasing light in both species ($R^2=0.59$ for *F. excelsior* and $R^2=0.62$ for *A. platanoides*, Fig. 2D). Slopes were not significantly different. For a given light environment, leaves of *A. platanoides* contain more phenolic compounds per unit of leaf area than those of *F. excelsior*.

Correlation between epidermal UV absorbance, UV absorbance of leaf extract, and composition of phenolics

A_{UV} values were highly correlated with A_{375} in both species (Fig. 3A; $R^2=0.95$ for *F. excelsior* and $R^2=0.79$ for *A. platanoides*). The root mean square error was also lower for *F. excelsior* (0.35) than for *A. platanoides* (0.42). Slopes were not significantly different from each other, but both were significantly higher than one, with 95% confidence intervals of [1.51, 1.86] for *F. excelsior* and [1.05, 1.66] for *A. platanoides*. The y-intercept was significantly negative for *F. excelsior* while it was negative but not different from zero for *A. platanoides* ($P=0.08$).

A_{UV} values were also well correlated with A_{305} for *F. excelsior* ($R^2=0.91$) but correlation was weaker for *A. platanoides* ($R^2=0.52$; Fig. 3B). The root mean square error was again lower for *F. excelsior* (1.85) than for *A. platanoides* (2.97). Slopes were not significantly different from each other, but were 3–4 times higher than with A_{375} because of the higher absorbance of leaf extracts in the UV-B region than in the UV-A region (Fig. 4). The y-intercept was significantly positive for *A. platanoides* while it was significantly negative for *F. excelsior*. The positive intercept on the A_{305} indicates that the extract contained UV-B absorbers coming from the mesophyll that do not vary with A_{UV} *in vivo*, which is restricted to epidermal absorbance.

A_{UV} values were highly correlated with the content of phenolic compounds in the leaf extract (Fig. 3C; $R^2=0.85$ and $R^2=0.65$, for *F. excelsior* and *A. platanoides*, respectively). Slopes were significantly different from each other and the root mean square error was lower for *F. excelsior* (0.86) than for *A. platanoides* (1.73).

UV absorbance spectra of leaves of sun and shade saplings

An average spectrum (250–400 nm) of leaf extract of three saplings growing in shade ($GSF < 10\%$, full points) and an average spectrum of leaf extract of three saplings

Dualex-derived UV absorbance (the sum of the abaxial and adaxial sides values, A_{UV} , A) and leaf phenolics content (PHE, D) for *A. platanoides* (closed symbols) and *F. excelsior* (open symbols). Determination coefficients (R^2) and linear regression lines (solid line for *A. platanoides* and dotted line for *F. excelsior*) are given when significant ($P < 0.05$).

