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Photoprotective implications of leaf variegation in *E. dens-canis* L. and *P. officinalis* L.

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Summary

Variegated leaves occur rarely in nature, but there are some species, primarily in the forest understory, that possess this characteristic. We recently studied two variegated plants: *Erytronium dens-canis* L., which is characterised by a pattern of red patches and *Pulmonaria officinalis* L., with light green spots. These non-green areas could attenuate light reaching mesophyll cells with respect to green sections. The aim of the study was to verify whether such red and light green parts are more photoprotected than green ones and if this trait could be of adaptive value. Red patches in *E. dens-canis* were due to a single layer of red cells in the upper parenchyma, which accumulated anthocyanins. Light green spots in *P. officinalis* were caused by the presence of loosely arranged cells instead of a well-established layer of packed cells in the palisade parenchyma. Chlorophyll fluorescence imaging was performed under light treatment, showing a greater decrease of photochemical efficiency in red and light green patches than in green sections. Differences in the extent of photochemical efficiency among patches were not attributable to different activation of the xanthophyll cycle. These observations failed to confirm our initial hypothesis, but they questioned the physiological reason for this higher sensitivity in red and light green patches of photosynthetic tissues. Chlorophyll fluorescence imaging was therefore performed in the field. The same pattern of photochemical efficiency was maintained only in *E. dens-canis*. The current results demonstrate that in both species the benefits of variegation, if any, are different from enhanced photosynthetic performance.

Abbreviations: α-Toc, α-tocopherol; A, antheraxanthin; β-car, β-carotene; Chl, chlorophyll; L, lutein; N, neoxanthin; F_o, initial fluorescence level; F_m, maximum fluorescence level; PPFD, photosynthetic photon flux density; ROS, reactive oxygen species; V, violaxanthin; Z, zeaxanthin.

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Introduction

In natural plant canopies, leaves are continually exposed to contrasting light conditions, ranging from shaded habitats, such as in the forest understory (Chazdon et al., 1996), to highly irradiated as in outer forest canopies (Pearcy, 1987). In addition, the light environment is far from being stable, with photon flux densities fluctuating during the day because of changes in the solar incidence angle, changes in leaf position, cloud cover or in the heterogeneous distribution of canopy gaps that lead to sunflecks of varying duration and intensity (Pearcy, 1990). Under such conditions, plants should adjust and regulate the utilisation of light to prevent damage that may occur whenever the light energy absorbed surpasses the amount that can be used. This excess energy must be dissipated to avoid the overexcitation of the photosynthetic apparatus, which may lead to the formation of reactive oxygen species (ROS), causing photodynamic bleaching and perturbation of cellular metabolism (Foyer et al., 1994). In such strongly fluctuating environments, species with higher acclimation capacities would be favoured (Yamashita et al., 2000; Gamper et al., 2001). Plants employ multiple photoprotective strategies, which include avoidance of light capture, down-regulation of the efficiency of light energy conversion and activation of antioxidant mechanisms. The first strategy is based on structural and morphological modifications that include changes in leaf orientation or accumulation of reflective structures of molecules, such as hairs (Morales et al., 2002), waxes (Shepherd and Griffiths, 2006) and anthocyanins (Gould et al., 2000). The second strategy seems to be in connection with the operation of the so-called xanthophyll cycle (VAZ) (Müller et al., 2001), and the third includes a set of enzymatic and non-enzymatic mechanisms that directly detoxify ROS (Mittler, 2002).

