Mesophyll distribution of ‘antioxidant’ flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance

Giovanni Agati1, Giovanni Stefano2, Stefano Biricolti2 and Massimiliano Tattini3,*

1Istituto di Fisica Applicata ‘Carrara’, IFAC, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy, 2 Dipartimento di Ortoflorofrutticoltura, Università di Firenze, Viale delle Idee 30, I-50019, Sesto Fiorentino, Firenze, Italy and 3 Istituto per la Valorizzazione del Legno e delle Specie Arboree,IVALSA, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy

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**Background and Aims** Flavonoids have the potential to serve as antioxidants in addition to their function of UV screening in photoprotective mechanisms. However, flavonoids have long been reported to accumulate mostly in epidermal cells and surface organs in response to high sunlight. Therefore, how leaf flavonoids actually carry out their antioxidant functions is still a matter of debate. Here, the distribution of flavonoids with effective antioxidant properties, i.e. the orthodihydroxy B-ring-substituted quercetin and luteolin glycosides, was investigated in the mesophyll of *Ligustrum vulgare* leaves acclimated to contrasting sunlight irradiance.

**Methods** In the first experiment, plants were grown at 20 % (shade) or 100 % (sun) natural sunlight. Plants were exposed to 100 % sunlight irradiance in the presence or absence of UV wavelengths, in a second experiment. Fluorescence microspectroscopy and multispectral fluorescence microimaging were used in both cross sections and intact leaf pieces to visualize orthodihydroxy B-ring-substituted flavonoids at inter- and intracellular levels. Identification and quantification of individual hydroxycinnamates and flavonoid glycosides were performed via HPLC-DAD.

**Key Results** Quercetin and luteolin derivatives accumulated to a great extent in both the epidermal and mesophyll cells in response to high sunlight. Tissue fluorescence signatures and leaf flavonoid concentrations were strongly related. Monohydroxyflavone glycosides, namely luteolin 4′-O-glucoside and two apigenin 7′-O-glycosides were unresponsive to changes in sunlight irradiance. Quercetin and luteolin derivatives accumulated in the vacuoles of mesophyll cells in leaves growing under 100 % natural sunlight in the absence of UV wavelengths.

**Conclusions** The above findings lead to the hypothesis that flavonoids play a key role in countering light-induced oxidative stress, and not only in avoiding the penetration of short solar wavelengths in the leaf.

Key words: Confocal laser scanning microscopy (CLSM), flavonoid glycosides, fluorescence microimaging, fluorescence microspectroscopy, hydroxycinnamates, intra-cellular flavonoid localization, *Ligustrum vulgare*, photoprotection, UV stress.

**INTRODUCTION**

The idea that flavonoids may counter oxidative damage, in addition to attenuating the highly energetic UV-B wavelengths reaching sensitive targets in a leaf, in response to high solar irradiance, may be inferred from several lines of evidence. Firstly, it is unlikely that the widely reported UV-B-induced increase in the flavonoid to hydroxycinnamate ratio (Ollson *et al.*, 1999; Burchard *et al.*, 2000; Tattini *et al.*, 2004) depends on their relative abilities to absorb the UV-B wavelengths. Hydroxycinnamic acid derivatives (εmax between 310 and 325 nm) have a greater ability to absorb over the UV-B waveband with respect to most flavonoids (εmax > 330 nm and εmin around 300 nm; Sheahan, 1996; Harborne and Williams, 2000; Tattini *et al.*, 2004).

Secondly, in high light-treated leaves, the biosynthesis of orthohydroxy B-ring-substituted flavonoids is strongly favoured over that of its monohydroxy B-ring-substituted counterparts (Markham *et al.*, 1998; Ryan *et al.*, 1998; Gould *et al.*, 2000; Tattini *et al.*, 2004, 2005). Nevertheless, the molar extinction coefficient, over the 300–390-nm waveband, of the monohydroxy flavone apigenin 7-O-rutinoside (12.2 mM−1 cm−1) considerably exceeds that of the orthohydroxy flavonol quercetin 3-O-rutinoside (9.8 mM−1 cm−1; Tattini *et al.*, 2004).

Finally, UV-B radiation is not a pre-requisite for flavonoid biosynthesis (Christie and Jenkins, 1996; Brosché and Strid, 1999; Jenkins *et al.*, 2001; Ibdaa *et al.*, 2002). An increase in the concentration of leaf flavonoids was observed in grapevine in response to visible light (Kolb *et al.*, 2001), and the very same flavonoids increased in *Lemma gibba* in response to both natural sunlight and visible light and excess copper ions (Babu *et al.*, 2003). Bilger *et al.* (2007) observed an increase in UV-screening compounds in response to low-temperature stress in the absence of UV radiation.