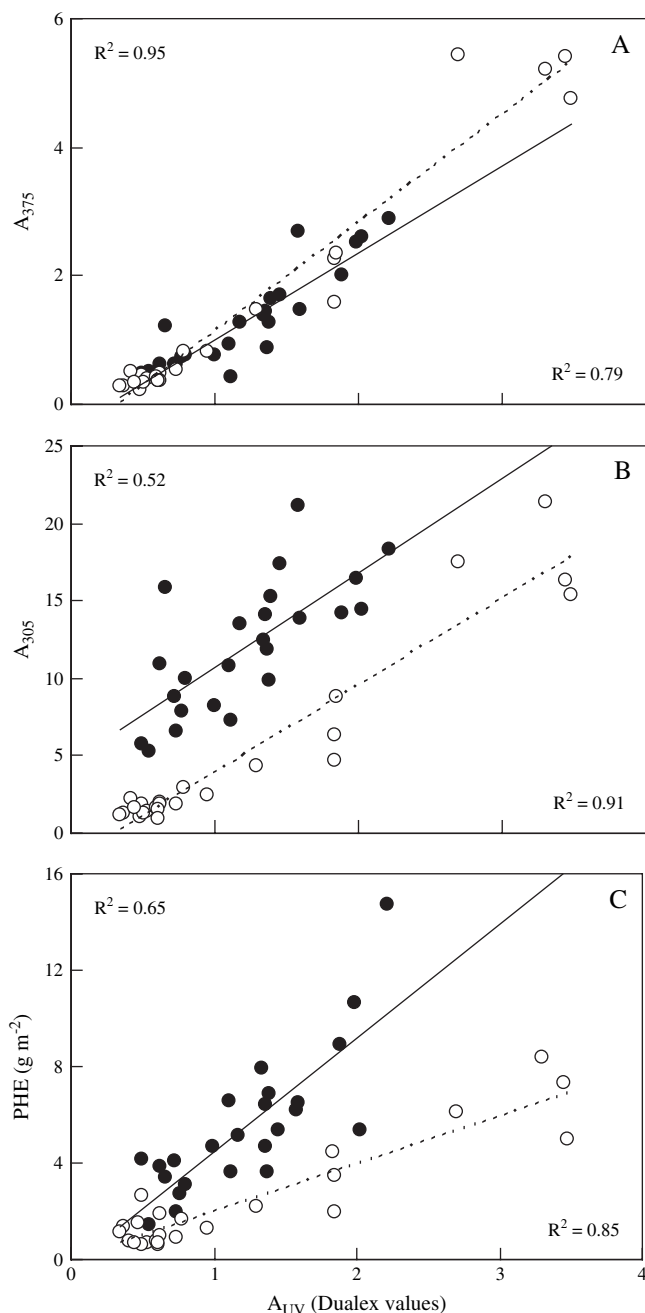


Fig. 3. Relationships between Dualex-derived UV absorbance (sum of the abaxial and adaxial sides values, A_{UV}) and absorbance of leaf extract at 375 nm (A_{375} , A) and 305 nm (A_{305} , B), and leaf phenolics content (PHE, C) for *A. platanoides* (closed symbols) and *F. excelsior* (open symbols). Determination coefficients (R^2) and linear regression lines (solid line for *A. platanoides* and dotted line for *F. excelsior*) are given when significant ($P < 0.05$).

growing in more open conditions ($GSF > 40\%$, open points) were computed for each species, and were plotted in Fig. 4A for sun leaves and in Fig. 4B for shade leaves. For both species, spectra of sun leaves showed a higher content of UV-absorbing compounds than those of shade leaves. Spectra of sun-exposed *F. excelsior* L. exhibited