Photosynthetic tissues are usually characterised by uniform coloured surfaces, but some species produce leaves with an irregular pattern of colour distribution (the so-called variegated leaves) as a result of their genotypic characteristics, somatic mutations or virus infections. Variegated leaves occur rarely in nature, usually in the forest understory (Tsukaya et al., 2004), but they are more common among ornamentals. Variegation is characterised by white, yellow, red or purple sections over the surfaces of the leaves (depending on the presence or absence of chlorophyll (Chl), carotenoids or anthocyanins). These ''non-green'' leaf sections have different optical properties that, in some cases, could increase photoprotection; in particular, reddish parts could reflect radiation 630–690 nm out of the leaves (Woodall et al., 1998), while absorbing blue wavelengths (Feild et al., 2001) and attenuating green light (Nishio, 2000; Kytridis and Manetas, 2006). Moreover, leaves with anthocyanins have a significantly greater antioxidant potential (Neill et al., 2002). Light green sections would be more protected by increasing the light reflectance of all wavelengths (Holmes and Keiller, 2002).

Variegated leaves are clear examples of the metabolic heterogeneity of cells within an organ (Bartley and Scolnik, 1995), rendering it possible to compare the responses of the different tissues to high light in the same organ. In the present work, we focused our study on two different variegated species with contrasting colour patterns: *Erytronium dens-canis* L., which is characterised by a pattern of red patches and *Pulmonaria officinalis* L., a species with light green spots, as examples of red and light green variegation. Both species represent models to test the hypothesis that red and light green parts of leaves are more photoprotected than are green sections, as light intensity intercepted by photosynthetic tissues of those parts could be attenuated. For this purpose, green and non-green sections of the leaves were investigated by Chl fluorescence imaging under dark and light conditions in the laboratory. Chl fluorescence imaging has been widely used to show heterogeneous distribution of light utilisation and photosynthetic activity over the leaf surface (Nedbal and Whitmarsh, 2004; Calatayud et al., 2006). The lower light interception by Chl in non-green patches may increase photoprotection, but it could also decrease photosynthetic rates, a disadvantage in extreme shade environments. The ecological meaning of such variegation patterns could be interpreted as maintaining photoprotective (non-green) parts of the leaves that could be useful during sunflecks or high light episodes, and more effective (green) parts under shade conditions. Variegated leaves could confer a drawback for photosynthetic efficiency; however, the presence of variegation in nature suggests an adaptive role. The use of variegated leaves in comparative studies is not new and has been previously employed to characterise physiological processes under controlled assays, such as stomatal function (Zeiger et al., 1980; Aphalo and Sánchez, 1986), antioxidant metabolism (Peltzer et al., 1999), air pollution (Niewiadomska and Miszalski, 1997), carbohydrate metabolism (Madore, 1990), anthocyanin function (Burger and Edwards, 1996), oxidative stress (Niewiadomska and Miszalski, 1997; Peltzer et al.,

1999) and enhanced UV-B radiation (Gaberscik et al., 2001). Studies of an anatomical basis for variegation (Tsukaya et al., 2004) have also been conducted. To our knowledge, however, this is the first study aiming to characterise the responses to light of this morphological trait and to assess the ecophysiological significance under field conditions. We speculate that higher reflectance of light green sections of *P. officinalis* or the light-filtering effect by red cells in the second *E. dens-canis* should protect photosynthetic tissues located more deeply in the leaf.

Materials and methods

Plant material, experimental design and sampling

The field study was carried out in Ozaeta (Alava, Spain) (lat. 42°55′N; long. 2°30′W) and in the surroundings at the University of the Basque Country campus in Leioa (Bizkaia, Spain) (lat. 43°17′N; long. 2°55′W). Plant material was collected in spring (April 2006 and May 2007). *E. dens-canis* plants were growing at Ozaeta in the understory of an oak (*Quercus pyrenaica* Willd.) forest and *P. officinalis* at Leioa in the understory of an oak (*Quercus robur* L.) forest. For light treatments, leaves were illuminated in the laboratory with a photosynthetic photon flux density (PPFD) of 300 μmol photons $m^{-2} s^{-1}$ for 30 min. This light dose was enough to induce a significant decrease in photochemical efficiency in shade plants. Illumination was provided by a 500 W metal halogen projector (model 906609, Massive, Barcelona, Spain). Infrared radiation was reduced using a thick glass covered with water. Field measurements were performed during the course of a sunny and bright day (during sunflecks, at noon direct PPFD at the leaf level was 481–1080 μmol photons $m^{-2} s^{-1}$) over intact plants growing in the understory of the same oak forests used for plant material collection. When sampling, leaves were separated into green and non-green sections before and after the treatment. Leaf discs (3 mm diameter) were then collected from the same leaves used for fluorescence measurements, frozen in liquid nitrogen and stored at −80 °C until biochemical analyses were performed.