These findings lead to the hypothesis that flavonoids may actually serve a key function to counter UV-induced oxidative stress (Close and McArthur, 2002; Winkel-Shirley, 2002; Tattini *et al.*, 2005), as concluded by Landry *et al.* (1995): ‘*Arabidopsis thaliana* responds to UV-B as to an oxidative stress, and sunscreen compounds reduce the oxidative damage caused by UV-B’. Orthodihydroxy B-ring-substituted hydroxycinnamates...
flavonoids have the potential to effectively inhibit the generation of, as well as to quench, reactive oxygen species (ROS), and structural/antioxidant relationships are well-established (Saskia et al., 1996; Rice-Evans et al., 1997; Brown et al., 1998; Nguyen et al., 2006). It has been suggested previously that metal-chelating and quenching properties, not just the UV-screening features of flavonoids, need to be considered to conclusively explain their roles in land-plant evolution (Swain, 1986; Cooper-Driver and Bhattacharya, 1998; Cockell and Knowland, 1999).

However, it is a matter of fact that flavonoids accumulate mostly in surface organs and epidermal cells because of high-light stress (Schnitzler et al., 1996; Fishback et al., 1997; Tattini et al., 2000, 2007). Yamasaki et al. (1997) proposed that orthodihydroxy B-ring-substituted flavonoids help quench hydrogen peroxide freely diffusing from the mesophyll to enter the vacuoles of the epidermal cells (i.e. serving as substrates for class III peroxidases; Takahama and Oniki, 1997; Pérez et al., 2002). By contrast, few data are available on light-induced accumulation of ‘potentially antioxidant’ flavonoids in chlorophyll-containing tissues, which is a pre-requisite for flavonoids to actually carry out antioxidant functions within the sites of ROS generation (Gould et al., 2002; Kytridis and Manetas, 2006; Agati et al., 2007).

To gain new insights on the complex issue of the multiple functional roles, particularly the antioxidant one, of ‘UV-absorbing’ flavonoids in photoprotection, experiments were performed aimed at visualizing them in ROS-generating cells of leaves exposed to different solar irradiances. For this purpose, ‘new’ techniques of fluorescence microspectroscopy and multispectral fluorescence microimaging were used. Leaves of *Ligustrum vulgare*, acclimated to (a) 20 % or 100 % natural sunlight and (b) to full sunlight irradiance by cutting or not cutting the UV waveband, were studied. The distribution of hydroxycinnamates and flavonoids was estimated in the leaves paying special emphasis to the mesophyll tissues. Finally, the intracellular distribution of orthodihydroxy B-ring-substituted flavonoids in mesophyll cells was analysed using confocal laser scanning microscopy (CLSM) analysis in intact leaves. As far as is known this is the first report on this subject.

**MATERIALS AND METHODS**

**Plant material and growth conditions**

In the first experiment, *Ligustrum vulgare* L. plants were grown outside under 20 % (shade) or 100 % natural sunlight (sun) over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period. Plants at the full-sun site received a daily dose of 12.1 and 1.05 MJ m$^{-2}$ over a 5-week period.

In the second experiment, *L. vulgare* plants were grown in greenhouses constructed with the roof and walls made from plastic foil with specific transmittances, over an 8-week experimental period. The greenhouses were north–south exposed, with small shielded openings below the front roof at the north-east and north-west corners to permit air circulation. Solar UV radiation was excluded by LEE 226 UV foils (LEE Filters, Andover, UK) in the PAR100 treatment, whereas in the PAR100 + UV treatment plants were grown under a 100-µm ETFE fluoroplastic film (NOWOFLO® ET-6235; NOWOFLO Kunststoffprodukte GmbH & Co. KG, Siegsdorf, Germany). Control plants were grown under 25 % PAR irradiance (PAR25), which was obtained by adding a proper black polyethylene frame to the LEE 226 UV foil. UV irradiance (280–400 nm) and PAR inside the greenhouses were measured with a SR9910-PC double-monochromator spectroradiometer (Macam Photometric Ltd, Livingston, UK) and a calibrated Li-190 quantum sensor (Li-Cor Inc., Lincoln, NE, USA), respectively. Total UV irradiance was 24.5 or 1.1 W m$^{-2}$ in the PAR100 + UV and PAR100 treatments, respectively, at midday on a clear day (PAR was 1450 ± 46 µmol m$^{-2}$ s$^{-1}$ at both sites).

