# Nondestructive assessment of leaf chemistry and physiology through spectral reflectance measurements may be misleading when changes in trichome density co-occur

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## Summary

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• Reflectance indices are frequently used for the nondestructive assessment of leaf chemistry, especially pigment content, in environmental or developmental studies. Since reflectance spectra are influenced by trichome density, and trichome density displays a considerable phenotypic plasticity, we asked whether this structural parameter could be a source of variation in the values of the most commonly used indices.

• Trichome density was manipulated in detached leaves of three species having either peltate (*Olea europaea* and *Elaeagnus angustifolius*) or tubular (*Populus alba*) trichomes by successive removal of hairs. After each dehairing step, trichome density was determined by light or scanning electron microscopy and reflectance spectra were obtained with a diode-array spectrometer.

• Although species-specific differences were evident, most of the indices were considerably affected even at low trichome densities. In general, the less-affected indices were those using wavebands within the visible spectral region. The index that could be safely used even at very high hair densities in all species was the red edge index ($\lambda_{RE}$) for chlorophyll.

• The results indicate that changes in reflectance indices should be interpreted cautiously when concurrent changes in trichome density are suspected. In this case, the red edge for chlorophyll content may be the index of choice.

**Key words:** indices, leaf optical properties, pigments, reflectance, trichome density, water index.

## Introduction

Light falling on a leaf is either reflected, absorbed or transmitted. Both total and spectral reflectance (and transmittance) are inversely correlated to absorbance (i.e. 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. Thus, reflectance indices have been developed for the nondestructive estimation of chlorophylls (Gitelson & Merzlyak, 1994; Curran *et al.*, 1995; Blackburn, 1998; Datt, 1998; Adams *et al.*, 1999;

Richardson *et al.*, 2002), the ratio of carotenoids to chlorophylls (Peñuelas *et al.*, 1995) and water content (Peñuelas *et al.*, 1997) at both leaf and canopy levels. The importance of finding reliable spectral reflectance indices for these pigments lies on the fact that long- or medium-term changes in the above compounds can be related to stress imposed or to the environmental history of the test plants. Thus, chlorophyll levels have been correlated to light levels during growth (Larcher, 1995), water stress (Kyparissis *et al.*, 1995) and nitrogen nutrition (Filella *et al.*, 1995). Moreover, short-term changes in the intensity of reflectance at some bands could be

indicative of the metabolism of some compounds and thus correlated to physiology. For example, reflectance at *c.* 530 nm is sensitive to zeaxanthin formation during the light-dependent development of photoprotective, nonphotochemical quenching and the accompanying decrease of photosystem II (PSII) photochemical efficiency (Gamon *et al.*, 1992). However, reflectance depends also on leaf anatomical features such as surface relief and/or internal architecture. For this reason, corrections are applied by measuring reflectance at some distant bands in which the investigated compounds do not absorb (Peñuelas & Filella, 1998).

With the introduction of portable, sensitive and reliable spectrometers, reflectance spectroscopy has become popular in ecophysiological studies because of its simplicity, rapidity and nondestructive nature. In particular, the effects of stress on various plants have been assessed through seasonal measurements (Stylinski *et al.*, 2002), along environmental gradients (Filella & Peñuelas, 1999; Richardson *et al.*, 2001; Richardson & Berlyn, 2002), or after manipulation of environmental parameters (Carter & Knapp, 2001). In addition, these indices have been used for the nondestructive assessment of pigment changes during leaf development (Gamon & Surfus, 1999; Winkel *et al.*, 2002). In most of these cases, simultaneous measurements of photosynthesis and/or the levels of corresponding compounds were used for an empirical correlation with the measured reflectance indices. The investigations cited were based on the silent assumption that the structural features of leaf surfaces do not change to any appreciable level that could be considered as a source of variation in the measured values of reflectance indices.

A structural feature that dramatically changes leaf relief is the presence of trichomes. Trichome density has a considerable influence on leaf reflectance (Ehleringer & Björkman, 1978). Moreover, not only interspecies, but also intraspecies variation in trichome density are known along environmental gradients or during leaf development (Ehleringer, 1982; Karabourniotis *et al.*, 1995; see References in the Discussion section). The aim of this study was to investigate the possible effects that the trichome density might have on some reflectance indices obtained from the same leaf, on which trichome density was rapidly manipulated by successive, artificial removal of hairs. Under these conditions of physiological equilibrium and chemical stability, the possible contribution of hair density in the variation of reflectance indices could be evaluated. Ideally, constant index values should indicate independence from trichome density. Yet, our results showed considerable deviations from this expectation for most of the indices and the range of leaf trichome cover that each index can tolerate is given.

## Materials and Methods

Mature leaves from three species were used. *Olea europaea* L. (Oleaceae) and *Elaeagnus angustifolius* L. (Elaeagnaceae) with peltate hairs and *Populus alba* L. (Salicaceae) with tubular hairs. The leaves were harvested late in the afternoon, put in small airtight bags to avoid desiccation, kept in the dark and used the following morning. Except for dark adaptation, all manipulations related to dehairing were made under dim laboratory light (< 1 µmol m$^{-2}$ s$^{-1}$, LI-185 Quantum sensor; Li-Cor, Lincoln, NE, USA) to avoid changes in the photochemical reflectance index (PRI), which is light dependent. Dehairing was performed by gently pressing a transparent adhesive tape on the surface. Three consecutive applications with fresh tape were enough for complete hair removal. Spectral reflectance was measured on the intact leaf as well as immediately after each tape application, with a portable spectrometer (Unispec; PP Systems, Haverhill, MA, USA) equipped with an internal halogen source and a bifurcated fiber optic cable (internal diameter 2.3 mm) directly attached on the leaf surface with the help of a leaf clip. Three spots were measured (four scans per spot) and the average was taken as representative for the leaf and its state (i.e. intact, first tape application, second tape applications, complete dehairing). Each spot was illuminated for 2 s during measurement and preliminary trials indicated that this short illumination was unable to cause any change in PRI. The leaves as well as the tapes after each application were viewed with an Axioplan-Zeiss (Oberkochen, Germany) light microscope in order to determine the corresponding hair density. This applies only for the species bearing peltate hairs (*O. euporaea* and *E. angustifolius*). The experiment was repeated with 10 leaves from each species.

