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The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light

PANAGIOTA KARAGEORGOU[1] and YIANNIS MANETAS[1,2]

[1] *Laboratory of Plant Physiology, Department of Biology, University of Patras, GR-26500 Patras, Greece*

[2] *Corresponding author (y.manetas@upatras.gr)*

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Summary Young leaves of many plants are transiently red because of the accumulation of anthocyanins, with the redness disappearing as leaves mature. Among the many hypothetical functions of foliar anthocyanins, two are tested in this field study: the sunscreen photoprotective function against excess visible light and the handicap signal against herbivory. We took advantage of intraspecies variation in anthocyanin concentrations of young leaves of *Quercus coccifera* L. to compare in vivo chlorophyll fluorescence parameters, reflectance spectra, total phenolics and the extent of herbivory of leaves of red- and green-leaved phenotypes occupying the same habitat. Photosystem II (PSII) photochemical efficiencies obtained at various photon fluence rates of red light were similar in green and red leaves. In white light, PSII efficiencies were slightly higher in red leaves than in green leaves, indicating a slight photoprotective role of anthocyanins in the field. However, compared with red phenotypes, green phenotypes suffered greater herbivore damage, as judged by the number of leaves attacked and the area lost to herbivory. In addition, there was a positive correlation between the concentrations of anthocyanins and total phenolics. We suggest that the importance of a photoprotective anthocyanic screen is low in thin, young leaves with low chlorophyll concentrations because the green light attenuated by anthocyanins is less significant for chlorophyll excitation. However, the decreased reflectance in the green spectral band and the concomitant leveling of reflectance throughout the 400–570 nm spectral range may either make red leaves less discernible to some insect herbivores or make insect herbivores more discernible to predators, or both. Moreover, excessive herbivory may be additionally discouraged by the high phenolic concentrations in red leaves.

Keywords: handicap signal, herbivory, insect vision, phenolics, photoinhibition.

Introduction

It is generally accepted that anthocyanins (together with other pigments) in flowers and fruits provide optical guides to animals facilitating pollination and seed dispersal. Anthocyanins are also present in leaves and in some cases their concentrations are high enough to mask the green coloration due to chlorophyll. Because anthocyanins absorb visible light without participating in photosynthesis, their presence in leaves should reduce the probability of photon capture by chlorophylls thereby lowering photosynthesis, an effect that may be of adaptive significance. However, the possible role(s) of anthocyanins in leaves is obscure as is reflected in the numerous hypotheses that have been proposed concerning their function(s). Foliar anthocyanins have been correlated with resistance to biotic and abiotic agents like fungi, herbivores, cold and excess radiation, both UV and visible (see reviews by Chalker-Scott 1999, Gould et al. 2000, Hoch et al. 2001, Gould et al. 2002, Steyn et al. 2002, Close and Beadle 2003).

In most cases, the coloration of leaves by anthocyanins is transient, being expressed in young, developing leaves or old, senescing leaves. In addition, it can be induced in mature leaves of some plants by nutrient deficiency (Hodges and Nozzolillo 1996), UV-B radiation (Mendez et al. 1999) and high light (Krol et al. 1995), especially when temperatures are low (Christie et al. 1994). Abiotic stresses predispose mature leaves to photoinhibition of photosynthesis (Long et al. 1994). The finding that developing leaves may be susceptible to photoinhibition because of their immature photosynthetic machinery (Miranda et al. 1981) and senescing leaves may be susceptible because of the risk of reactive oxygen species production during chlorophyll breakdown (Matile 2000) led to the hypothesis that an anthocyanic sunscreen affords protection against excess light. The photoprotective hypothesis has been tested experimentally several times, yet with conflicting results. Thus, a correlation between tolerance to photoinhibition and anthocyanin accumulation was suggested in some studies (Gould et al. 1995, Krol et al. 1995, Mendez et al. 1999, Gould et al. 2000, Feild et al. 2001, Manetas et al. 2002) but not in others (Burger and Edwards 1996, Lee et al. 2003). Moreover, Manetas et al. (2003), working with the same species used in

the present study, found red leaves were more tolerant to photoinhibition than green leaves under laboratory conditions, yet the extent of photoinhibition was similar in these leaf types in the field, as judged by predawn measurements of PSII photochemical efficiency.