**Identification and quantification of phenylpropanoids, and analysis of their spectral features**

Hydroxycinnamates and flavonoids were extracted, identified and quantified by HPLC-DAD as reported previously in Tattini et al. (2004, 2005). Hydroxycinnamates were p-coumaric acid and echinacoside (a caffeic glycoside ester), and flavonoids were identified as the orthodihydroxy B-ring-substituted quercetin 3-O-rutinoside and luteolin 7-O-glucoside, and the monohydroxy B-ring-substituted luteolin 4′-O-glucoside and apigenin 7-O-glycosides (both glucoside and rutinoside; Tattini et al., 2004). P-coumaric acid was calibrated at 310 nm, echinacoside at 330 nm and flavonoids at 350 nm, using calibration curves of individual compounds operating in the range 0–30 µg. Echinacoside was isolated with semi-preparative HPLC using protocols previously reported in Romani et al. (2000, 2002). The extinction coefficient spectra of 40 µmol phenolic solutions (authentic standards purchased from Extrasynthese, Lyon, Nord, Genay, France) with or without the addition of 200 µmol of 2-amino ethyl diphenyl boric acid [0-1, w/v, Natursstoff reagent (NR)] in phosphate buffer (pH 6.8) with addition of 1 % NaCl (w/v), were recorded using a diode array spectrophotometer (HP8453, Agilent, Les Ulis, France). Fluorescence spectra of 10 µmol solutions (in phosphate buffer, pH 6.8) of echinacoside, luteolin 7-O-glucoside and quercetin 3-O-rutinoside with the addition of 50 µmol NR were recorded under UV-($\lambda_{exc}$ = 365 ± 5 nm) and blue-light excitation ($\lambda_{exc}$ = 488 ± 5 nm). A 1 x 1 cm quartz cuvette was horizontally positioned on the sample-holder of a Diaphot epifluorescence microscope (Nikon, Japan) equipped with a high-pressure mercury lamp (HBO 100 W; OSRAM, The Netherlands). Fluorescence spectra were recorded with a CCD multichannel spectral analyser, described in detail in the next section.

**Fluorescence microspectroscopy and multispectral fluorescence microimaging**

Fluorescence microspectroscopy and multispectral fluorescence microimaging were performed on cross-sections (approx. 100 µm thick) cut from leaves with a vibratory microtome (Vibratome 1100 Plus; St Louis, MO, USA), and stained with NR. The multispectral fluorescence microscope unit has
been detailed previously (Tattini et al., 2004; Agati et al., 2007). Fluorescence spectra were recorded using the 365-nm excitation wavelength, which was selected using a 10-nm bandwidth interference filter 365FS10-25 (Andover Corporation, Salem, NH, USA) and an ND 400 Nikon dichroic mirror. Fluorescence spectra (over the 400–800-nm waveband) were measured with a CCD multichannel spectral analyser (PMA 11-C5966; Hamamatsu, Photonics Italia, Arese, Italy) connected to the microscope through an optical fibre bundle, with a ×40 Plan Fluor objective. Fluorescence imaging (on a 400 × 400 μm area) was performed at 580 ± 5 nm using both the 365- and 488-nm excitation wavelengths using a ×10 objective, as described previously (Agati et al., 2007).

CLSM analysis was conducted on a leaf-half infiltrated with approx. 100 μL of NR solution using a plastic syringe without the needle, through a pinhole (made with a 100 μL pipette tip), on the upper leaf end. A leaf piece of approx. 5 × 5 mm, was cut at a distance of 4–5 mm from the pinhole, mounted in the staining buffer, and observed from the adaxial surface. Images were recorded using a Leica TCS SP5 confocal microscope (Leica Microsystems CMS, Wetzlar, Germany) equipped with an acusto-optical beam splitter, and an upright microscope stand (DMI6000). A 246 × 246 μm area was imaged using a ×63 objective (HCX PL APO lambda blue 63.0 × 1.40 OIL UV), and image spatial calibration was 0.5 μm pixel⁻¹. The pinhole was set to one ‘Airy unit’. A microscope was used in the sequential scan mode to detect (a) flavonoids: \( \lambda_{\text{exc}} = 488 \text{ nm} \), \( \lambda_{\text{em}} \) over the 560–600 nm spectral band; and (b) chlorophyll: \( \lambda_{\text{exc}} = 514 \text{ nm} \), \( \lambda_{\text{em}} \) over the 670–750 nm spectral band. Fluorescence spectra of NR-stained mesophyll cells were recorded (over the 500–640 nm waveband, using the Leica LAS-AF software package) through measurements in the λ-scan mode with a detection window of 10 nm.