Assessing of trichome viability was made using the propidium iodide test: dead cells fluoresce red when stained with propidium iodide (100 µg ml$^{-1}$) and viewed with green excitation filter under epifluorescence microscopy, according to Hawes & Satiat-Jeunemaitre (2001). The green filter set 530–585 was used (BP 530–585 as exciter filter, FT 600 as chromatic beam splitter and LP 615 as barrier filter; Zeiss, Oberkochen, Germany). For scanning electron microscopy (SEM) micrographs, leaves were sputter coated with gold and viewed under a JEOL 6300 (JEOL, Tokyo, Japan) scanning electron microscopy.

The determined reflectance indices were the following, with $Rn$ indicating reflectance at $\lambda = n$:
1 Normalized difference index, NDI = $(R750 - R705)/(R750 + R705)$ (Gitelson & Merzlyak, 1994).
2 Red edge of reflectance, $\lambda_{RE}$ (i.e. the wavelength in the red band where the rate of change in reflectance – assessed from the first derivative of the reflectance vs wavelength curve – is maximum) (Curran *et al.*, 1995).
3 Yellowness index, YI = $(R580 - 2R624 + R668)/(44\ nm)^2$, which is in practice a three-point approximation of the second derivative of spectra in the decreasing, right side of the green reflectance maximum, found to be correlated with chlorophyll concentration (Adams *et al.*, 1999).
4 Pigment specific simple ratio for chlorophyll *a*, PSSRa = $R800/R675$ (Blackburn, 1998).

5 'Datt' vegetation index, $R624/(R550 \times R708)$ (Datt, 1998). All the above indices have been used as a measure of chlorophyll concentration.

6 Photochemical reflectance index, PRI = $(R531 − R570)/(R531 + R570)$, proposed as a measure of photosynthetic efficiency (Gamon *et al.*, 1992). Some recent studies have indicated that PRI is also correlated with the carotenoid to chlorophyll ratio (Sims & Gamon, 2002).

7 Structure-independent pigment index, SIPI = $(R800 − R445)/(R800 + R445)$, a measure of carotenoid to chlorophyll ratio (Peñuelas *et al.*, 1995). Note, however, that the usefulness of this index has recently been questioned (Sims & Gamon, 2002).

8 Water index, WI = $R900/R970$, a measure of water content (Peñuelas *et al.*, 1997).

In addition, the differences in PRI between the dark and the light-adapted state (ΔPRI) were compared in intact and completely dehaired leaves. Such a comparison requires the mesophyll of the two leaf groups to receive the same actual photon fluence rates. This was ascertained by adjusting the incident photosynthetically active radiation (PAR) in each case allowing for the trichome transmittance. For transmittance measurements, the three tapes bearing the complete hair layer were appressed, covered an illuminated quantum sensor and light penetration was compared with that of a similar three-tape configuration without hairs. In all species, a transmittance of *c.* 0.2 was found. Accordingly, intact and dehaired leaves were illuminated for 20 min with 1200 µmol m$^{-2}$ s$^{-1}$ and 960 µmol m$^{-2}$ s$^{-1}$ PAR, respectively, provided by a quartz-halogen lamp. During illumination the leaves were located in covered Petri dishes on top of moistened filter paper. Preliminary trials indicated that ΔPRI was saturated after *c.* 10 min in all cases.

Significance of differences in reflectance indices between treatments (paired-*T*-test) was assessed with the SPSS 10.0 statistical package (SPSS, Chicago, IL, USA).