A second hypothetical function of foliar anthocyanins is that they participate in defense mechanisms against herbivory. Insects may show preferences for healthy green leaves for food or oviposition (Prokopy and Owens 1983). In addition, anthocyanin-rich leaves may contain high concentrations of other phenolic compounds, as found in young leaves of some tropical plants (Lee and Lowry 1980) and *Eucalyptus* seedlings (Close et al. 2001), because anthocyanins share common initial steps with other phenolics in the phenylpropanoid biosynthetic pathway (Winkel-Shirley 2002). Some phenolics serve as potent deterrents against generalist herbivores (Feeny 1970, Bennett and Wallsgrove 1994) and it has been proposed that the presence of anthocyanins in old, senescing leaves may be an optical warning signal (handicap signal) against consumers, indicating the co-occurrence of potentially defensive phenolic compounds (Hamilton and Brown 2001, Lev-Yadun 2001). Although evidence from the colonization preferences of aphids on senescing red or yellow-orange leaves of *Acer palmatum* Thunb. (Furuta 1986, 1990) and insect damage on leaves of the following season in *Betula pubescens* J. F. Ehrh. (Hagen et al. 2003) provide support for this hypothesis, its validity has been questioned on several grounds, including the ability of folivorous insects to perceive red as humans do (Wilkinson et al. 2002, Schaefer and Wilkinson 2004). Numata et al. (2004) reported preferential damage by insects to young, fast-greening leaves compared with slow-greening red and pink leaves of tropical species within the genus *Shorea*.

We conducted field tests of both the photoprotective and the handicap signal hypotheses in the Mediterranean evergreen sclerophyll *Quercus coccifera* L., which displays intraspecies variation in anthocyanin concentrations in young, developing leaves, whereas mature leaves are invariably green. Red- and green-leaved individuals occupy the same habitat, providing the opportunity to compare aspects of photosynthesis as well as the extent of herbivory and phenolic concentrations in plants subjected to the same set of environmental parameters. We predicted that, if the primary role of anthocyanins were photoprotective, red leaves would have higher PSII photochemical efficiencies than green red leaves. Alternatively, less damage by insects to red leaves than to green leaves would favor the antiherbivore hypothesis.

Materials and methods

Plant material and sampling sites

Quercus coccifera is a characteristic Mediterranean evergreen sclerophyll tree. Individuals within this species can be distinguished by the redness of developing leaves, which display a full range of tints from dark red to green. In red leaves, anthocyanins reside in two adaxial and abaxial sub-epidermal layers (Manetas et al. 2003). Although in some cases green and reddish twigs co-occur on the same individual, the developmental pattern of leaf coloration in each plant is relatively constant (author's unpublished observations). New leaves expand during the spring (early April to early June depending on the altitude and prevailing temperatures) at the apex of older branches, and are thus fully exposed to direct solar radiation.

We used two populations for our measurements and observations, one at a lowland site (38.14° N, 21.44° E, 350 m a.s.l.) and one at a montane site (38.01° N, 22.13° E, 1500 m a.s.l.). Both sites are open and sampling areas were about 500 × 500 m. The sites were repeatedly visited during the springs of 2002, 2003 and 2004 (at least once per week at the lowland site and once per month at the montane site) to confirm the constancy of the pattern of leaf coloration of tagged individuals and to make preliminary, qualitative (naked eye) assessments of the extent of herbivory. Detailed quantitative assessment of leaf anthocyanins, phenolics and herbivory were performed at both sites during 2004. Fluorescence measurements were made at the lowland site during the springs of 2003 and 2004. The two sites were un-grazed by domestic mammals, but insect herbivory was apparent. For all measurements, the leaves were sampled 1.5–2.0 m aboveground.

Chlorophyll fluorescence measurements

Effective PSII efficiency (as $\Delta F/F_m' = (F_m' - F)/F_m'$ where F is initial fluorescence yield and F_m' is maximal fluorescence yield during a saturating light pulse according to Genty et al. (1989), light-adapted state) was measured around midday (1100–1300 h) during clear days, with a pulse-amplitude modulated fluorometer (MINI-PAM, Walz, Effeltrich, Germany). Because young leaves of *Q. coccifera* are small (about 0.5 cm^2) with short petioles, it was not possible to use the 2030-B leaf clip holder of the fluorometer. Hence, the leaves were detached and immediately (within seconds) inserted into a dark clip. The dark clip was used in conjunction with the built-in function of the so-called "rapid light curves." The program allows the recording of fluorescence responses to steps of increasing and predetermined actinic irradiances of 10-s duration, separated by 0.8-s saturated pulses. Actinic light was generated by the internal white light source of the instrument. Light curves were recorded in white light and in red light. For red light, a colored cellulose filter was inserted in the dark clip between the leaf and the instrument's light guide. We assumed that by using red actinic light (which is not absorbed by anthocyanins), we provided mesophyll chloroplasts of both green and red leaves with the same photon fluence rates. This assumption is based on studies by Karabourniotis et al. (1999) and Neill and Gould (1999) who characterized the internal light environment of intact leaves differing in anthocyanin and chlorophyll concentrations and concluded that only the green spectral band was affected. Spectral transmittance of the red filter was measured with an Optronic (Orlando, FL) spectrometer equipped with a Taylor-type integrating sphere. The filter was located in the entrance port and a Spectralon standard (reflectance > 0.97) covered the exit port. For comparative purposes, a transparent cellulose acetate filter (transmittance

> 90% throughout the visible spectral band) was similarly inserted in the dark clip during recording of light curves recorded in white light.