Leaf reflectance

Leaf reflectance, defined as the proportion of incident light that is reflected from the leaf, was measured in whole leaves on five individual leaves from each species with a spectroradiometer (UNISPEC™, FieldSpec UV/NIR portable spectral system, PP Systems, Amesbury, USA) with the optic fibre, leaf-clip holder and reference provided by the manufacturer. Illumination was provided by a halogen light source supplied with an integral 7.0 W halogen lamp. Three replicates per plant and per section (green and non-green sections) were made at 3.3 nm intervals over a range from 425 to 725 nm. Standard error of these measurements represents, on average, 3.9% of the mean.

Microscopy

Transverse hand-cut sections of fresh leaves were taken following the method of Manetas et al. (2003). Samples were examined under a bright-field microscope (LEICA DMRB, Germany). Photographs were taken with a digital camera (Nikon Coolpix 4500, Nikon Corporation, Japan) joined to the microscope.

Fluorescence

Chl fluorescence imaging was measured at room temperature using a commercial imaging fluorometer (FluorCam, P.S.I., Brno, Czech Republic, http://www.psi.cz), as described in Nedbal et al. (2000), to study the spatial heterogeneity of photoinhibitory responses in *E. dens-canis* and *P. officinalis*. Images of photochemical efficiency were captured before and immediately after light treatment in the variegated leaves. Fluorescence was detected by a high-sensitivity charge coupled device camera equipped with an F 4.5–10 mm, 1:1.6 objective that produced images in a 12-bit grey scale. Pixel value images of the fluorescence parameters were displayed as a false colour code ranging from black (0.4) through grey to white (0.8). The instrument is driven by the FluorCam software package (FluorCam 6). First, images of the dark-adapted fluorescence level, F_o, were determined using non-actinic measuring flashes. Next, an 800 ms duration pulse of saturating light radiation (2000 μmol photon $m^{-2} s^{-1}$) was given. The maximum fluorescence level, F_m, was measured during the saturating light pulse using 12 measuring flashes. To improve the signal to noise ratio, both F_o and F_m were averaged. The maximal photochemical efficiency of photosystem II was estimated by the ratio $F_v/F_m = (F_m - F_o)/F_m$. Images of photochemical efficiency were captured before and immediately after light treatment in the variegated leaves. Each experiment was repeated five times with five different leaves.

Pigment and α-tocopherol analysis

Lipophilic antioxidants (carotenoids and tocopherols) and photosynthetic pigments were extracted and measured by reverse-phase HPLC following the method of García-Plazaola and Becerril (1999), with the modifications described in García-Plazaola and Becerril (2001). Retention times and conversion factors for carotenoids were the same as described by García-Plazaola and Becerril (1999, 2001). The de-epoxidation index was estimated by the ratio (A+Z)/(V+A+Z).

Anthocyanin determination

Six leaf discs (approximately 42 mm^2) were disrupted in liquid nitrogen and extracted in 1 mL of 3 M HCl:H_2O:MeOH

(1:3:16 by vol.) using a tissue homogeniser. The extracts were centrifuged and anthocyanin levels were estimated (as cyanindin-3-glycoside equivalents) using a molar extinction coefficient of 33,000 (Gould et al., 2000). Absorbance of anthocyanins at 524 nm was corrected by subtracting the interference by phaeophytin as A_{524}–$0.24A_{653}$ (Murray and Hackett, 1991).