RESULTS

Spectral features of hydroxycinnamates and flavonoids, and their tissue-specific localization

UV spectra of \( p \)-coumaric acid and apigenin derivatives, which have a monohydroxy substitution in the benzene ring or in the B-ring of the flavonoid skeleton, respectively, did not undergo a bathochromic shift upon treatment with Naturstoff reagent, in contrast to that observed with the orthodihydroxy structures (Fig. 1A, B). We propose the adduct formation for echinacoside (in blue) and for the orthodihydroxy B-ring-substituted...
luteolin 7-O-glucoside (in red) upon reaction with NR. It is likely that the same adduct is formed after reaction of quercetin 3-O-rutinoside with the fluorescence enhancer.

The fluorescence spectra of the mesophyll, not only those of the epidermal tissues, greatly differed between UV-excited cross-sections of *L. vulgare* leaves acclimated to contrasting sunlight irradiance (Fig. 2). The fluorescence intensity of the adaxial epidermis in sun leaves ($\lambda_{em} = 573$ nm) was three times greater than the fluorescence intensity in shade leaves ($\lambda_{em} = 559$ nm; Fig. 2A). Fluorescence spectra of the abaxial epidermises differed mostly for the shape, more than for the intensity, when comparing shade ($\lambda_{em} = 470$ nm) and sun leaves ($\lambda_{em} = 562$ nm). The fluorescence intensity of ‘sunny’ mesophyll tissues (both palisade and spongy parenchyma tissues) was more than four times greater than the fluorescence intensity of ‘shade’ mesophyll tissues (Fig. 2B).

These findings are consistent with the concentration and composition of the ‘soluble’ phenylpropanoid pool detected in shade or sun *L. vulgare* leaves. Indeed, the concentration of the ‘highly fluorescent’ (NR-treated) orthodihydroxy-substituted compounds (Fig. 3A), i.e. echinacoside (+75 %), and particularly luteolin 7-O-glucoside (+275 %) and quercetin 3-O-rutinoside (+520 %), mostly varied because of high sunlight (Table 1). In contrast, the concentrations of $p$-coumaric, apigenin 7-O-glycosides and luteolin 4’-O-glucoside, which have negligible fluorescence yields upon staining with NR (Agati *et al.*, 2002), decreased on average by 20 % passing from shade to sun leaves (Table 1).
B-ring-substituted kaempferol 3-dissolved in the cellular milieu. Indeed, the monohydroxy bound to proteins emits in the green-yellow waveband under or ‘dissolved’ in lipid bilayers, and quercetin non-covalently autofluorescent when conjugated to the epidermal cell walls (O-noids, i.e. quercetin 3-tissue-specific fluorescence signatures.

However, note that changes in tissue anatomy and in the tissue-specific content in wall-bound phenolics may contribute considerably to tissue fluorescence signatures under UV-light excitation. Furthermore, the phenolic content varies considerably to tissue fluorescence signatures under UV-light and echinacoside, was visualized by exciting cross-sections (Tattini et al., 2007). Changes in the relative intensities of the 365- and 488-nm excitation wavelengths may have partially contributed to slight variations in F(580) between UV-excited (λ(exc) = 365 ± 5 nm; Fig. 3B) and blue light-excited cross-sections (λ(exc) = 488 ± 5 nm; Fig. 4A). These data are consistent with a preferential accumulation of quercetin and luteolin derivatives in the adaxial palisade cells, as echinacoside, if present in appreciable amounts, would have enhanced greatly F(580) emitted from those cells (fluorescence intensity of echinacoside is greater than that of luteolin 7-O-glucoside and above all of quercetin 3-O-rutinoside, under UV-excitation; Fig. 3A). Changes in the relative intensities of the 365- and 488-nm excitation wavelengths may have partially contributed to slight variations in F(580) between UV- and blue light-excited cross-sections (Tattini et al., 2004). Finally, it is noted that fluorescence quenching, as a consequence of re-absorption and dimer annihilation processes (Ferrer et al., 2003; Rodriguez et al., 2004), which is of increasing significance as tissue flavonoid concentration increases, is more likely to have underestimated, rather than enhanced, light-induced increase in F(580).