Because fluorescence measurements were performed in the field, there was an unavoidable decrease in lamp output with decreasing battery voltage. Therefore, we calibrated the actinic light under the same conditions for each step and for each filter by inserting the quantum sensor of the 2030-B leaf clip into the dark clip and triggering series of virtual light curves covering the whole range of useful battery voltages. The incident photosynthetically active radiation (PAR) for each step of the light curves was corrected according to the battery voltage monitored by the instrument.

Yield measurements were used to calculate the linear electron transport rate (ETR) along PSII according to the formula $ETR = \Delta F/F_m' (PAR) A 0.5$ (Genty et al. 1989), where A is the fraction of incident photons absorbed by the photosynthetic pigments (i.e., absorptance) and 0.5 holds for an assumed equal distribution of absorbed photons between the two photosystems. This formula is valid only in the absence of non-photosynthetic pigments absorbing in the photosynthetically active band (i.e., green leaves in white or red light) or when the spectral band of incident light is not absorbed by anthocyanins (red leaves in red light). Because the small leaf size precluded actual absorptance measurements, we used a value of 0.8 for green leaves in white light and 0.85 for both green and red leaves in red light. In the case of red light, we arbitrarily used a slightly greater value for absorptance because the filter mainly transmits red photons, which are better absorbed by chlorophyll. Chlorophyll concentrations in the two leaf types were similar (Manetas et al. 2003).

Fluorescence parameters on each sampling date were measured in south-facing, exposed leaves of similar size from 8–10 trees per phenotype and 3–6 leaves per individual tree (green or red) and actinic light quality (white or red). Red and white light curves were completed on each tree before proceeding to the next tree. For fluorescence measurements, only trees whose young leaves were at one or the other end of the color gradient (either dark red or green) were used.

Spectral reflectance of leaves

Spectral reflectance of intact leaves was recorded with a diode array spectrometer (Unispec, PP Systems, Haverhill, MA) equipped with a small diameter (2.3 mm) bifurcated fiber optic cable, an internal halogen source and an appropriate leaf clip. A spectralon (reflectance > 97%) standard was used as the reference and the spectra were dark corrected for stray light with the internal source off.

Sampling for phenolics and herbivory

Sampling was performed 2–3 weeks after leaf burst (i.e. during late April or late May from the lowland and montane site, respectively). Young shoots were cut, put into air-tight plastic bags and transferred to the laboratory for analysis within 15 and 120 min from the low and high altitude site, respectively.

For assessing whether the presence of anthocyanins was associated with high phenolic concentrations, the whole-leaf color gradient was used, i.e., shoots were harvested not only from dark red or green individuals, but from plants displaying intermediate redness as well. Southeast-facing shoots with no signs of damage by herbivores were used. The 3rd to 4th leaves from the tops of shoots from each individual were pooled and, after determination of their areas (LI-3000 leaf area meter, LI-COR, Lincoln, NE), extracted as described below, and similar leaves were used for the determination of leaf dry mass per area. For dry mass, leaves of known size were dried in an oven at 80 °C to constant mass.

To assess the extent of herbivory, plants whose young leaves were at one or the other end of the color gradient were used (i.e., either dark red or green). Thirteen and 26 plants per phenotype were sampled from the lowland and the montane site, respectively. Ten shoots facing southeast were randomly removed from each plant and brought to the laboratory, five of them were selected by a random process for further measurements and the rest were discarded.

Extraction and determination of anthocyanins and phenolics

Extraction of total phenolics was performed in boiling methanol:H_2O:HCl (90:1:1 v/v/v) for 10 min (Day 1993). After centrifugation, absorbance spectra of the clear supernatants were recorded with a Shimadzu UV-160A double beam spectrophotometer (Shimadzu Deutschland GmbH, Duisburg, Germany) from 250 to 700 nm. The relative amounts of anthocyanins were estimated in the same extracts from their peak absorbance (530 nm) after correction for interference by chlorophyll pigment at this wavelength (Mancinelli et al. 1975). Values were normalized for a 1-cm light path and 1 cm^3 of extract obtained from 1 cm^2 of leaf area. For total phenolics, the method of Levizou and Manetas (2002) was used. Regression equations correlating absorbance of leaf methanolic extracts at 300 nm to chemically determined total phenolics (as tannic acid equivalents) according to the Folin-Ciocalteu method (Waterman and Mole 1994) were prepared for a subset of the sample ($y = 0.0405x - 0.019$, $r^2 = 0.878$, $P < 0.0001$, where y denotes total phenolics and x denotes the absorbance at 300 nm, both on a leaf area basis). Subsequently, total phenolics in the rest of the sample were assessed based on their absorbance at 300 nm, in conjunction with the same regression equation.

To assess the absorbance spectrum of anthocyanins in the absence of interference by photosynthetic pigments, the crude methanolic extract was partitioned with diethylether and diluted with water. Chlorophyllous pigments and carotenoids migrated into the ether layer, whereas anthocyanins and other phenolics remained in the methanol:water phase.