## Results

The result of three successive tape applications on adaxial and abaxial leaf surfaces of *O. europaea* are shown in Fig. 1a,c and b,d, respectively; (a) and (b) show an intact, fully haired leaf, while (c) and (d) indicate a practically dehaired surface after the third tape application. Intermediate hair densities were observed after the first and second tape application (not shown). Corresponding pictures of the abaxial leaf surface of *E. angustifolius* and *P. alba* are shown in Fig. 2a,c and (b,d), respectively. As expected (Ehleringer & Björkman, 1978), hair removal in *O. europaea* caused a decrease in visible
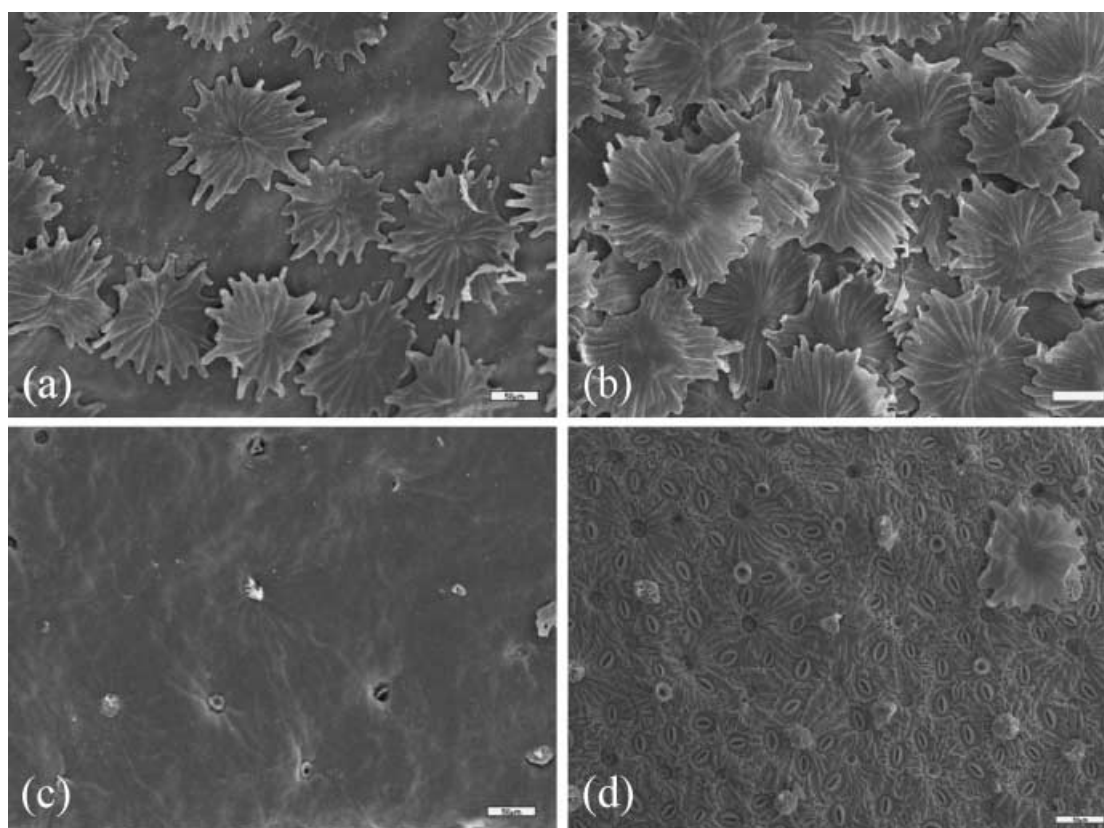


**Fig. 1** Scanning electron photomicrographs of *Olea europaea* leaf surfaces: (a,c) adaxial and (b,d) abaxial surface. (a,b) Intact leaves; (c,d) practically dehaired leaves after three successive tape applications. Bar, 50 µm.
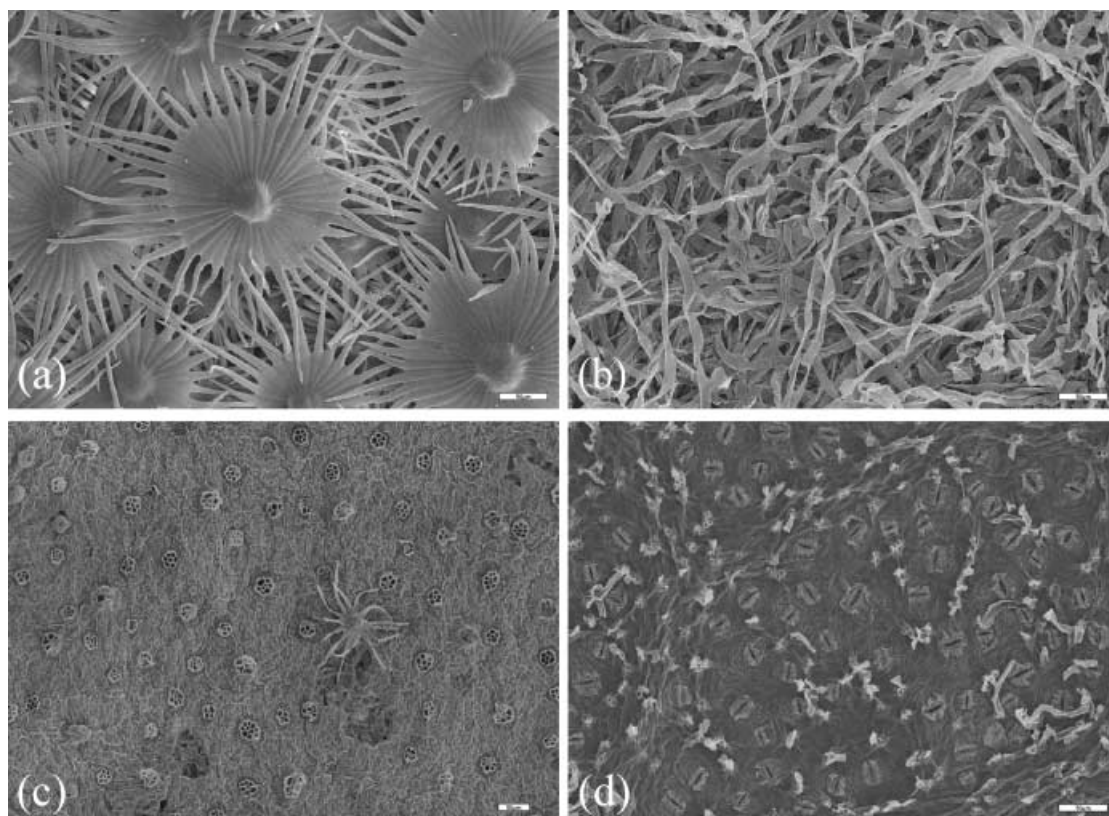
**Fig. 2** Scanning electron photomicrographs of *Elaeagnus angustifolius* (a,c) and *Populus alba* (b,d) abaxial leaf surface. (a,b) Intact leaves; (c,d) practically dehaired leaves after three successive tape applications. Bar, 50 µm.

reflectance (Fig. 3a,b), especially in the abaxial surface. However, a corresponding increase was observed in the infrared, indicating that in this species the epidermis reflects more than the peltate hair layer. In the other two species, dehairing caused a decrease in reflectance throughout the 400–1000 nm spectral range (Fig. 3c,d).