Statistics

Differences between green and non-green tissues were analysed using Student's *t*-test (SPSS 11.0 statistical package), assuming statistical significance at $p<0.05$ (*) or at $p<0.001$ (**).

Results

Characterisation of variegated leaves

Pigment compositions of green and non-green patches of *P. officinalis* and *E. dens-canis* were analysed (Table 1). Carotenoid content was not significantly different between green and non-green regions of either species. The only difference found in pigment composition was the Chl content in *P. officinalis*, which was 30% lower in light green regions of the leaf ($p<0.001$). However, the Chl *a*/*b* ratio was similar among species and leaf regions. We also found that α-tocopherol (α-Toc) content was lower in the red regions of *E. dens-canis* ($p<0.05$). Anthocyanin content was significantly different between red and green regions of *E. dens-canis* ($p<0.001$).

To study leaf optical properties, the reflectance spectrum over the range 425–700 nm of green and non-green regions in leaves of both species was analysed (Figure 1). Reflectance in red patches of *E. dens-canis* was lower and more uniform than in green sections. In *P. officinalis*, reflectance was higher in light green spots, following the same pattern as in green parts.

To characterise the morphological histological structure that leads to the formation of variegated leaves, transverse sections of fresh leaves from both species were hand-cut for microscope analysis. Red patches in *E. dens-canis* were due to a single layer of red cells, located in the upper parenchyma of the adaxial surface, which did not modify leaf thickness (Figure 2A). Only the accumulation of anthocyanins accounted for the reddish colour of the patches. Surprisingly, light green spots in *P. officinalis* were attributed to the presence of loosely arranged cells instead of a well-established layer of packed cells in the palisade parenchyma (Figures 2B and C). However, this characteristic did not significantly modify the thickness of the light

Table 1. Pigment composition of *E. dens-canis* and *P. officinalis* in green and non-green tissues ($n = 5$ replicates ± SE)

Species	Tissues	Carotenoids and tocopherol (mmol mol^{-1} Chl *a*+*b*)					Chl *a*/*b*	Chl *a*+*b* (μmol m^{-2})	Anthocyanin (μmol m^{-2})
		N	V+A+Z	L	β-Car	α-Toc			
E. dens-canis	Red	40.9 ± 0.65	81.9 ± 5.19	127.3 ± 0.78	116.9 ± 10.91	49.6 ± 2.57	2.5 ± 0.02	365 ± 9.3	194.76 ± 2.54
	Green	39.4 ± 0.62	87.4 ± 5.68	128.9 ± 1.73	111.8 ± 1.85	61.3 ± 3.18*	2.5 ± 0.03	339 ± 13.5	49.19 ± 6.37***
P. officinalis	Light green	41.1 ± 1.24	65.6 ± 5.45	137.6 ± 5.13	116.6 ± 1.31	119.4 ± 29.3	2.4 ± 0.08	170 ± 7.6	
	Green	42.3 ± 0.91	66.3 ± 6.20	135.2 ± 4.51	114.9 ± 1.24	92.8 ± 17.9	2.4 ± 0.06	249 ± 14**	

Asterisks indicate significant differences between green and non-green tissues within the same species (Students's *t*-test: *$p<0.05$; **$p<0.01$; ***$p<0.001$).

green regions (350 μm light green regions and 370 μm green regions), but it certainly modified the density of the tissue, which could potentially have an effect on light penetration.

Effect of light treatment

To study the photoprotection mechanisms in green and non-green sections of the two species (Figure 3A), leaves were exposed to PPFD 300 μmol photons $m^{-2} s^{-1}$ for 30 min and Chl fluorescence imaging was carried out before (Figure 3B) and after light treatment (Figure 3C). Before light treatment, no differences were observed between leaf regions of the two species. However, after light exposure, red and light green parts showed a greater decrease in F_v/F_m than their respective green parts (Table 2), pointing to greater light sensitivity in non-green tissues. Changes in xanthophyll composition were measured in parallel in green and non-green sections of both species. We did not detect significant differences in the de-epoxidation index (A+Z/VAZ) after light treatment between green and non-green sections of the two species (Table 3).

