Assessment of oak forest condition based on leaf biochemical variables and chlorophyll fluorescence

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Summary  Pedunculate oak forests (Quercus robur L.) in the Ticino Regional Park, Italy, are declining as a result of insect attacks, summer droughts and air pollution. The assessment and monitoring of forest condition can provide a basis for managing and conserving forest ecosystems and thereby avoid loss of valuable natural resources. Currently, most forest assessments are limited to ground-based visual evaluations that are local and subjective. It is therefore difficult to compare data collected by different crews or to define reliable trends over years. We examined vegetation variables that can be quantitatively estimated by remote observations and, thus, are suitable for objective monitoring over extended forested areas. We found that total chlorophyll (Chl) concentration is the most suitable variable for assessing pedunculate oak decline. It is highly correlated with visual assessments of discoloration. Furthermore, Chl concentration can be accurately estimated from leaf optical properties, making it feasible to map Chl concentration at the canopy level from satellite and airborne remote observations.

Keywords: foliar pigments, leaf reflectance, optical indices, photosynthetic efficiency, Quercus robur, stress detection.

Introduction

Although forests have always experienced periods of decline in response to a variety of causes, there is a growing awareness that atmospheric pollution, i.e., global climate change in combination with traditional pests and pathogens, may pose a serious threat to forest ecosystems (UNECE 2004a). Assessment and monitoring of forest condition over time are of crucial importance for developing optimal management practices (Ferretti 1997). International monitoring programs have been undertaken in Europe to detect suspected changes in forest condition (e.g., the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP-Forests), the ICP on Integrated Monitoring on Air Pollution Effects (ICP-IM) and the International Union of Forest Research Organization (IUFRO)). In Europe, much work in forest monitoring has concentrated on the transparency and discoloration of the crown as indicators of forest condition. These indices are visually estimated by field surveyors, with the aid of binoculars, and provide a subjective evaluation, making it difficult to compare data collected by different crews or to define reliable trends over years (Ferretti 1998). Transparency and discoloration indices both rely on the difference of the observed crown characteristic (leaf color or foliar density) from a “reference tree.” A reference tree is a conceptual tree, defined as the best tree with full foliage that could grow at a particular site, taking into account factors such as altitude, latitude, tree age, site conditions and social status (UNECE 1998). The definition of a reference tree for discoloration assessment is straightforward (i.e., a tree with green leaves). However, the definition of a reference tree for defoliation assessment is not trivial when the investigated forest is heterogeneous. The field crew must determine the potential amount of foliages, given the biotic (e.g., population density, mean diameter at breast height, ecological competition) and abiotic (e.g., soil type, hydrologic regime) characteristics of the stand. Furthermore, because these visual methods are too time consuming to allow monitoring of extended areas, forest conditions on a regional scale are inferred by a statistical approach. Reliability of the statistical approach is based on the number and size of sampling sites selected and on the method of selection employed (Innes 1995, Ferretti 1997).

Forest decline is the result of complex physiological processes that change tree biochemical and structural variables, such as the concentration, composition and efficiency in light harvesting of foliar pigments, and leaf area index (LAI). Monitoring these physiological processes may help to describe forest conditions. Under stress-inducing conditions, reductions in total chlorophyll concentration (Chl; chlorophyll a + chlorophyll b), and variations in chlorophyll a/chlorophyll b ratio (Chl a/b) and in chlorophyll/carotenoid ratio (Chl/Car) can occur (Young and Britton 1990, Biswal 1995, Merzlyak and Gitelson 1995, Demmig-Adams and Adams 1996, Taiz and Zeiger 1998, Gitelson et al. 2002, Lorenzini and Nali 2005). Variation in photosystem II (PSII) efficiency, which can be detected by estimation of chlorophyll fluorescence (CF), is also induced by stress-inducing conditions. Chlorophyll fluorescence occurs in competition with photochemistry and heat dissipation; hence, variation in the yield of CF may provide information about variation in the efficiency of photosynthetic activity (Papageorgiou and Govindjee 2004). The initial and
maximal fluorescence intensities \((F_o \text{ and } F_m, \text{ respectively})\), the PSII photochemical efficiency in the dark \((F_v/F_m; \text{ Kitajima and Butler 1975})\) and performance index \((PI; \text{ Strasser et al. 1999, Tsimillii-Michael et al. 2000})\) are widely used to diagnose plant stress (e.g., Méthy et al. 1996, Clark et al. 2000, Strasser et al. 2000, Hermans et al. 2003). At the canopy level, LAI is a structural variable related to crown transparency and tree vigor; therefore, deviation in LAI from an expected trend may provide additional information about forest condition.