Upon dehairing, not only the direction, but also the extent of reflectance changes in the visible (where the sampling wave bands) and the infrared (where the reference wave bands in some indices) displayed species-specific differences. As shown in Table 1, considerable decreases in the visible reflectance (ranging from 73% in *E. angustifolius* to 16% in the adaxial surface of *O. europaea*) were accompanied by slight decreases in the infrared reflectance (13% in *E. angustifolius* and 8% in *P. alba*) or even increases (4% in the abaxial surface of *O. europaea*).

In Fig. 4, plots of the various reflectance indices vs trichome density are given for the adaxial surface of *O. europaea* leaves. As shown, $\lambda_{RE}$, yellowness, SIPI, PRI and water index perform well within the density range of 0–31 peltate trichomes $mm^{-2}$ observed in these samples. Yet, at the higher end of this range, a departure from expectations was shown for NDI, Datt and PSSRa indices. Although small, these index changes may correspond to a significant magnitude of chlorophyll differences (Richardson *et al.*, 2002). In order to

**Table 1** Reflectance changes in the visible (400–700 nm) and the infra-red (700–1000 nm) part of the spectrum during successive leaf dehairing

| | 400–700 nm | % Difference | 700–1000 nm | % Difference |
|---|---|---|---|---|
| *Olea europaea* (adaxial surface) | | | | |
| Intact | 6.42 ± 1.3 | | 60.57 ± 1.0 | |
| 1st | 5.69 ± 0.9 | −11.5 | 61.35 ± 1.2 | 1.3 |
| 2nd | 5.45 ± 0.9 | −15.1 | 60.75 ± 0.8 | 0.3 |
| 3rd | 5.38 ± 0.9 | −16.2 | 60.71 ± 1.1 | 0.2 |
| *O. europaea* (abaxial surface) | | | | |
| Intact | 28.34 ± 4.4 | | 64.85 ± 3.1 | |
| 1st | 22.91 ± 4.3 | −19.1 | 66.12 ± 2.1 | 2.0 |
| 2nd | 17.73 ± 4.0 | −37.4 | 66.87 ± 1.4 | 3.1 |
| 3rd | 14.75 ± 1.6 | −47.9 | 67.20 ± 1.6 | 3.6 |
| *Elaeagnus angustifolius* | | | | |
| Intact | 24.46 ± 2.0 | | 51.80 ± 1.6 | |
| 1st | 16.48 ± 2.4 | −32.6 | 48.19 ± 1.7 | −7.0 |
| 2nd | 9.17 ± 2.4 | −62.5 | 46.26 ± 1.5 | −10.7 |
| 3rd | 6.62 ± 0.7 | −72.9 | 45.20 ± 1.6 | −12.7 |
| *Populus alba* | | | | |
| Intact | 31.28 ± 4.2 | | 52.39 ± 2.4 | |
| 1st | 24.02 ± 3.6 | −23.2 | 51.02 ± 1.8 | −2.6 |
| 2nd | 17.30 ± 2.9 | −44.7 | 48.82 ± 2.0 | −6.8 |
| 3rd | 14.56 ± 1.5 | −55.1 | 48.31 ± 1.5 | −7.8 |

Data are means ± SE from 10 leaves per species. 1st, 2nd and 3rd denote the stages of dehairing.
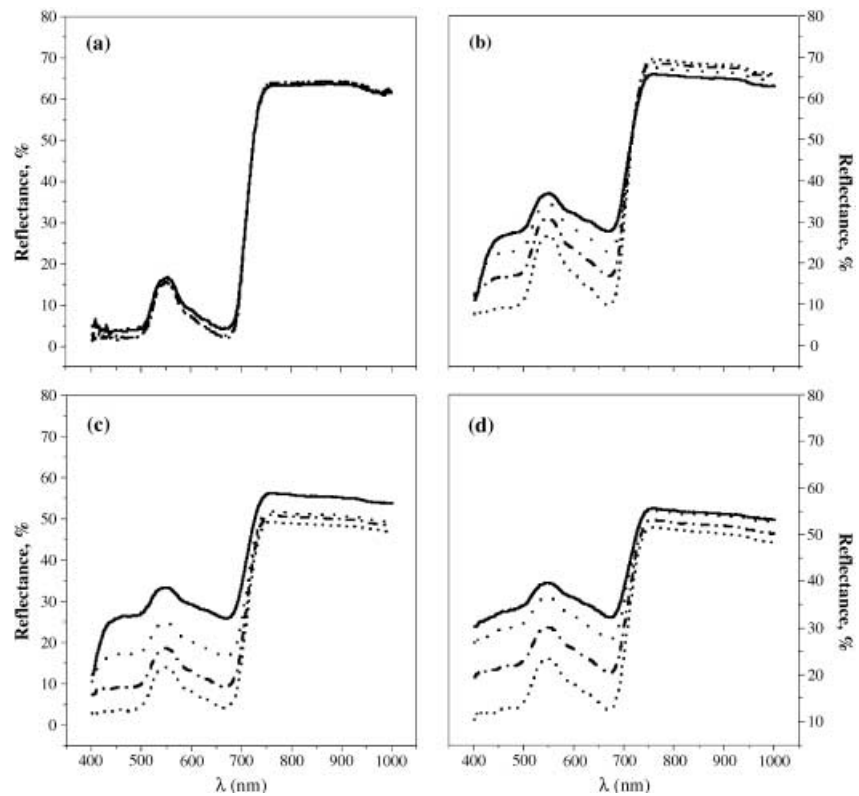
Fig. 3 The effects of hair removal on reflectance spectra. (a) Adaxial and (b) abaxial leaf surfaces of *Olea europaea*. (c,d) Abaxial surfaces of *Elaeagnus angustifolius* and *Populus alba*, respectively. Solid line, intact leaves; dotted line, thick dashed and dotted line and thin dashed line, after first, second and third tape application, respectively; λ, wavelength. Data from a characteristic leaf among 10 showing similar changes.