Herbivory

After counting the number of attacked leaves, the leaves were cut and scanned with a Hewlet-Packard scanner (Scanjet 6200C, Houston, TX, USA). The area lost to herbivores was computed on both a per shoot and per attacked leaf basis with the Image-Pro Plus 4.5 program.

Statistics

For each measured parameter, a mean value for each individual was established and the number of individuals used are reported in the figure and table legends. The PSII yield versus PAR curves were treated as exponential decays and the null hypothesis of one curve fitting all data sets (Motulsky and Ransnas 1987) was assessed by an *F* test. Differences in extent of herbivory between red and green leaves were assessed by a one-way analysis of variance (ANOVA). All of the statistical analyses were performed with the SPSS v.12.0 statistical package (SPSS, Chicago, IL).

Results

PSII efficiency and electron transport rates of red leaves in the field

Partially purified extracts of anthocyanins from young leaves absorbed maximally in green light, less in blue light and were almost transparent to red light (Figure 1), indicating that light entering the mesophyll of red leaves is red-enriched, because photons in the green and, to a lesser extent, in the blue band are attenuated by the anthocyanins located in the sub-epidermal layers. Young green leaves displayed considerable in vivo reflectance in green light (about 20% at the peak), whereas in red light the green peak disappeared and maximum reflectance shifted to a broad band of lower reflectance (about 7%) in the orange–red (610–640 nm) region of the spectrum (Figure 2). These data indicate that the presence of anthocyanins in young leaves of *Q. coccifera* affected reflectance mainly in the green–yellow region and, to a much lesser extent, in the orange region, whereas reflectance in the blue and red regions were almost unaffected by the presence of anthocyanins.

Figure 1 also shows the transmittance spectrum of the red cellulose filter used to illuminate the leaves during chlorophyll fluorescence measurements. As indicated, the filter had considerable transmittance in the red, less transmittance in the blue, and virtually none in the green. The two spectra show an apparent complementarity, implying that illumination of a red or green leaf through a red filter results in similar irradiances in the mesophyll, because anthocyanins do not absorb in the red band. Accordingly, this red-enriched, green-depleted light can be used to compare the inherent photosynthetic properties of red and green leaves.

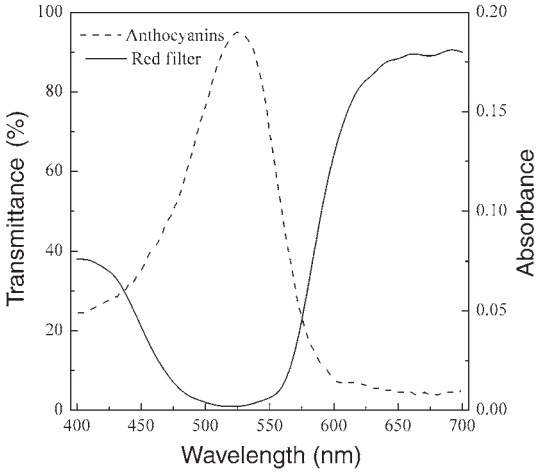

Figure 1. Spectral absorbance of *Quercus coccifera* leaf anthocyanins (dashed line) and transmittance (%) of the red filter (solid line) used to illuminate leaves during determination of photosystem II yield, along the photosynthetically active band of the spectrum.

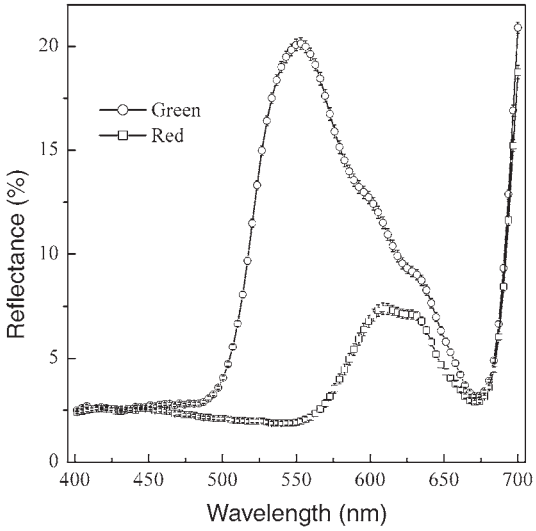

Figure 2. Spectral reflectance of green (○) and red (□) young leaves of *Quercus coccifera* along the photosynthetically active band of the spectrum. Data are means ± SEM of 25 leaves.

