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Photosynthetic pigment contents in twigs of 24 woody species assessed by in vivo reflectance spectroscopy indicate low chlorophyll levels but high carotenoid/chlorophyll ratios

Efi Levizou [a,b,*], Yiannis Manetas [a]

[a] *Laboratory of Plant Physiology, Department of Biology, University of Patras, Patras GR-265 00, Greece*
[b] *Laboratory of Botany, Department of Biological Applications and Technology, University of Ioannina, Ioannina GR-451 10, Greece*

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Abstract

We have examined whether spectral reflectance indices used to non-destructively assess photosynthetic pigment levels and their ratios in leaves, could also be used for the same purpose in peridermal twigs. Regression lines of selected indices versus actual pigment levels, obtained from leaves and twigs of five species, suggested that semi-quantitative assessments are safe, provided that twig periderms could be easily removed. Given that, we proceeded to our next objective of screening a large number of species (24), in order to characterize their photosynthetic pigment profiles. Index comparisons between twigs and corresponding leaves indicated that twigs are characterized by lower levels of total chlorophyll and, unexpectedly, higher carotenoid/chlorophyll ratios. Moreover, the exposed and shaded sides of twigs displayed similar values for both indices in 80% of the species, suggesting that shade may not be the only factor shaping pigment levels and ratios. We discuss our results arguing that the distinct microenvironment within a twig may pose additional needs to the photosynthetic machinery, necessitating elevated carotenoid/chlorophyll ratios.

Keywords: Cortex; Periderm; Reflectance indices; Shade acclimation

1. Introduction

Although leaves are the main plant organs optimized for photosynthesis, active chloroplasts abound in many other organs like petioles, twigs, stems and even flowers and roots, primarily designed for other functions (Aschan and Pfanz, 2003). Thus, the so-called corticular photosynthesis occurs in the chlorenchyma behind the periderm of stems and twigs. Since periderms lack stomata and possess a high diffusive resistance to gases, corticular photosynthesis practically re-fixes respiratory CO_2 or that coming up with the transpiration stream (Pfanz et al., 2002). The reported gross CO_2 assimilation rates in the cortex are low and seldom surpass those of CO_2 production by respiration (Wittmann et al., 2001). Accordingly, corticular photosynthesis is considered as a mechanism of CO_2 recycling and O_2 enrichment in the interior of the stem, facilitating the avoidance of protoplasm acidification and reducing the risk of anoxia or hypoxia (Pfanz et al., 2002). Periderm displays considerable absorbance of visible radiation, transmitting only 10–50% of incident light, depending on species and age of twigs (Kauppi, 1991; Aschan et al., 2001; Manetas and Pfanz, 2005). Accordingly, corticular photosynthesis should display characteristics of the shade acclimation syndrome. Indeed, light curves for both net CO_2 assimilation (Wittmann et al., 2001) and electron transport rates (Manetas, 2004a) attain saturation at low PAR and display low maximum values.

Concerning photosynthetic pigments in a photon limited environment, a general rule predicts that pigments engaged in light capture are favored under shade, while those engaged in protection against over-excitation may be down-regulated. Thus, chlorophyll (chl) a/b ratios in shade leaves are low due to the high ratios of light harvesting complexes per reaction centers (Anderson, 1986). Moreover, the pool sizes of the potentially photoprotective β-carotene and the components of the xanthophyll cycle tend to decrease in the shade (Demmig-Adams et al., 1989; Thayer and Björkman, 1990; Rosevear et al., 2001). Since the contents of photoselective xanthophylls (neoxanthin

* Corresponding author. Tel.: +30 26510 97364; fax: +30 26510 97061.
E-mail address: elevizou@cc.uoi.gr (E. Levizou).

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and lutein) are not appreciably affected by the light environment (Demmig-Adams et al., 1989; Rosevear et al., 2001; Hansen et al., 2002), the total carotenoid to chlorophyll ratios are reduced in leaves under low light.

Low chl a/b ratios have been consistently reported for twigs of many plants (Pfanz et al., 2002). Yet, carotenoid to chl ratios, estimated in crude extracts form three species, did not follow the predictions of a shade acclimation hypothesis. In one case (Pilarski, 1999), the ratio was higher in twigs compared to exposed leaves, while in two other cases the ratios were similar (Wittmann et al., 2001). Moreover, a recent report on chromatographically analyzed photosynthetic pigments from twigs and leaves of five species (Levizou et al., 2004b) indicated higher carotenoid to chl ratios in twigs of four species and similar ratios in the fifth species. These findings contradict expectations and indicate that, in spite of the low light incident on corticular chlorenchyma, there may be other environmental conditions within twigs that shape the unusual carotenoid to chl profiles.