further assess the effect of higher trichome densities, similar measurements were performed on abaxial surface of *O. europaea* leaves, displaying densities of up to 180 peltate hairs mm$^{-2}$. As shown in Fig. 5, only the yellowness index remained constant throughout, while the rest departed considerably from expectations starting from lower (NDI, Datt, PSSRa and PRI) or higher (SIPI, $\lambda_{RE}$ and WI) trichome densities.

In Table 2, a summary of the dehairing effects on the performance of various indices for *E. angustifolius* and *P. alba* is given. As shown, $\lambda_{RE}$ performed well up to the highest trichome density (i.e. that of the intact leaf) for both species, while NDI, PSSRa, Datt and yellowness index were completely unacceptable. The effects of trichome density on SIPI were controversial for both species as it was PRI for *E. angustifolius*. The water index performed relatively well in *E. angustifolius* but less well in *P. alba*. In general, the effects of trichome density were species-specific and probably depended on trichome morphology.

In the cases of *O. europaea* and *E. angustifolius*, which possess peltate hairs, the minimum trichome density and/or per cent covering of leaf surface by hairs for the safe of each index can be calculated (Fig. 6). In *O. europaea* for example, the often used NDI for chlorophyll assessment is useless at densities above 7 peltate trichomes mm$^{-2}$ (corresponding to a surface covering of 13%), while the $\lambda_{RE}$ can be safely used for up to 120 peltate hairs mm$^{-2}$ (i.e. a 99% covering). In *E. angustifolius*, however, the toleration of indices to trichome

**Table 2** Summary of the dehairing effects on the performance of various indices for *Elaeagnus angustifolius* and *Populus alba*

|  | *Elaeagnus angustifolius* | | | | *Populus alba* | | | |
|---|---|---|---|---|---|---|---|---|
|  | Intact | 1st | 2nd | 3rd | Intact | 1st | 2nd | 3rd |
| NDI | a | b | c | d | a | b | c | d |
| $\lambda_{RE}$ | a | b | b | b | a | b | b | b |
| YI | a | b | c | c | a | b | c | d |
| PSSRa | a | a | b | c | a | b | c | d |
| Datt | a | a | b | c | a | b | c | d |
| PRI | a | b | c | b | a | b | c | c |
| SIPI | a | b | a | c | a | b | a | c |
| WI | a | b | b | b | a | b | c | c |

NDI, normalized difference index; $\lambda_{RE}$, red edge of reflectance YI, yellowness index; PSSRa, pigment specific simple ratio for chlorophyll *a*; Datt, Datt, vegetation index; PRI, photochemical reflectance index; SIPI, structure-independent pigment index; WI, water index. Different letters denote statistically significant differences ($P < 0.05$) in indices found between the intact leaf and after the first, second and third tape application.

density changes was negligible, with the exception of $\lambda_{RE}$ and water index which could stand densities up to 72 peltate hairs mm$^{-2}$, corresponding to surface covering of 86%.