**Table 1. The concentration of soluble individual phenylpropanoids in Ligustrum vulgare leaves exposed to 20% or 100% natural sunlight irradiance over a 5-week period**

<table>
<thead>
<tr>
<th>% Sunlight</th>
<th>p-coumaric</th>
<th>echinacoside</th>
<th>que 3-O-rut</th>
<th>lut 7-O-glc</th>
<th>lut 4’-O-glc</th>
<th>api 7-O-gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>12.7 ± 1.6 a</td>
<td>10.8 ± 1.2 b</td>
<td>2.4 ± 0.2 b</td>
<td>3.2 ± 0.3 b</td>
<td>3.9 ± 0.5</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>100</td>
<td>9.8 ± 1.5 b</td>
<td>18.9 ± 2.0 a</td>
<td>14.9 ± 2.3 a</td>
<td>12.1 ± 2.1 a</td>
<td>3.3 ± 0.4 ns</td>
<td>3.0 ± 0.4 ns</td>
</tr>
</tbody>
</table>

Data are means ± s.d., n = 6.
Values in a column not accompanied by the same letter are significantly different at P < 0.05 based on a least significant difference (LSD) test. ns, Not significant.

Abbreviations: que 3-O-rut, quercetin 3-O-rutinoside; lut 7-O-glc, luteolin 7-O-glucoside; lut 4’-O-glc, luteolin 4’-O-glucoside; api 7-O-gly includes apigenin 7-O-glucoside and apigenin 7-O-rutinoside.

**Fig. 4. Distribution of the fluorescence at 580 nm, F(580) as false-colour surface plots, in leaves of Ligustrum vulgare acclimated to full-sunlight irradiance. Cross-sections were stained with NR and excited with (A) far blue- (λ(exc) = 488 ± 5 nm) or (B) UV light (λ(exc) = 365 ± 5 nm).**
Distribution of ‘antioxidant’ flavonoids in mesophyll cells

Quercetin and luteolin glycosides did not accumulate in the mesophyll of leaves acclimated to 25% natural sunlight in the absence of UV wavelengths (PAR25; Fig. 5C). Noticeably, the green-yellow fluorescence due to these ortho-dihydroxy flavonoids did not vary substantially in intensity when comparing the adaxial palisade cells of leaves exposed to 100% natural sunlight in presence (PAR100 + UV; Fig. 5A) or absence (PAR100; Fig. 5B) of UV radiation. These findings are consistent with the leaf concentrations of quercetin 3-O-rutinoside and luteolin 7-O-glucoside that increased from 1·1 ± 0·17 μmol g⁻¹ d. wt under PAR25 (means ± s.d., n = 4) to 8·6 ± 0·8 and 11·3 ± 1·9 μmol g⁻¹ d. wt, under PAR100 or PAR100 + UV treatments, respectively. The fluorescence spectrum of blue-light excited (λexc = 488 nm) adaxial palisade cells in PAR100 leaves, which peaked at 565 nm (Fig. 5D), conclusively confirmed the occurrence of these orthodihydroxy B-ring-substituted flavonoids in the cell vacuole (Fig. 5A, B). Chlorophyll-induced attenuation of the excitation wavelength is unlikely to have affected the imaging of flavonoid distribution, as chlorophyll fluorescence did not differ in the adaxial palisade cells of leaves exposed to contrasting sunlight irradiance (Fig. 5A–C).
DISCUSSION

In the present experiments, the ‘new’ techniques of fluorescence micro-spectroscopy and multispectral fluorescence microimaging, aimed to visualize leaf flavonoids at inter- and intracellular levels, were used to give new insights on their antioxidant functions in photoprotective mechanisms.

It is shown that antioxidant flavonoids occurred to a great extent in the adaxial (both epidermal and mesophyll) cells of leaves acclimated to high sunlight. This finding, taken together with the preferential distribution of hydroxycinnamates (potentially, the best UV-B screening compounds; Landry et al., 1995; Sheahan, 1996; Harborne and Williams, 2000) in deeper leaf tissues (Fig. 4; Ollson et al., 2004), suggest that the functional roles of flavonoids in photoprotective mechanisms, do not depend merely on their UV-absorbing features (Markham et al., 1998; Gould et al., 2000).