Interest in the use of these foliar and canopy variables for forest assessment has recently increased (Carter and Knapp 2001, Zárco-Tejada et al. 2002, Sampson et al. 2003, Coops et al. 2004) because they can be indirectly estimated from remotely sensed data at the leaf and canopy levels. These variables directly influence vegetation optical properties particularly in the visible and infrared wavelengths, as has been confirmed with numerous optical indices acquired at narrow spectral resolution (in the order of 5–10 nm) and known as “hyper-spectral” data. These optical indices have been correlated to Chl concentration, Car concentration and Chl/Car (Carter 1994, Datt 1998, Zárco-Tejada et al. 2001, 2005, Sims and Gamon 2002, Dash and Curran 2004, Le Maire et al. 2004), photosynthetic performance (Gamon et al. 1992, 1997, Richardson et al. 2001, Sims and Gamon 2002, Stylinski et al. 2002, Winkel et al. 2002, Peñuelas et al. 2004) and LAI (Brogé and Leblanc 2001, Lee et al. 2004). Detailed investigation of these relationships has been encouraged by the recent availability of data from airborne and space-borne spectrometers with high spectral resolution which make it feasible to map canopy variables that are useful in forest monitoring on local and regional scales. Among these, MERIS (Medium Resolution Imaging Spectrometer), onboard the European Space Agency’s Envisat, and Hyperion, onboard the NASA’s Earth Observing-1 (EO-1), are potentially valuable sensors in terrestrial environment studies (Gong et al. 2003, Dash and Curran 2004, Curran and Steele 2005). Thus, it has become possible to introduce quantitative variables that can be estimated from remote observations, such as Chl and Car concentration, CF and LAI, to forest monitoring strategies and thereby overcome the drawbacks of the visual methods.

The primary objective of this research was to compare quantitative leaf and canopy variables with visual discoloration and defoliation indices for the assessment of forest condition. In particular, biochemical variables (i.e., Chl, Car, Chl/Car and Chl \(a/b\)) and CF variables (i.e., \(F_o, F_m, F_v/F_m\) and PI) were correlated to discoloration classes, and LAI was correlated to defoliation classes. Second, we focused on the relationships between these foliar quantitative variables and leaf optical properties acquired by high-resolution field spectroradiometry. The research was conducted in oak forests in the Ticino Regional Park, Italy. Forest decline in this area is probably the result of a combination of a severe infestation of oak processionary caterpillar \((Thaumetopoea processionea \text{ L.).})\) that occurred at the end of the 1990s, with periodic intense defoliations until 2001–2002 and the climatic extremes recorded in the same years. An intensive field campaign was conducted in summer 2003 concurrently with an airborne campaign with the airborne hyperspectral MIVIS (Multispectral Infrared and Visible Imaging Spectrometer) sensor. Estimation of quantitative variables at the leaf level from leaf optical properties is seen as a first step for further studies at the canopy scale by means of the aerial observations and for monitoring variation in the biophysical variables across the Ticino Park oak forests.

Materials and methods

Study area and sampling scheme

The Ticino Regional Park (8°84’ E, 45°44’ N), which belongs to the Ticino Valley Biosphere Reserve (UNESCO-Man and Biosphere Programme), is one of the largest and most important residual areas of the Po River plain in northern Italy. The Park is 91.14 ha and includes a flat alluvial landscape where the natural forest (22 kha) and the agricultural element (47 kha) coexist within an urbanized area. The site encompasses a mosaic of ecosystems with large river habitats, wetlands, riparian woods and conspicuous patches of primary plain forest that covered the entire valley during Roman colonization. The area is characterized by a traditional rural landscape with semi-natural ecosystems of rice paddies, cornfields and permanent grasslands.

Ten oak stands (coded Q1 to Q10) were randomly selected (stratified sampling), within the forested area, characterized by a high oak presence (>80%), closed canopy and fairly uniform defoliation and discoloration (Figure 1). Stands ranged from 0.2 to 2 ha. None of the stands were infested by oak processionary caterpillar at the time of the study, but signs of its presence were observed in the Ticino Park area until 2002. The stands suffered frost damage at the beginning of the growing season (May 2003), which was followed by a severe summer drought. The most severe frost-induced damage occurred in the southern area of the Park, where oaks had already budded by early May.