In red light, the PSII yield versus PAR curves of fully light-adapted leaves (i.e., measurements made at midday on clear days) were similar for both leaf types (Figure 3a). Similar PSII yields in red leaves and green leaves implies similar ETR versus light curves, provided that the *A* of the two leaf types is similar. This is a reasonable assumption because *A* in red actinic light is defined by chlorophyll concentrations, which do not differ between the two leaf types (Manetas et al. 2003). Accordingly, the corresponding light saturation curves (Figure 3b) displayed the same initial slope at low photon fluence rates for ETR saturation and maximum ETR. In addition, a tendency for decreasing ETRs was evident above about 800 μmol m^{-2} s^{-1}. We predicted that, in white light, apparent PSII yield would be higher in red leaves than in green leaves, because part of the incident light is attenuated by anthocyanins before it penetrates the mesophyll (Pietrini et al. 2002). This was observed over the entire range of photon fluence rates tested (Figure 3a) and the differences between leaf types were statistically significant ($P < 0.05$).

Red leaves and green leaves differ in susceptibility to damage by herbivores and in phenolic concentration

Young green leaves were frequently attacked by herbivores, with almost 60% of the leaves showing signs of damage at both sampling sites (Figure 4), whereas corresponding values for red leaves were 37 and 19% for the low altitude site and

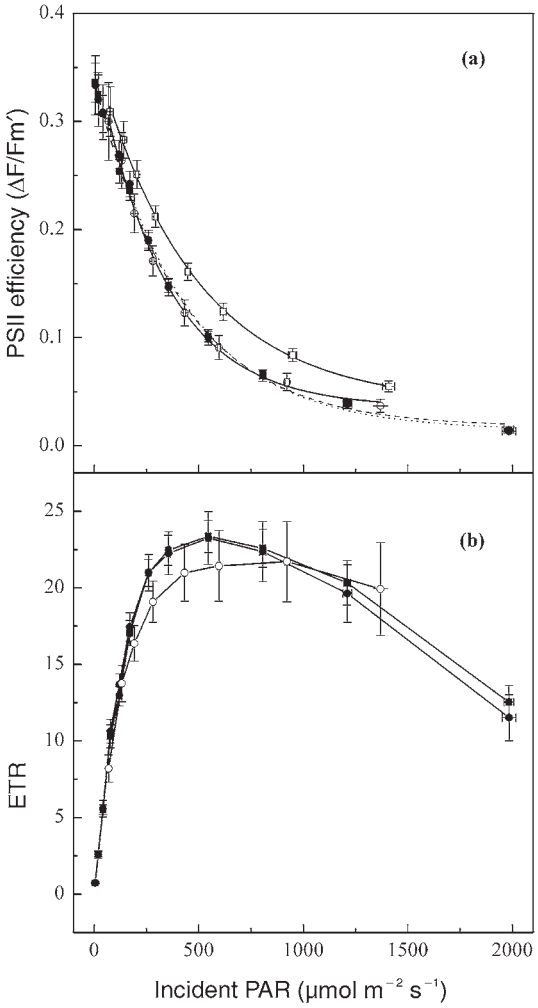

Figure 3. Light dependence of photosystem II (PSII) photochemical efficiency (a) and corresponding electron transport rates (ETR; b) of young green (○, ●) and red (□, ■) leaves of *Quercus coccifera*, illuminated with white (empty symbols) and red (filled symbols) light. Values are means ± SEM of nine individuals (with 3–6 leaves measured per individual). The experiment was performed around midday on a clear day (April 21, 2004) with leaves exposed to ambient photosynthetically active radiation (PAR) of about 1400 μmol m^{-2} s^{-1} (i.e., with leaves adapted to high irradiance). Similar experiments performed on different dates during 2003 and 2004 gave similar results. The differences in the data sets between red leaves treated with white light and the rest of the treatments were significant ($P < 0.005$). The ETR versus PAR curve for red leaves in white light is missing because of uncertainties in the contribution of anthocyanins to absorbed PAR.

montane site, respectively. In addition, the area consumed per attacked leaf was significantly higher in green leaves than in red leaves at the montane site (18 versus 13%), a similar trend was observed at the low altitude site but the difference was not statistically significant (Figure 4). When the area lost to herbivores was expressed on a branch basis (i.e., taking into account the intact leaves), the differences between leaf types were statistically significant for both sampling sites and the area lost in green leaves compared with red leaves was almost 2-fold higher at the low altitude site and more than 3-fold higher at the high altitude site (Figure 4). Hence, it seems that red leaves were not only less frequently attacked than green leaves, but also less severely attacked.

A reason why red leaves subject to herbivory were less severely attacked than green leaves may be their high phenolic concentration. Regression analysis of the concentration of anthocyanins versus total phenolics indicated that red leaves had high phenolic concentrations at both sampling sites, although the statistical significance of the difference was weaker at the montane site. The result is not an artifact of anthocyanin absorbance at 300 nm because extinction coefficients of non-acylated anthocyanins are lower in the UV region compared with in the green part of the spectrum (Woodall and Stewart 1998). When acylated, extinction coefficients in the two spectral bands are about equal (Giusti et al. 1999). Assuming that anthocyanins in our test plant were acylated, their calculated contribution to A_{300} was less than 2%.