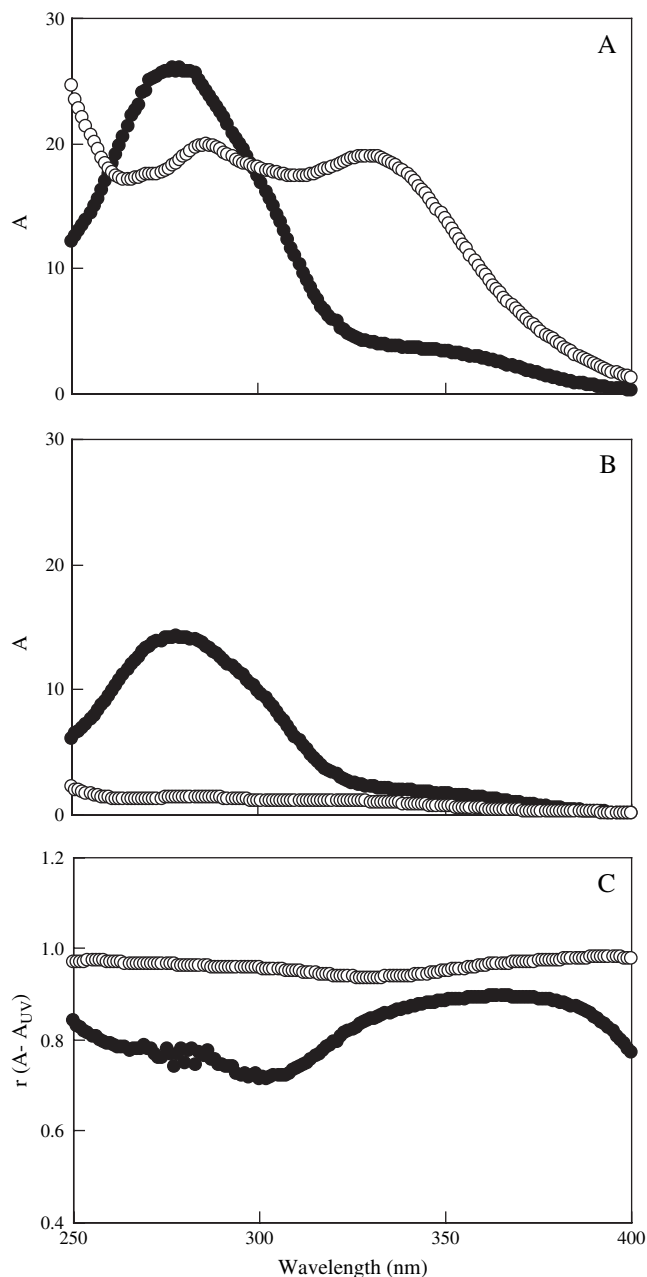


Fig. 4. Absorbance spectra in the UV spectral region of methanolic extracts sun leaves (A) and shade leaves (B) for *A. platanoides* (closed symbols) and *F. excelsior* (open symbols). Spectra of five saplings were averaged for each group. Correlation coefficients (r , C) between absorbance of methanolic extracts and Dualex-derived UV absorbance [sum of the abaxial and adaxial sides values, A_{UV} for *A. platanoides* (closed symbols, $n=24$) and *F. excelsior* (open symbols, $n=24$)].

high absorbance at all wavelengths with two distinguishable maxima at 286 nm and 329 nm. The absorbance spectra of *A. platanoides* exhibited a broad band with a maximum at 279 nm and most of the difference between spectra of sun and shade saplings ranged around this maximum. For *Acer platanoides* L. half of the spectrum amplitude, i.e. half of the UV-B absorbers contained in

the leaf extract, was already present in leaves of shade saplings. Correlation coefficients between A_{UV} and absorbance of the leaf extract at each wavelength (between 250 nm and 400 nm) are reported in Fig. 4C. A_{UV} values were well correlated with the absorbance of the leaf extract for *F. excelsior* at all wavelengths (R ranging between 0.94 and 0.98; Fig. 4C) while correlation was weaker for *A. platanoides* at all wavelengths, but especially in the UV-B spectral region.

Discussion

The level of sun irradiance, as estimated from the GSF values, influenced leaf absorbance in the UV region of field-grown saplings of *A. platanoides* and *F. excelsior*. Increase in sun irradiance enhanced the UV-screening capacity of the leaf epidermis as already reported (Krause *et al.*, 2003; Goulas *et al.*, 2004). The light-induced variation was more pronounced in *F. excelsior* leaves than in *A. platanoides* leaves, but in the shade, leaves of *A. platanoides* contained higher amounts of UV-absorbing compounds than leaves of *F. excelsior*. The accumulation of UV-absorbing compounds in the epidermal cells is thought to protect the mesophyll cells and the photosynthetic apparatus against UV-induced damage (Mazza *et al.*, 2000; Kolb *et al.*, 2001) and shade leaves are probably more prone to UV stress when exposed to full sunlight, even during short periods of time (Krause *et al.*, 2003). The biosynthesis and accumulation of flavonoids, hydroxycinnamic acids, and other related phenylpropanoid compounds are among the most commonly reported responses of plants to UV-B radiation (Caldwell *et al.*, 1995; Cockell and Knowland, 1999). The adaxial UV-absorbance was three times higher than the corresponding abaxial value for both species. Similar results have been reported on many species (Bilger *et al.*, 1997; Barnes *et al.*, 2000; Markstädter *et al.*, 2001; Krause *et al.*, 2003; Kolb and Pfündel, 2005) and, in this case, the difference between the two sides of the leaves was probably related to the horizontal foliage orientation of these species, with adaxial surfaces receiving a greater amount of UV radiation than abaxial surfaces. As already pointed out, UV-absorbing substances are particularly protective when they are accumulated in the adaxial epidermis of planophile leaves (Markstädter *et al.*, 2001) while opposite results or less pronounced differences between both sides were reported for plants with more erect leaves (Burchard *et al.*, 2000; Barnes *et al.*, 2000; Kolb and Pfündel, 2005).