In an additional experiment, the effect of dehairing on the changes in PRI during induction of photosynthesis and photoprotection by actinic light was examined. Although care
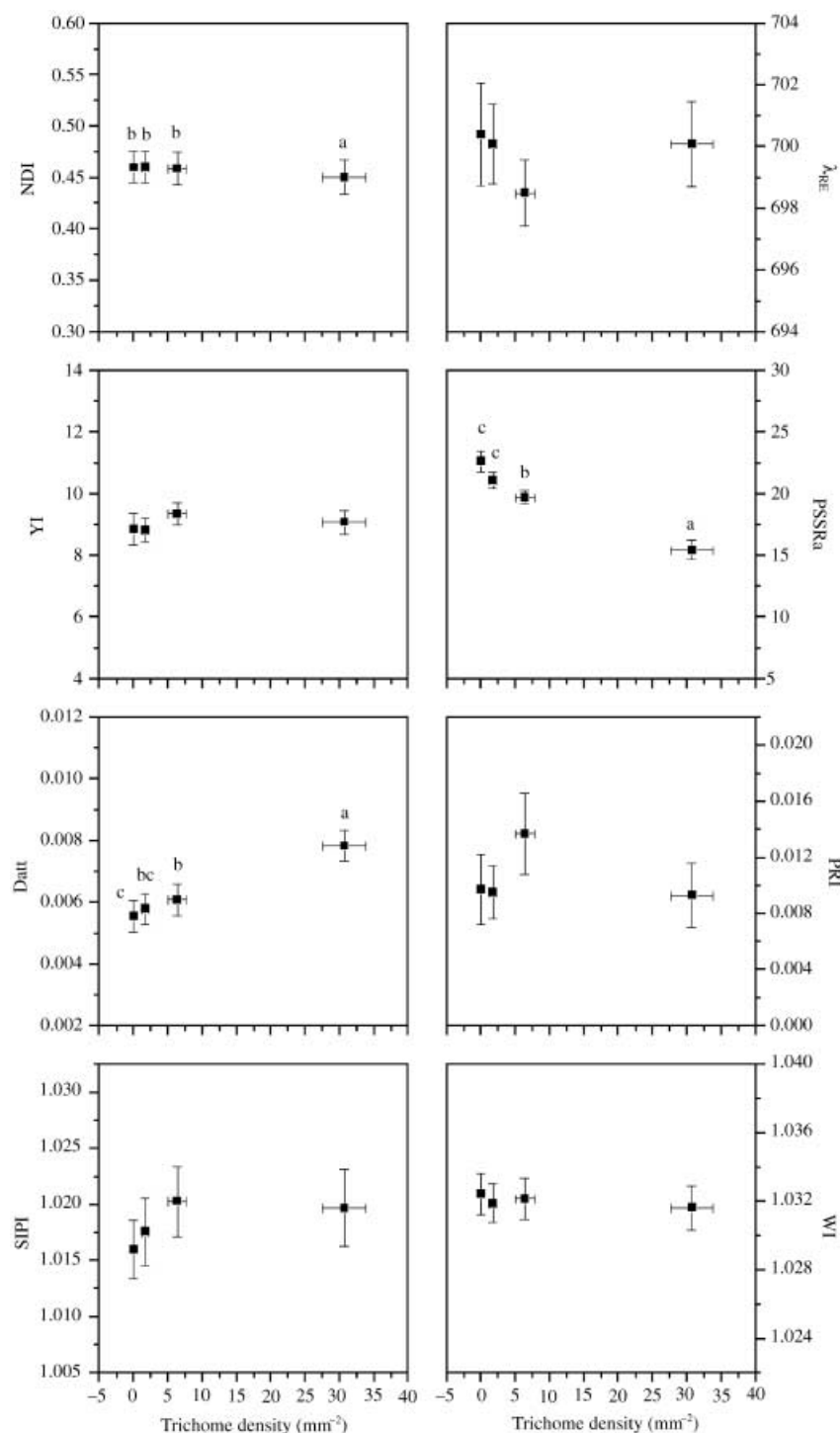
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**Fig. 4** Plots of the various reflectance indices vs trichome density for the adaxial leaf surface of *Olea europaea*. The *x*- and *y*-axis values are means ± SE from 10 independent leaves. Different letters, where present, denote statistically significant differences (*P* < 0.05) between index values at various trichome densities. NDI, normalized difference index; YI, yellowness index; Datt, Datt, vegetation index; SIPI, structure-independent pigment index; $\lambda_{RE}$, red edge of reflectance; PSSRa, pigment specific simple ratio for chlorophyll *a*; PRI, photochemical reflectance index; WI, water index.

was taken to provide the same actual actinic intensity to the mesophyll of both intact and dehaired leaves (see the Materials and Methods), the ΔPRI between dark- and light-adapted leaves was considerably higher in dehaired samples from all three species (Table 3).

Finally, we should note that the results of the present investigation were not artifactually induced by tape application

*per se*, since similar applications on glabrous leaves did not change anyone of the above measured indices (not shown).

## Discussion

Much effort has been devoted to the study of leaf optical properties, aimed at finding reflectance indices that correlate
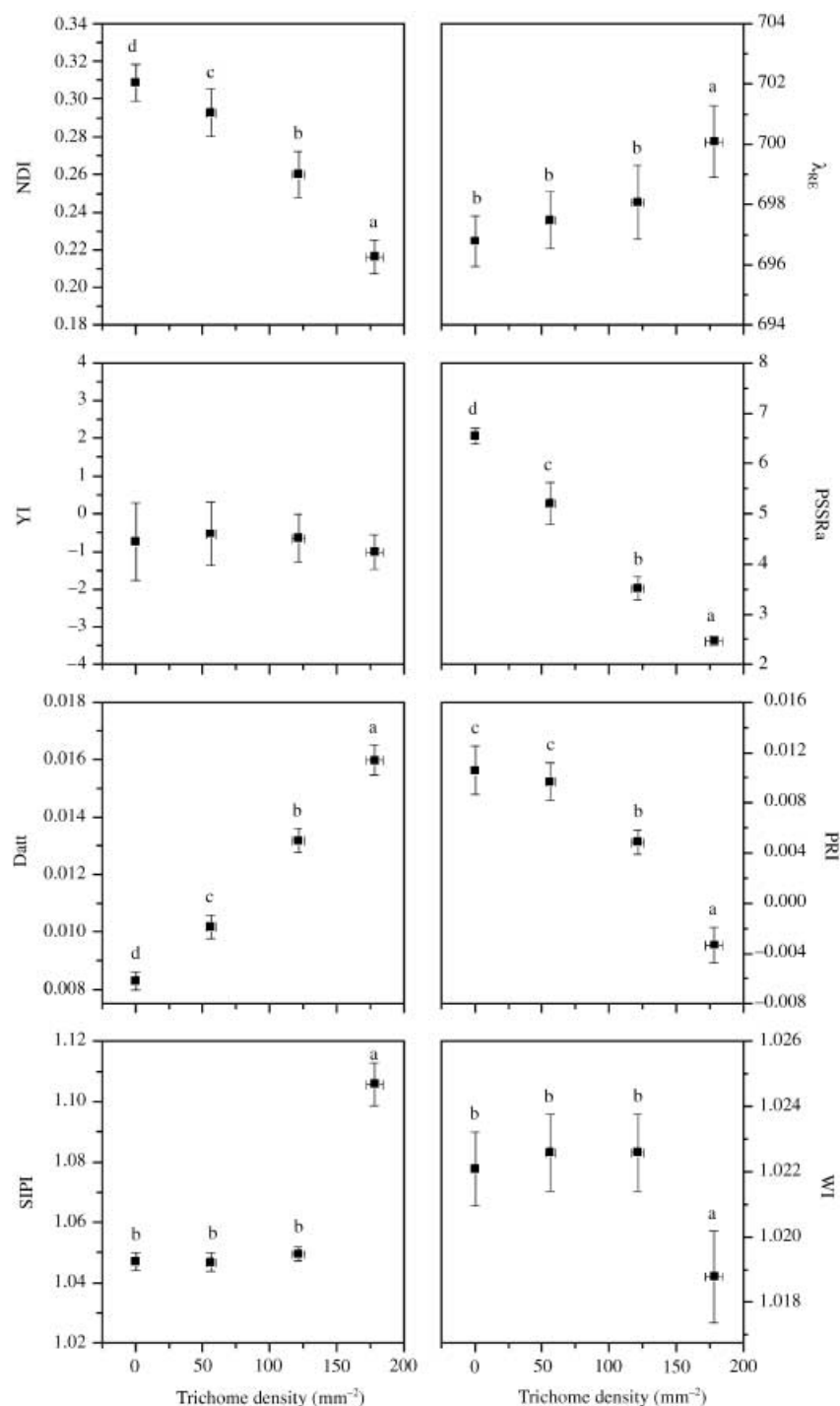
**Fig. 5** Plots of the various reflectance indices vs trichome density for the abaxial leaf surface of *Olea europaea*. The x- and y-axis values are means ± SE from 10 independent leaves. Different letters, where present, denote statistically significant differences ($P < 0.05$) between index values at various trichome densities. NDI, normalized difference index; YI, yellowness index; Datt, Datt vegetation index; SIPI, structure-independent pigment index; $\lambda_{RE}$, red edge of reflectance; PSSRa, pigment specific simple ratio for chlorophyll *a*; PRI, photochemical reflectance index; WI, water index.