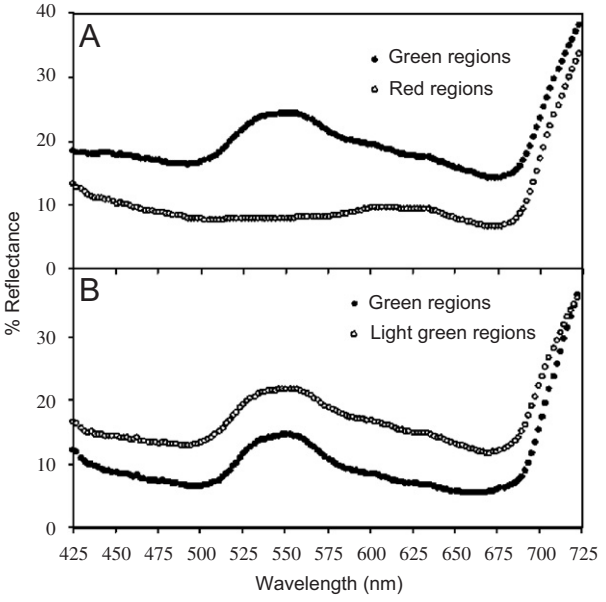

Figure 1. Spectral reflectance over the range 425–725 nm for green and non-green regions of *E. dens-canis* (A) and of *P. officinalis* (B). Each line is the average of three different scans on five different leaves.

Field experiments

It could be argued that the higher sensitivity to light excess of non-green sections may be a laboratory effect without any ecological relevance. To evaluate whether this difference is also maintained in the field, Chl fluorescence imaging was performed on intact plants growing in the understory of an oak forest during the course of a sunny and bright day. Interestingly, the leaf pattern of F_v/F_m in *E. dens-canis* (Figure 4A and B) imitated that which was observed under controlled conditions (Table 2 and Figure 3C), with greater decrease of photochemical efficiency in red patches. However, in *P. officinalis* (Figure 4C and D) photochemical efficiency was similar in green and light green sections.

Discussion

Characterisation of variegated leaves

In the present study, we evaluated photoprotective strategies in green and non-green sections of two species with different patterns of variegation: *P. officinalis*, with light green circular portions, and *E. dens-canis* with red irregular sections. Variegated leaves of both species were initially characterised in detail. In *P. officinalis*, light green variegation occurred due to the presence of loosely packed cells, not affecting the thickness of the leaves (Figure 2C), while green tissues showed a typical layer of densely packed palisade

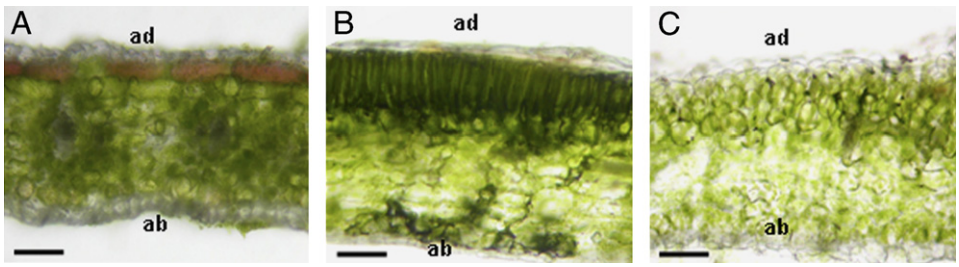

Figure 2. Bright-field micrograph showing a transverse section of a fresh leaf of *E. dens-canis* (A), green (B) and light green (C) leaf portions of *P. officinalis*. Bars = 100 μm.