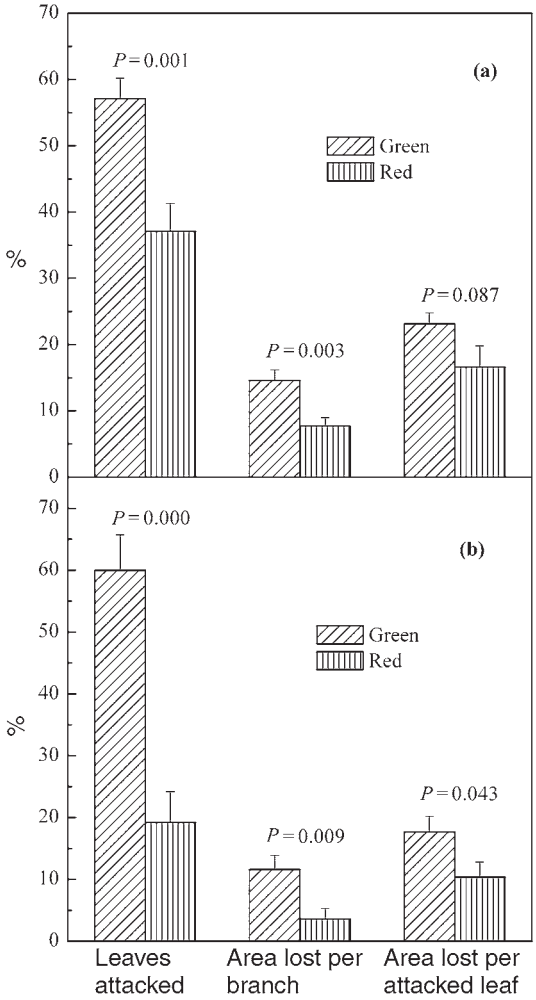

Figure 4. Percentages of leaves subject to herbivory (attacked) and area lost to herbivores on young green and red leaves of *Quercus coccifera* at the low (a) and high (b) altitude sites. Data are means ± SEM of 13 and 26 individuals per phenotype at the low and the high altitude site, respectively. The significance (*P*) of the differences between green shoots and red shoots is shown above each data set.

Discussion

Anthocyanins as sunscreens

An ideal sunscreen against photoinhibition should attenuate light at the wavelengths of maximum chlorophyll absorption. Anthocyanins, however, absorb in the green region, i.e., in the spectral region where the probability of photon capture by chlorophylls is minimal, indicating that anthocyanins do not have ideal optical properties for a sunscreen against photoinhibition, unless green light is photosynthetically harmful. However, action spectra for photoinhibition do not support this notion (Jones and Kok 1966). In addition, the contribution of green light to photosynthesis becomes increasingly important with leaf depth because blue and red photons are absorbed by chlorophylls in the first mesophyll cell layers (Nishio 2000), whereas green light is absorbed and used photosynthetically deeper in the leaf. For example, in a thick (about 750 µm) spinach leaf, the contribution of blue or red light to CO_2 fixation is much higher compared with green light in the first 150 µm of depth, whereas the situation is reversed beyond a depth of about 250 µm extending to the lower epidermis (Sun et al. 1996). Apparently, the contribution of green light to total leaf photosynthesis increases with increasing leaf thickness and decreasing area-based chlorophyll concentration. Leaf absorptance in the green band decreases considerably with leaf thickness and chlorophyll concentration, whereas leaf absorptance in the blue and the red bands is not appreciably changed (Moss and Loomis 1952). Thus, the attenuation of green light by anthocyanins in thin leaves with low chlorophyll concentrations may be insufficient to affect photosynthesis or protect against photoinhibition. The effect of leaf thickness (or chlorophyll cross section) on the effectiveness of green light has been overlooked in previous studies of leaf anthocyanins and may underlie some contradictions in the literature. For example, photosynthesis in red leaves compared with green leaves was found to be either lower (Choinski and Johnson 1993, Burger and Edwards 1996, Dodd et al. 1998), higher (Gould et al. 1995) or similar (Pietrini et al. 2002). Furthermore, the risk of photoinhibition in anthocyanic leaves may be lower (Gould et al. 1995, Krol et al. 1995, Mendez et al. 1999, Feild et al. 2001, Pietrini et al. 2002) or similar (Burger and Edwards 1996, Dodd et al. 1998, Havaux and Kloppstech 2001, Lee et al. 2003) to that in green leaves. The works of Dodd et al. (1998) and Lee et al. (2003) were performed with low chlorophyll, juvenile or senescing leaves, respectively. In the *Q. coccifera* young leaves that we studied, leaf thickness was less than 200 µm and chlorophyll concentration was less than 11 µg cm^{-2}, whereas corresponding values for a mature leaf were 2-fold and 4-fold higher, respectively (Manetas et al. 2003). We argue that the extent of photoprotection by green-light-absorbing anthocyanins may depend on leaf thickness and the depth of penetration of green light. Accordingly, the importance of anthocycanins as photoprotectants would diminish with leaf thickness.