well with pigment levels in a variety of species and functional groups. Evidence to date indicates that the regression equations of the measured parameter vs the corresponding index depend on the species tested (Gamon & Surfus, 1999; Richardson *et al.*, 2002). This has been ascribed to species-specific leaf structural attributes. Accordingly, it is assumed that the indices should work well with leaves of the same species. This assumption disregards intraspecies variation in leaf structure resulting from developmental stage, stress or influence of environmental parameters, both biotic and abiotic. Leaf hair density, being a major determinant of leaf surface relief, displays a considerable phenotypic plasticity. In some plants, trichomes are transient only in young leaves, to be abscised later or just diluted as the leaf increases in size (Uphof, 1962;
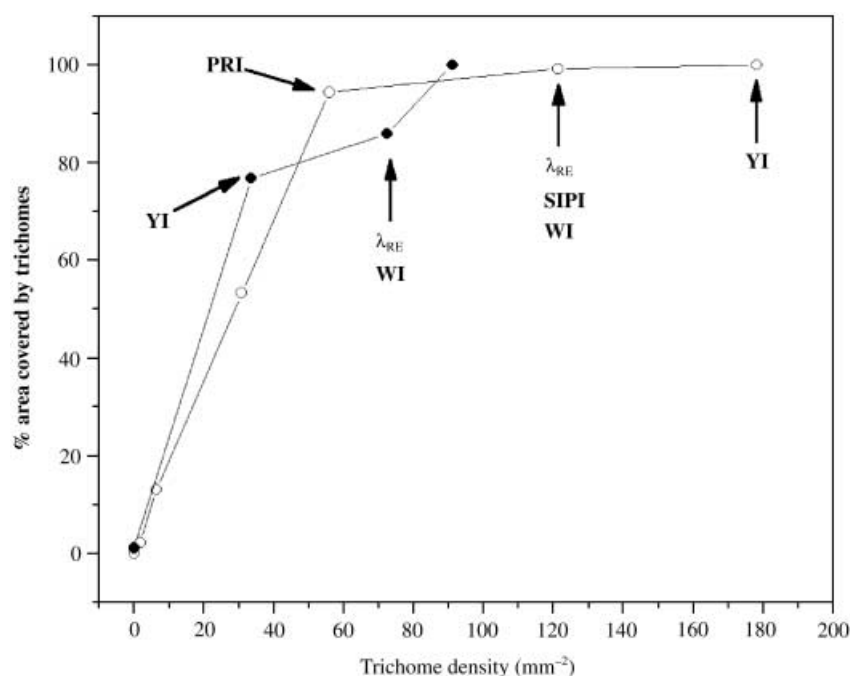
**Fig. 6** Per cent cover of leaf surface by hairs vs trichome density for *Olea europaea* (open circles) and *Elaeagnus angustifolius* (closed circles). Arrows indicate the maximum trichome density and/or per cent cover by hairs for the safe use of each index for each plant. Indices that do not appear in this plot show a negligible toleration to trichome density changes. YI, yellowness index; SIPI, structure-independent pigment index; $\lambda_{RE}$, red edge of reflectance; PRI, photochemical reflectance index; WI, water index.