An intensive field campaign was conducted during the period July 2–4, 2003, corresponding to the period of maximum vegetation growth, with the aim of estimating visual indices (discoloration and defoliation) and quantitative variables (Chl, Car, CF and LAI). In each stand, leaves were sampled from three dominant oak trees, representative of the stand condition, located in the stand center. Branches on the south side of the upper crowns were detached with a shot gun and the branch ends were immediately stored in sealed plastic bags and kept in an ice chest until transported to the laboratory. Leaf optical properties and CF measurements were conducted within a few hours after branch excision. Following the measurements, the leaves were stored at –80 °C until Chl and Car were extracted. Because certain spectral characteristics are known to change rapidly with irradiance (Gamon et al. 1997, Evain et al. 2004, Louis et al. 2005), optical properties and CF were measured in a cool, darkened room on dark-adapted leaves to standardize measurements acquired on different days.
Visual indices of crown condition

Visual assessment of forest condition was conducted by means of a terrestrial survey following the crown visual assessment protocol (UNECE 1998, 2004b). This consisted in visual assessment of defoliation and discoloration of single tree crowns. The proportions of leaves discolored (yellowed) or lost in individual tree crowns were evaluated by the same observer with the aid of binoculars and categorized in four classes: class 0 = discoloration/defoliation < 10%; class 1 = 11–25%; class 2 = 26–60%; and class 3 = 61–100%. Based on the UNECE (1998) double entrance table (Table 1), defoliation and discoloration classes were then combined in four damage classes: undamaged (class 0); slightly damaged (class 1); moderately damaged (class 2); and severely damaged (class 3).

Leaf area index and forest stand measurements

To derive the effective LAI, five hemispherical photographs were taken at 1 m above ground level with a horizontally leveled digital camera (Nikon Cool Pix 4500) with a fish-eye lens (Nikon FE-E8 8mm), at the approximate stand center and 10 m from the center of each of the four cardinal directions. All photographs were taken under homogeneous illumination to ensure a good contrast between the canopy and the sky. Images were analyzed semi-automatically by Can Eye free share software Version 3.6 (http://www.avignon.inra.fr/can_eye/) to compute the gap fraction value at different zenith angles and derive the corresponding canopy structural characteristics (LAI and mean leaf inclination angle) (Jonckheere et al. 2004, Weiss et al. 2004).

Forest stand measurements were made within a 10 × 10 m plot located in the center of each stand. In each plot, number of trees, tree species, diameter at breast height and heights were recorded.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured on 10 leaves per tree. Chlorophyll a fluorescence transients were measured with a portable fluorimeter (PEA, Hansatech Instruments, King’s Lynn, U.K.) on leaves dark-adapted for 30 min with leaf clips. Fluorescence emission was induced on a 0.5 cm² leaf area with red light (peak at 650 nm) at 3000 µmol m⁻² s⁻¹ provided by an array of six light-emitting diodes. Following the JIP-test procedure (Strasser et al. 1995, 2000), analysis of transients took into consideration fluorescence values at 50 µs ($F_o$).

<table>
<thead>
<tr>
<th>Defoliation class</th>
<th>Discoloration class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1. Double entrance table that combines discoloration and defoliation classes into damage classes (UNECE 1998).
300 µs, 2 ms and $F_m$ to investigate: $F_o$, $F_m$, $F_o/F_m$ and PI. Data were analyzed with the software Biolalyzer (Maldonado Rodríguez 1998–2002).

**Pigment extractions**

Five leaves per tree were used to determine pigment concentrations in a total of 150 extractions. Leaf pigments were extracted with N,N-dimethylformamide (DMF) from a leaf disk of 2.3 cm diameter. The tissue samples were crushed by adding liquid nitrogen, ground in 5 ml of DMF for 3 h and then centrifuged at 3000 g at 4 °C for 30 min. Absorbance of the supernatant was measured at 663.8, 646.8 and 480 nm with a Thermo Spectronic Unicam UV 500 spectrophotometer (Spectronic Analytical Instruments, U.K.). The Chl a, Chl b and Car concentrations per unit leaf mass (µg g⁻¹) and per unit leaf area (µg cm⁻²) were calculated based on the extinction coefficients derived by Porra et al. (1989).