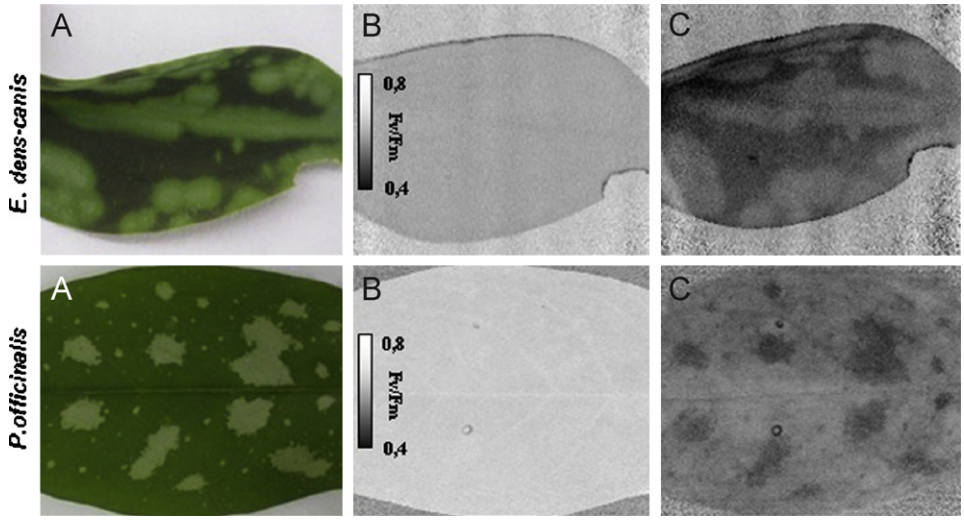

Figure 3. Effect of illumination (300 μmol photons $m^{-2} s^{-1}$) on photochemical efficiency in variegated leaves *E. dens-canis* and *P. officinalis*. Panel (A) (first column) shows the colour pattern photograph of a typical leaf of both species. Panels (B) (second column) and (C) (third column) show the results of chlorophyll fluorescence imaging (F_v/F_m) performed in the same leaves before (B) and after (C) light treatment. The false colour code depicted at the left of the panel (B) ranges from 0.8 (white) to 0.4 (black). Note the correspondence between non-green parts and photoinhibited (lower F_v/F_m) sections. The experiment was repeated five times for each species and the same correspondence between non-green patches and photoinhibited portions was observed in all samples.

Table 2. Effect of illumination (300 μmol photons $m^{-2} s^{-1}$) on photochemical efficiency in green and non-green tissues of variegated leaves *E. dens-canis* and *P. officinalis*

Species	Tissues	Photochemical efficiency	
		Before treatment	After treatment
E. dens-canis	Red	0.773 ± 0.004	0.568 ± 0.013
	Green	0.781 ± 0.004	0.653 ± 0.012**
P. officinalis	Light green	0.709 ± 0.005	0.473 ± 0.013
	Green	0.709 ± 0.005	0.577 ± 0.014**

Data have been calculated from the fluorescence images by the software Fluorcam 6. Each value represents the mean of 15 values ± SE. Asterisks indicate significant differences between green and non-green tissues within the same species (Students's *t*-test:*$p < 0.05$; **$p < 0.01$).

Table 3. Effect of light treatment (300 μmol photons $m^{-2} s^{-1}$) on de-epoxidation index in green and non-green tissues of *E. dens-canis* and *P. officinalis* ($n = 5$ replicates ± SE)

Species	Tissues	(A+Z)/(V+A+Z)	
		Before light treatment	After light treatment
E. dens-canis	Red	0.06 ± 0.01	0.39 ± 0.07
	Green	0.07 ± 0.02	0.40 ± 0.07
P. officinalis	Light green	0.00 ± 0.00	0.67 ± 0.04
	Green	0.00 ± 0.00	0.63 ± 0.05

Asterisks indicate significant differences between green and non-green tissues within the same species (Students's *t*-test: **$p < 0.01$).