**Table 3** Photochemical reflectance index ($\Delta$PRI) between dark- and light-adapted intact or completely dehaired leaves for all species tested

| | $\Delta$PRI(PRI$_{dark}$ − PRI$_{light}$) | | |
| --- | --- | --- | --- |
| | Intact leaf | Completely dehaired leaf | % Difference |
| *Olea europaea* | $0.0115 \pm 0.0009$ | $0.0275 \pm 0.0024$ | 140.3 |
| *Elaeagnus angustifolius* | $0.0107 \pm 0.0004$ | $0.0159 \pm 0.0027$ | 48.5 |
| *Populus alba* | $0.0051 \pm 0.0009$ | $0.0119 \pm 0.0019$ | 134.5 |

PRI, photochemical reflectance index.
Data are means ± SE from 10 leaves per species. Differences between intact and dehaired leaves are statistically significant ($P < 0.05$) in the cases of *O. europaea* and *P. alba*, while the trend observed in *E. angustifolius* is not.

Karabourniotis *et al.*, 1995). Trichome density is positively correlated to prevailing air temperatures and negatively correlated to leaf water potential. As a result, trichome density varies along temperature and aridity gradients (Ehleringer, 1982). Trichome density increases considerably at low nutrient availability, possibly through a negative effect on leaf size (Roy *et al.*, 1999). A positive correlation to photon exposure has also been reported, with canopy leaves bearing more trichomes compared to internal leaves (Liakoura *et al.*, 1997; Filella & Peñuelas, 1999). In addition, UV-B radiation increases hair density (Barnes *et al.*, 1996; McCloud & Berenbaum, 2000) and may change their morphology (Yiannopoulos *et al.*, 2001). Finally, a clear correlation of salt spray incidence and trichome abundance has been found in *Borrichia arborescens* (Morrison, 2002). In all cases, a corresponding adaptive significance to this extensive phenotypic plasticity was ascribed (i.e. protection against photoinhibition of photosynthesis, overheating, UV-B radiation damage, desiccation avoidance and prevention of stomatal clogging by salt spray).

With regard to biotic interactions, insect feeding induced a considerable increase in leaf trichome density of *Brassica rapa* (Traw & Dawson, 2002) or even a change from glabrous to heavily pubescent leaves in *Alnus incana* (Baur *et al.*, 1991).

As shown in the present investigation, trichome density can have a profound influence on the measured reflectance indices. The observed changes are considerable. For example, hair removal in the abaxial leaf surface of *O. europaea* results in an increase in NDI from c. 0.2–0.3, giving the false impression that chlorophyll levels considerably increase within seconds after dehairing (Fig. 5). Considerable changes were found for most indices in all test plants. According to the results of Richardson *et al.* (2002), such an increase in NDI corresponds to a doubling of chlorophyll on a leaf area basis.

Therefore, we may assume that index changes can result from an interaction of both pigment and trichome density variations. The direction of changes caused by the two variables can be parallel or antiparallel. We may use the light/shade acclimatization as an example. Shade leaves are thinner, therefore having lower surface based chlorophyll levels, expecting a lower NDI as well. Yet, shade leaves also display a lower trichome density (Liakoura *et al.*, 1997; Filella & Peñuelas, 1999), driving NDI to higher values. Therefore, the alleged chlorophyll changes can be masked by the antiparallel influence of decreasing trichome density on NDI. Conversely, it is

known that some plants respond to water stress by a decrease in chlorophyll levels, reducing the risk of photoinhibition (Kyparissis *et al.*, 1995). Yet, water stress also affects positively the density of trichome (Ehleringer, 1982; Kyparissis & Manetas, 1993). In this case, both the decrease in chlorophylls and the increase in trichome density cooperate in parallel to decrease NDI and can lead to an overestimation of chlorophyll change. One may speculate similarly for the rest of the indices.

A question can be raised concerning which index could perform best in case of a suspected, co-occurring change in trichome density. Although our results indicated that the morphology of hairs can have a profound influence (i.e. peltate vs. tubular hairs), there is a trend for those indices that do not use infrared spectral bands to perform better. Among them, the red edge ($\lambda_{RE}$) index appears to tolerate an almost complete hair covering of leaf surface, while the frequently used NDI seems to be completely unacceptable for hairy leaves, even at low trichome densities. Table 1 and Fig. 3 may offer an empirical explanation for the better performance of indices within the visible spectral range, indicating a differential influence of trichome density on reflectance changes in the visible compared with the infrared. In particular, while reflectance in the visible region decreases upon dehairing in *O. europaea*, the corresponding reflectance in the infrared increases.

A final note should be added for the behavior of water index, where both the sample and the reference bands lie in the infrared at a comparatively short spectral distance (900 nm vs 970 nm). Typically, one should expect independence of the index in the case of living hairs, where water content differences between hair and epidermal cells should be negligible. Yet, hairs of our test plants seem to be dead, as judged from their red fluorescence obtained when immersed in propidium iodide and viewed under epifluorescence microscope (see the Materials and Methods section). In this case, the reflectance signal of an intact leaf is mainly received from a dry object. Subsequent dehairing should reveal the wet epidermal cells, causing an increase in water index. Such an increase, however, was observed only at the highest trichome densities. This result does not necessarily indicate that the index is safe, since its apparent independence on trichome density could result from low sensitivity of this index to changes in water content (Peñuelas & Inoue, 1999).

We conclude that changes in trichome density can distort reflectance spectra and considerably affect reflectance indices independently of corresponding changes in pigment contents. Among the most frequently used indices, those using wavelength bands within the visible part of the spectrum (for example the red edge index) perform better and withstand considerable changes in trichome density. Although not addressed in this study, similar biases in reflectance indices may result from changes in epicuticular waxes, the levels of which display considerable phenotypic plasticity as well (Kolattukudi, 1996; Holmes & Keiller, 2002).

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