**Leaf radiometric measurements**

Radiometric measurements were made with a SPAD 502 (Minolta, Tokyo, Japan) leaf chlorophyll meter and a Li-Cor 1800-12 integrating sphere (Li-Cor, Lincoln, NE) coupled to an ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices, USA). The SPAD is a nondestructive, hand-held instrument that measures light attenuation at 650 nm (i.e., close to the maximum total Chl absorption) and 950 nm from a 0.06 cm² leaf area. Five readings between major veins were taken on each of 15 leaves per tree. Because SPAD values are not a direct measure of Chl concentration, a regression between SPAD units and foliar Chl concentration was calculated (Markwell et al. 1995).

The ASD FieldSpec FR is a high-resolution spectroradiometer that was coupled to the Li-Cor integrating sphere to measure leaf bi-hemispherical reflectance and transmittance at closely spaced wavelengths across the spectral range 350–2500 nm (i.e., a spectral resolution of 3 nm at 700 nm, and a sampling interval of 1.4 nm) from a 1.63 cm² leaf area. Leaf optical properties were measured in the laboratory on five leaves per tree from the same leaves used for the SPAD measurements and for pigment extractions.

Leaf reflectance ($\rho$) and transmittance ($\tau$) were calculated following the methodology described in the Li-Cor 1800-12 user manual (Li-Cor 1983) based on the following four measurements (Equations 1 and 2): radiation reflected from the sample (RSS); radiation transmitted from the sample (TSP); radiation reflected from the sphere internal standard (RTS); and the "stray" radiation due to the non-perfectly collimated beam of the illuminating source (RSA). Reflectance and transmittance were calculated as:

$$\rho = \frac{\text{RSS} - \text{RSA}}{\text{RTS} - \text{RSA}} \rho_{\text{BaSO}_4},$$

(1)

$$\tau = \frac{\text{TSP}}{\text{RTS}} \rho_{\text{BaSO}_4},$$

(2)

where $\rho_{\text{BaSO}_4}$ is the reflectance of the internal barium sulfate standard.

**Computation of optical indices**

We tested several narrowband reflectance indices to estimate leaf pigment concentration and CF variables from leaf reflectance. Optical indices that correlate well with Chl concentration are based on wavelengths located in the "red edge" region, which corresponds to the sharp transition from low reflectance in the red region to high reflectance in the near infrared. Estimation of leaf Car concentration from reflectance is more difficult than Chl concentration because of the overlap of the Chl and Car absorption peaks in the blue region and the high Chl concentration in green leaves. Chlorophyll a and chlorophyll b were not investigated individually because they are highly correlated and their absorption peaks overlap.

Indices based on simple mathematical combinations were used for Chl and Car estimation such as the ratios $R_{709}/R_{710}$ (Zarco-Tejada et al. 2001) where $R$ is the reflectance at the specified wavelengths (nm), $R_{710}$ and $R_{555}$ (Gitelson and Merzlyak 1994), $R_{672}/(R_{550} \times R_{708})$ (Datt 1998), the double difference ($R_{749} - R_{730})/(R_{701} - R_{672})$ (DD; Le Maire et al. 2004) and the normalized differences ($R_{750} - R_{705})/(R_{750} + R_{705})$ (Gitelson and Merzlyak 1994), ($R_{750} - R_{445})/(R_{705} - R_{445})$, ($R_{755} - R_{705})/(R_{750} + R_{705} - 2R_{445}$) (Sims and Gamon 2002). Indices based on derivative spectrum were also tested such as the red edge position (Horler et al. 1983, Curran et al. 1990), which is the wavelength corresponding to the maximum slope of the reflectance spectrum between 690 and 740 nm. The red edge position ($\lambda_{RE}$, nm) was determined as the local maximum of the first derivative spectrum smoothed by means of the Savitzky and Golay (1964) method. The MERIS terrestrial chlorophyll index (MTCI; Dash and Curran 2004), in our study ($R_{754} - R_{705})/(R_{705} - R_{431})$, was also tested. This index is strongly correlated with $\lambda_{RE}$, but unlike red edge position, it is sensitive to high values of foliar chlorophyll concentration.