parenchyma cells (Figure 2B). As a consequence, Chl content in light green parts of *P. officinalis* was 30% lower than in green tissues (Table 1), as has also been shown by Gaberscik et al. (2001). This pattern of variegation significantly reduced photoprotective molecules (α-Toc, β-carotene and VAZ pigments) by unit area in light green tissues, but ratios to Chl remained unchanged. This contrasts with other cultivated species for which there is a complete lack of Chl in white regions, as is the case of variegated leaves of *Hedera helix* (Aphalo and Sánchez, 1986) and *Hedera canariensis* (Soldatini et al., 1998). Hara (1957) described an "air space" variegation type in leaves of many plant genera, such as *Arisaema*, *Begonia*, *Clematis*, *Cyclamen*, *Ornithogalum*, *Pyrola*, *Saxifraga* and *Viola*. This variegation is produced through variations in palisade-cell development. In this situation, fully green leaf parts have tightly arranged, column-shaped palisade cells, while greyish-green parts have rounded palisade cells with air spaces between them. The higher reflectance of light

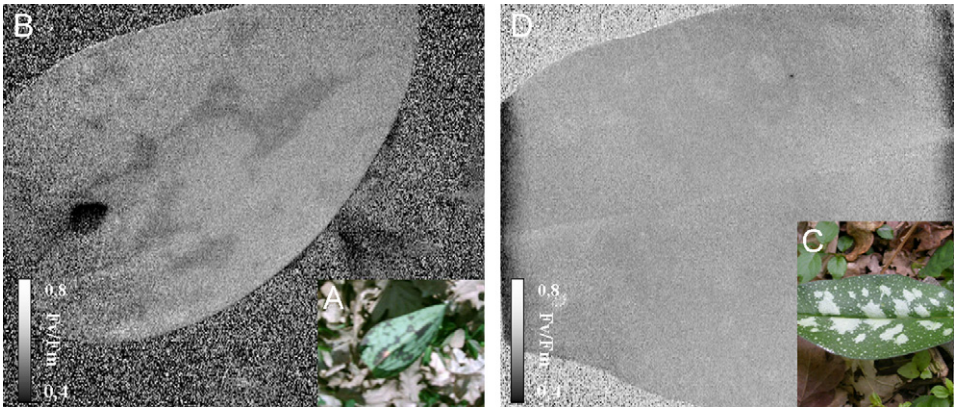

Figure 4. Field appearance of variegated leaves and chlorophyll fluorescence imaging, showing F_v/F_m of an intact leaf of the same plant, performed in the field. Panels (A) and (C) show the colour pattern photograph of a typical leaf of *E. dens-canis* and *P. officinalis*, respectively. Panels (B) (*E. dens-canis*) and (D) (*P. officinalis*) show the results of chlorophyll fluorescence imaging (F_v/F_m) performed in the same leaves. The false colour code depicted at the left of panel (B) ranges from 0.8 (white) to 0.4 (black). Note the correspondence between non-green parts and photoinhibited (lower F_v/F_m) sections in *E. dens-canis*. The experiment was repeated five times with the same result.

green surfaces (Figure 1) would be generated by air spaces due to the presence of loosely arranged round cells (Figure 2C) (Tsukaya et al., 2004). Gaberscik et al. (2001) concluded that, under UV-B, light green spots of *P. officinalis* became less transparent to PAR, likely due to a structural change in the mesophyll. The case of *E. dens-canis* is, to a certain extent, different because the reddish variegation was due to the presence of a single layer of red cells in the upper parenchyma, which accumulated anthocyanins in the vacuole (Figure 2A). The presence of a layer of red-coloured anthocyanic cells together with the lower α-Toc content of red sections support a role for red pigments as antioxidants (Kytridis and Manetas, 2006) or attenuating visible light, as has been shown in several studies (Burger and Edwards, 1996; Woodall et al., 1998).