One may argue that the sample size of the examined species is low for generalization. However, screening many plant species for photosynthetic pigment contents is impeded by the difficulties in extracting the hard twig material and the possibility of co-extracting high amounts of phenolics, which may interfere with carotenoid estimation in the crude extracts at 470 nm (Levizou et al., 2004a). On the other hand, chromatography can bypass this problem, yet it is not recommended for large sample sizes in ecophysiological investigations as costly and time consuming.

Pigments in an intact leaf can be non-destructively estimated through optical methods. Thus, spectral reflectance is inversely correlated to absorbance and, hence, to the chemical constituents of a leaf and their concentrations. Accordingly, the intensity of reflectance at specific spectral bands can give information on leaf chemistry. Several reflectance indices have been developed for the estimation of total chls and the carotenoid/chl ratio (Gitelson and Merzlyak, 1994; Peñuelas and Filella, 1998; Sims and Gamon, 2002; Richardson et al., 2002). Reflectance spectroscopy has recently become popular in ecophysiological studies due to its simplicity, sensitivity, rapidity and non-destructive nature (Fillela and Peñuelas, 1999; Carter and Knapp, 2001; Richardson et al., 2001; Stylinski et al., 2002). Yet, it has not been used with twigs up to now.

The present investigation had a dual scope. First, to examine whether corticular pigment contents and their ratios could be reliably assessed by in vivo reflectance measurements in twigs. Provided that the method was indeed credible, we proceeded to our second scope, i.e. screening a large number of plant species in order to confirm (or reject) the unusual photosynthetic pigment profiles of twigs hitherto reported.

2. Materials and methods

The criteria used in preliminary trials to identify plant species suitable for this study were a well-developed periderm that could be removed, revealing intact green underlying tissues. In addition, the green window after periderm removal should be wide enough to accommodate the optical fibers used for spectral reflectance measurements. Twenty-four species of woody trees and shrubs were selected on the basis of the above prerequisites. The sampling site was within or in the vicinity of the Patras University campus. On each sampling date (September/October 2004), 15–27-month-old leafy twigs were cut after labeling the exposed (upper or south facing) side of the twig. At this age periderm is developed as judged by its brownish color and the visual presence of lenticels. Care was taken to sample leafy twigs from the crown perimeter. The material was sealed in plastic bags, transferred to the laboratory and kept in the dark for at least 3 h before analysis. The following species were used: *Arbutus adrachne* L., *Arbutus unedo* L., *Citrus aurantium* L., *Cupressus sempervirens* L., *Elaeagnus angustifolius* L., *Ficus carica* L., *Hedera helix* L., *Laurus nobilis* L., *Ligustrum japonicum* Thunb., *Melia azedarach* L., *Nerium oleander* L., *Phyllirea latifolia* L., *Pinus halepensis* Mill., *Pinus nigra* Arn., *Pistacia lentiscus* L., *Populus deltoides* Bartr., *Prunus cerasus* L., *Punica granatum* L., *Pyrus piraster* Burgsd., *Quercus coccifera* L., *Robinia pseudacacia* L., *Sorbus folgneri* (C.K. Schneid.) Rehder, *Tamarix parviflora* DC., *Vitex agnus castus* L.

2.1. Measuring spectral reflectance

All manipulations were performed under dim laboratory light (PAR < 1 μmol m^{-2} s^{-1}, LI-185 Quantum sensor; Li-cor, Lincoln, NE, USA). In most cases, engraving the twig with a razor blade facilitated periderm peeling off by hand. In more resistant species, the periderm was carefully scratched by the razor blade until opening a window of suitable dimensions. Spectral reflectance was measured with a portable diode-array spectrometer (Unispec; PP-Systems, Haverhill, MA, USA) equipped with an internal halogen source and bifurcated fiber optic cables directly attached to the sample (peeled twig or leaf). A spectralon standard (reflectance > 0.97 for the whole 400–1100 nm range) was used as a reference and the obtained spectra were dark-corrected for stray light after closing the instrument shutter. Measurements were performed on 24 species, 10 spots per twig (equally divided between exposed and shaded side), 5 twigs/individual and 3 individuals/species. In leaves (5 spots/leaf, 5 leaves/individual, 3 individuals/species), only the upper (exposed) surface was scanned.

The determined reflectance indices were the following, with *Rn* denoting reflectance at $\lambda = n$:

1. Normalized difference index, NDI $= \frac{R_{750}-R_{705}}{R_{750}+R_{705}}$. This index is positively correlated with chl concentrations (Gitelson and Merzlyak, 1994).
2. Photochemical reflectance index, PRI $= \frac{R_{531}-R_{570}}{R_{531}+R_{570}}$. It is proposed as a measure of photosynthetic efficiency (Gamon et al., 1992) and it is negatively correlated to the carotenoid/chl ratio (Sims and Gamon, 2002).

2.2. Correlating reflectance indices with actual pigment levels and ratios

A sub-sample consisting of five species (i.e. *A. unedo*, *P. lentiscus*, *P. deltoides*, *P. cerasus*, *Q. coccifera*) was used to