Dualex readings were in accordance with the absorbance at 375 nm of the leaf extract. This result was expected since measurements of Dualex are based on the excitation at 375 nm (Goulas *et al.*, 2004) and the linear relationships observed in this study contrasted with the curvilinear relationship reported by others (Kolb *et al.*,

2001; Kolb and Pfündel, 2005). For the two species, the negative intercept can be explained by the presence of UV-absorbing compounds in the epidermal cells that are not extractable by methanol (Ounis *et al.*, 2001). The slopes of the linear regression between A_{UV} and A_{375} were similar between the two species, demonstrating that the non-invasive technique of the Dualex system provides a useful assessment of leaf protection against solar UV-A radiation (at 375 nm). However, the slopes were slightly higher than 1 for both species, suggesting that additional phenolics are present in mesophyll and vascular tissues of leaves and contribute to the additional A_{375} extract (Barnes *et al.*, 2000; Kolb *et al.*, 2001; Kolb and Pfündel, 2005). Dualex senses only epidermal UV-absorbers whereas the extracts are obtained from the entire leaf. Therefore, the absorbance values (per unit leaf surface) in extracts are larger than the corresponding Dualex measurement.

The slopes were much higher when A_{UV} was regressed against A_{305} . This was expected since most of the UV-absorbing compounds have a much greater absorption in the UV-B region than in the UV-A. This is well illustrated by the absorbance spectra of the leaf extracts. A_{305} values were higher than A_{375} values, as already reported (Krause *et al.*, 2003). Differences between spectra obtained on the two species highlighted that different classes of phenolics are involved in light-induced variation in UV absorbance, in agreement with the observed difference in slopes of A_{UV} versus A_{305} relations. Sun leaves of *F. excelsior* seem to accumulate both UV-B and UV-A screening compounds; whereas *A. platanoides* accumulate only UV-B protective compounds. This difference between the two species is similar to the one found between woody and herbaceous plants, the latter tending to accumulate flavones (Day *et al.*, 1994). The nature of the phenolic compounds involved in UV protection in these two species has not been identified. The absorbance spectra of sun leaves of both species showed a broad band with a maximum around 270 nm and 290 nm which may correspond to simple phenols, phenolic acids, some flavonoids (isoflavones, flavanones), or anthocyanins that have only a weak absorbance in the UV-A region while *F. excelsior* exhibited a second broad band with a peak around 320 nm and 340 nm, corresponding to other flavonoids like flavone or hydroxycinnamic acids that absorb in both spectral regions (see Cerovic *et al.*, 2002, for a compilation of spectral regions of absorption maxima of major classes of phenolics). The difference in phenolic composition between both species probably explained the high positive intercept observed for *A. platanoides* and the negative one for *F. excelsior* when A_{305} is regressed against A_{UV} . In addition, the absorption spectrum of the extract containing total leaf-extractable constituents might not be representative of epidermal constituents. This large difference between the two species highlighted that the UV-A epidermal transmittance is not a reliable quantitative indicator of the total UV screening capacity of the leaf

epidermis, despite the fact that most of UV-B absorbing compounds also absorbed in the UV-A region. Other studies exist which disagree with (Barnes *et al.*, 2000) or corroborate (Krause *et al.*, 2003) this finding.

Total leaf phenolics are often assessed using the Folin-type colorimetric method that is based on the reducing properties of phenolic compounds, especially for the study of herbivore protection, energetic cost of defence investment, or decomposition (Appel *et al.*, 2001). These methods have been applied when the light-induced changes in these ecological properties are analysed within a canopy or across a light gradient (Cronin and Lodge, 2003; Covelo and Gallardo, 2004; Chacon and Armesto, 2006; Poorter *et al.*, 2006). But as far as we are aware, the relationship between changes in phenolic content as assessed by Folin-type methods (PHE) and changes in epidermal UV absorbance has never been studied. In the present work it was found that A_{UV} was correlated with PHE, but that the slopes are significantly different between the two species. Nevertheless, non-destructive *in vivo* measurements (Dualex) are better indicators of the light regime (*GSF*) than either the Folin-type method or the extract-absorbance method.

Conclusion

The most important finding of this study was the good correlation between soluble phenolics in methanolic extracts (determined by absorbance in the UV-A, in the UV-B, and with the Folin–Ciocalteu method) and the optical measurement of epidermal UV absorbance of leaves of *A. platanoides* and of *F. excelsior*. However, since the intercepts differ markedly between species when relating A_{UV} with the absorbance in the UV-B region of the leaf extract or with the amount of phenolics in the leaf extracts assessed by the Folin–Ciocalteu method, any quantitative estimate of UV-B screening capacity or energetic requirement for leaf construction would need a species-specific calibration.

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