Effect of light

Our initial hypothesis that non-green parts are more photoprotected than green ones was tested by a mild photoinhibitory treatment. Before treatment, F_v/F_m values were between 0.7 and 0.8 in green and non-green leaf areas of both species (Figure 3B and Table 2), indicating the absence of photoinhibited regions (Björkman and Demming, 1987). However, after light exposure, F_v/F_m decreased, indicating a sustained loss of PSII efficiency. Interestingly, this decrease was not uniform and there was a strong correspondence between non-green sections and photoinhibited portions (lower F_v/F_m) in both species (Figure 3C and Table 2). This unexpected result is partly consistent with a previous study by Soldatini et al. (1998), who showed that white sections of *Hedera canariensis* had lower F_v/F_m and F_m than green sections after ozone fumigation. The loss of PSII efficiency was not due to differential activation of the xanthophyll cycle, as the de-epoxidation index did not differ between sections in the two species (Table 3), suggesting the activation of other mechanisms in the reaction centre of the non-green tissues. This indicates that, in the case of *E. dens-canis*, anthocyanins did not increase photoprotection as has been found in red portions of variegated *Coleus* (Burger and Edwards, 1996) and in other non-variegated species where red leaves exhibited higher levels of chronic photoinhibition than green leaves (Dodd et al., 1998; Williams et al., 2003). Because the induction of anthocyanin synthesis has been described in plant tissues subjected to different stress conditions (Chalker-Scott, 1999; Hasegawa et al., 2001; Steyn et al., 2002; Close and Beadle, 2003, 2005; Manetas, 2006), we hypothesize that the presence of these compounds in the more photosensitive cells could be the result of an induction of photoprotective compounds. This would mean that the accumulation of red anthocyanins in this species, more than a preventive strategy, is the consequence of a higher susceptibility to photoinhibition. In the case of *P. officinalis*, the loosely packed cells, instead of a well-established palisade layer (Figure 2C), increased reflectance of the upper surface, but this attenuation did not manifest strongly enough to compensate the light filter as seen in green tissues by palisade to mesophyll cells located below (Figure 2B). As a result, light green sections were also more susceptible to photoinhibition since

mesophyll cells were consequently exposed to a higher irradiance.

Ecological relevance

These experiments clearly reject the initial hypothesis that non-green sections are more photoprotected than green sections, but questions the physiological reasons for their higher sensitivity and the ecophysiological implications of variegation. In order to address this question and to exclude the possibility that the observed higher light sensitivity of non-green portions could be the result of a laboratory effect, we performed fluorescence imaging on intact *E. dens-canis* and *P. officinalis* plants growing in the understory of oak forests during the course of a sunny day. Interestingly, the same pattern of a decrease of photochemical efficiency demonstrated in laboratory essays was observed in red patches of *E. dens-canis* (Figure 4A and B), meaning that variegation is maintained in these species at the expense of a decrease on photochemical efficiency. In the case of *P. officinalis* (Figure 4C and D), this effect was not observed, showing uniform F_v/F_m throughout the entire leaf surface. Thus, it implies that in this species the effect of variegation in photoprotection, if any, is negligible under most environmental conditions. However, in *E. dens-canis*, light-saturated rates of photosynthesis could be similar in green and variegated portions, and shifts to shade conditions should result in lower carbon assimilation in photoinhibited sections. Anthocyanins have likely been co-opted over the course of evolution to perform an array of tasks (Gould et al., 2002). Thus, apart from a photoprotective role, alternative explanations, such as defence from herbivores (Schaefer and Wilkinson, 2004; Karageorgou and Manetas, 2006), could also be considered. Since these species typically occur in the forest understory, changes in light conditions due to the occurrence of intermittent sunflecks characterise the light environment under which these plants developed. In consideration of the present data, we conclude that the red and light green variegation play different functions under field conditions and the benefits of variegation, if any, are different from the explanation of better photosynthetic performance.

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