Conclusive evidence is offered on the mesophyll distribution of the ‘antioxidant’ quercetin 3-O-rutinoside and luteolin 7-O-glucoside in leaves of L. vulgare exposed to natural sunlight in the presence or absence of UV irradiance. These compounds are vacuolar, which conforms to the findings of Neill et al. (2002) and of Kytridis and Manetas (2006) on the vacuolar compartmentation of ‘flavonoid anthocyanins’ in the mesophyll cells of Elatostema rugosum and Cistus creticus, respectively. It is suggested that mechanical pressure on the tonoplast membrane, using the present infiltration technique, allowed NR to enter the vacuole of mesophyll cells, although the pH gradient across the tonoplast would drive its exclusion from the vacuole (Sheahan et al., 1998). Our fluorescence imaging avoided the generation of artefacts during cross-sectioning (Hutzler et al., 1998), and conclusively localized the flavonoids in ROS-generating cells (Gould et al., 2002; Kytridis and Manetas, 2006). Hence, major criticisms on the localization/functional relationship of flavonoids in photoprotection, which mostly concerns their almost exclusive occurrence in epidermal cells (Yamasaki et al., 1997), have been addressed in the present experiment. With the multispectral fluorescence micro-imaging it was not possible to visualize the distribution of flavonoids in other cellular compartments (e.g. the cytoplasm and the chloroplasts; Sheahan et al., 1998; Agati et al., 2007), probably due to the relatively low resolution of fluorescence imaging.

We hypothesize that flavonoids with a catechol group in the B-ring may quench free radicals and hydrogen peroxide (i.e. serving as substrates for class III peroxidases; Yamasaki et al., 1997; Pérez et al., 2002; Pourcel et al., 2006) in mesophyll cells suffering from severe high-light stress, not only to help scavenge H$_2$O$_2$ freely diffusing from them to enter the vacuole of the epidermal ones. These scavenger activities against H$_2$O$_2$ have been previously reported to be effectively served by orthohydroxy substituted hydroxycinnamates, like echinacoside (Grace et al., 1998; Tattini et al., 2004).

As a consequence, the differential tissue-specific distribution of orthohydroxy B-ring-substituted flavonoids and echinacoside in high-light exposed leaves may merit a deep comment. Here it is highlighted that the flavonoid skeleton, and not only the presence of the catechol group in the B-ring, confers to flavonoids a greater ability to inhibit the generation of free radicals as compared with other phenylpropanoids. Metal-chelating properties are enhanced by the presence of the C = O group in the C-ring of the flavonoid skeleton, and metal-flavonoid complexes mimic superoxide dismutase activity (Morel et al., 1998; Kostyuk et al., 2004). As a consequence, the overall scavenger activity of ‘antioxidant’ flavonoids may exceed that of ‘antioxidant’ hydroxycinnamates. Also it is not excluded that the light-induced biosynthesis of caffeic derivatives from p-coumaric acid, as observed in the present experiment, may have enhanced lignin biosynthesis, more than increasing the concentration of soluble hydroxycinnamic intermediates in highly irradiated cells. This process may be of key significance in cells suffering from high light-induced oxidative damage, i.e. the adaxial palisade parenchyma cells. In wounded tissues of Arabidopsis thaliana, the unusual substrate used by CYP98A3, a 3′-hydroxylase of phe-nolic esters, which synthesizes caffeic from p-coumaric acid, was to give priority to the synthesis of flavonoids (Schoch et al., 2001), which further corroborates the idea of an involvement of flavonoids in both preventing and repairing light-induced oxidative damage. Nevertheless, the present experimental data do not allow the issue of tissue-specific accumulation of different phenylpropanoid classes because of high sunlight to be conclusively addressed. This matter has, however, great physiological and biochemical significance, and merits further and in-depth investigation.

The accumulation of mesophyll orthohydroxy B-ring-substituted flavonoids in leaves acclimated to natural sunlight in the absence of UV radiation, adds further evidence for an important role of flavonoids in countering the oxidative stress generated under excess-light conditions, not only to attenuate the highly energetic UV wavelengths from reaching ROS-generating cells. We hypothesize that UV stress (Gerhardt et al., 2008) does not differ from other stressful agents, of both biotic and abiotic origin (Wojtaszek, 1997; Schoch et al., 2001; Roberts and Paul, 2006; Agati et al., 2007; Lillo et al., 2008), in up-regulating the phenylpropanoid branch pathway leading to the biosynthesis of antioxidant flavonoids.

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LITERATURE CITED


Antioxidant flavonoids in the mesophyll of Ligustrum vulgaris