Light-adapted PSII yields in both young red and young green leaves of *Q. coccifera* were similar after exposure to red light of various photon fluence rates (Figure 3). Because measurements were performed around midday on clear days, we infer that anthocyanins do not appreciably reduce the risk of photoinhibition in young leaves in the field. This inference is strengthened by the earlier finding that predawn PSII efficiencies of red and green leaves of *Q. coccifera* are similar (Manetas et al. 2003). This may not be a peculiarity of *Q. coccifera*. In most species, new leaves develop during a period of the year when conditions for growth are favorable and the photoinhibitory risk is minimal. However, the photoprotective hypothesis may be valid in the case of the transient accumulation of anthocyanins in mature, thick and chlorophyll-rich leaves after a period of stress (Krol et al. 1995, Pietrini et al. 2002).

The enhanced PSII yields of red leaves in white light (Figure 3) does not necessarily indicate a higher electron transport rates. In fact, electron transport rates could not be calculated, because the actual irradiances penetrating the anthocyanic screen cannot be easily determined. Similarly the high PSII yields of red leaves in white light are not indicative of strong photoprotection. If they were, red leaves should display stronger dynamic photoinhibition in red light at midday and stronger chronic photoinhibition at predawn. The result simply shows that red leaves can maintain slightly higher PSII yields compared with green leaves at all incident photon rates in the field. Because red leaves of *Q. coccifera* have smaller amounts of xanthophyll cycle components than green leaves (Manetas et al. 2003), we may ascribe their similar dynamic and chronic photoinhibitory responses under natural conditions to a balance between photosynthetic light absorption and utilization. Thus, red leaf chloroplasts are exposed to less light because it is attenuated by anthocyanins, whereas green leaf chloroplasts are better equipped to dissipate excess light as heat. In addition to light attenuation, anthocyanins may participate in photoprotection through their anti-oxidant capacity (Neill and Gould 2003).

Anthocyanins as optical signals

Based on the theoretical requirements for an effective optical stimulus against herbivory, one may argue that such a stimulus should either be perceived by the consumers leading to an avoidance action or it should mask the optical cues that enable herbivores to locate a food source. Insect herbivores require leaves for food or for their offspring. Behavioral tests with artificial leaves indicate that many folivorous insect species in search of food or sites for oviposition are attracted to objects that appear green or, more often, yellow to humans (Prokopy and Owens 1983). Corresponding observations in the field showed that young leaves (which appear more yellow than mature leaves and which probably have higher N/C ratios and are less tough (Kelber 1999)) suffer greater herbivory damage. The concept of specific optical stimuli is based on the opposing interactions of specific photo-receptors. Most folivorous insects have at least two different receptor types with sensitivity maxima in the UV (about 350 nm) and the green (about 540 nm) band, enabling them to compare short and relatively long wavelength parts of the spectrum. An additional receptor (about 440 nm) enhances discrimination in the blue band. The resulting overall region of color discrimination lies between

about 300 and 620 nm in most folivorous insect species, with decreasing sensitivity at the margins (Kelber et al. 2003). Accordingly, most insects cannot see what humans perceive as red. In some taxa a fourth red receptor (Briscoe and Chittka 2001) extends the threshold of color vision into the red, but it is used as an opponent stimulus for the choice of green leaves for oviposition (Kelber 1999).

Based on the foregoing evidence, we argue that the presence of anthocyanins in leaves interferes with spectral discrimination by leaf-eating insects by evening leaf reflectance across the insect's entire visual range (Figure 2). Hence, a red leaf may not be easily distinguished because it affords no useful chromatic optical cue. Yet, one may argue that a red leaf, possibly looking black to insects, can be detected through its achromatic signal against a green background (Cuthill et al. 2005). Even in this case, an "insect black" (i.e., human red) leaf seems to be less attractive than a green leaf (Figure 4). Accordingly, our findings of less herbivory damage to red leaves than to green leaves may reflect the color choices of most folivorous insects and the physiology of insect vision.

Based on published studies and our own observations, we suggest that anthocyanins in leaves do not constitute a warning red signal. Instead, their accumulation may mask what would otherwise be a perceptible green signal. Stone (1979, see also Gould 2004, Shaefer and Wilkinson 2004) concluded that red leaves mimic dead or unhealthy leaves of low nutritive value; however, we found that red coloration does not provide absolute protection, because red leaves were attacked, although to a lesser extent than green leaves (Figure 4). It is possible that red leaves are approached by chance; however, the invaders seemed to abandon red leaves after a short-lived attack (Figure 4), perhaps because of the high phenolic content of red leaves (Figure 5). Anthocyanins are end products of the flavonoid biosynthetic pathway, which starts by using products of the phenylpropanoid pathway (Winkel-Shirley 2002). Together these pathways give rise to a vast array of phenolics, including phenolic acids, flavonoids and anthocyanins. In some cases, high concentrations of many of these phenolics co-occur in the same tissues or organs (Figure 5) (Lee and Lowry 1980, Close et al. 2001, Dominy and Lucas 2004, Jaakola et al. 2004). Hence, red coloration may signal a high defensive commitment to those insects able to see human red. Mimicry may also occur, however, because in at least one case the accumulation of anthocyanins is unaccompanied by corresponding increases in other phenolics (Gould et al. 2000).

The lower rate of damage in red leaves can also be explained on the basis of the recently proposed hypothesis of an undermining of insect camouflage by plant coloration (Lev-Yadun et al. 2004). According to this hypothesis, the usually greenish folivorous insects are more conspicuous to their predators on a red leaf than on a green leaf, to the benefit of the plant. Hence, red color in this case is a plant signal for specific insect predators.

Phenolic concentrations in young red leaves of *Q. coccifera* were extremely high (Figure. 5), probably constituting a considerable cost by removing building blocks that could be otherwise used for leaf growth. Overinvestment in defensive chemicals is a general trend in young leaves (Meyer and Montgomery 1987, Kouki and Manetas 2002), which are both valuable (Harper 1989) and vulnerable to herbivore attack (Choong et al. 1992). Although phenolics may be transformed into cell wall constituents at later stages of leaf development (Ossipov et al. 1997), excessive incorporation of imported or assimilated carbon into phenolics during early leaf expansion may result in a trade-off between growth and herbivory risk.

Our results do not permit a conclusion about the mechanism(s) of reduced herbivory in red leaves and the relative merits of a possible a priori choice of green leaves or an a posteriori abandonment of red leaves. Furthermore, our hypothesis cannot be generalized to other organisms. Most vertebrates, including many primates possess the ability to see human red (Robinson 1994). Because some folivorous primates prefer young red leaves, it has been suggested that their trichromatic

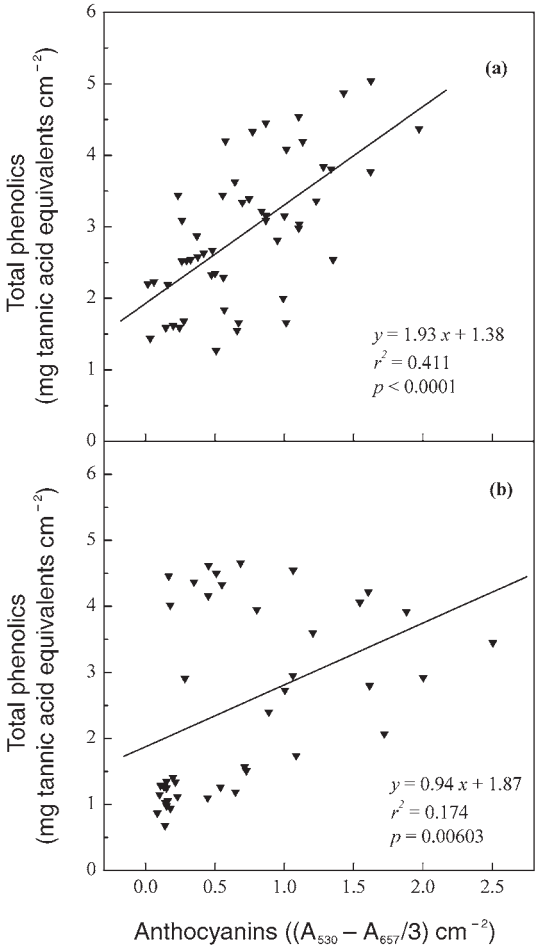

Figure 5. Regression lines of anthocyanin concentration versus total phenolic concentration in young leaves of *Quercus coccifera* sampled from the low (a; $n = 51$) and high altitude (b; $n = 42$) sites. In both cases, regressions were significant. The symbol A denotes the 1-cm light path absorbance at the corresponding wavelengths of 1 cm^3 of methanolic extract obtained from 1 cm^2 of leaf surface. The values for total phenolics correspond to a range from 50 to 550 mg tannic acid equivalents per g leaf dry mass.

vision evolved for the detection of such leaves (Lucas et al. 1998), which abound in the understory of tropical forests (Dominy et al. 2002).

In conclusion, young red leaves of *Q. coccifera* are attacked less by insect consumers than young green leaves. We hypothesize that this is because anthocyanin accumulation masks the strong green reflectance that acts as an optical cue for consumers or increases the risk of consumer recognition by a predator, or both. Incidental attack may be *a posteriori* discouraged by the high phenolic investment of red leaves. The anthocyanic screen only slightly affected photosynthesis and photoinhibition, probably because the importance of attenuated green photons to photosynthesis is greatly reduced in thin, young leaves with low chlorophyll concentrations. The reduced risk of insect attack probably compensates for the metabolic cost of chemical defense.

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