Chlorophyll fluorescence ($F_o/F_m$ and PI) was empirically related to the photochemical reflectance index (PRI; calculated as $(R_{531} - R_{570})/(R_{531} + R_{570})$; Gamon et al. 1992). The PRI is a highly dynamic index correlated with the epoxidation state of xanthophyll cycle pigments, non-photochemical quenching of fluorescence (NPQ) and thus, PSII efficiency ($F_o/F_m$) (Gamon et al. 1997, Richardson et al. 2001). Measurements of PRI were made under uniform conditions (i.e., dark adaptation, same illumination condition).

**Statistical analysis**

A comparison between the discoloration and defoliation indices and the quantitative variables (Chl, Car, CF and LAI) was performed by one-way analysis of variance (ANOVA), followed by the Tukey test. Correlation coefficient values ($r$) between visual indices and quantitative variables were calculated. Relationship between vegetation indices and foliar variables were evaluated by reduced major axis regression (RMA). We used RMA regression instead of standard OLS (ordinary least squares) regression because it assumes that er-
rors in measurement occur in both the dependent and independent variables (Curran and Hay 1986, Cohen et al. 2003) and therefore, fit properly to our experimental data. The determination coefficient \( r^2 \), the root mean square error in fitting (RMSE), the coefficient of determination in cross validation \( r^2_{cv} \) and the RMSE in cross validation (RMSE\(_{cv} \)) are reported to compare regressions accuracy between different vegetation indices and leaf variables.

**Results and discussion**

**Visual damage evaluation**

Table 2 reports stand mean defoliation, discoloration and resulting damage classes. Damage class 1 (slightly damaged) is poorly represented in the dataset because 10 stands were insufficient to represent the four damage conditions and because of difficulties in discriminating between undamaged crowns (class 0) and slightly damaged crowns (class 1) based on visual indices. Furthermore, incipient discoloration may occur in the top leaves not visible to the field crew who assessed discoloration on the portions of canopy visible from below.

**Quantitative variables estimation**

To evaluate the foliar quantitative variables and their variation among stands, a reference value for each variable was calculated as the mean of all the leaves sampled in the 10 stands (Table 3). The relative deviation of each variable value from the reference value was then calculated for each stand (Figure 2): positive and negative values identify stands that are above and below the mean, respectively.

Chlorophyll concentration and Chl a/b were the biochemical variables that showed highest variations among stands with deviations from the mean of >10%. These variables were inversely proportional, with higher Chl concentrations corresponding to lower Chl a/b ratios. This may indicate that Chl a/b increases in damaged leaves because Chl a is less susceptible to degradation than Chl b. Among the CF variables, PI had the highest variations among stands, with deviations from the mean of > 30%.

Chlorophyll concentration and PI sometimes showed opposite behaviors because they are the result of processes characterized by different temporal dynamics. In response to stress, variation in photosynthesis functioning precedes that in Chl concentration; hence, CF changes may be observed long before discoloration occurs (Zarco-Tejada et al. 2002), resulting in Chl concentrations above the mean and PI values below the mean (e.g., Q8). Conversely, Chl concentrations below the mean and PI values above the mean (e.g., Q4) may indicate that plants are recovering from stress or are compensating for a stress-induced reduction in Chl concentration. A high Chl concentration guarantees high light absorption, but it does not necessarily mean high photosynthetic activity and high biomass production, as indicated by the information on the efficiency of the photosynthetic processes provided by PI.

Among structural variables, basimetric stand area ranged from 25.08 to 52.87 m\(^2\) ha\(^{-1}\) and LAI ranged from 1.8 to 3.4, indicating heterogeneity in tree development and density.

**Comparison between visual indices and quantitative variables**

For each stand, visual indices and mean values of the quantitative variables were compared by correlation analysis. Biochemical variables (i.e., Chl, Car, Chl/Car and Chl a/b) and CF variables (i.e., \( F_o \), \( F_m \), \( F_o/F_m \) and PI) were correlated to discoloration classes. Leaf area index was correlated to defoliation classes. Data significantly correlated are plotted in Figures 3 and 4.

Leaf variables were tested by one-way ANOVA, with discoloration as the variability factor. Stands were grouped by discoloration classes. Descriptive statistics were calculated within groups for each quantitative variable investigated. The
Tukey test was performed to evaluate if quantitative variables differed significantly among discoloration classes (Table 4).

Chlorophyll concentration and Chl a/b ratio correlated well with discoloration class ($r = -0.857, P < 0.01$ and $r = 0.812, P < 0.01$, respectively) and provided a basis for distinguishing three discoloration classes out of the four visually evaluated. The Tukey test showed that differences in Chl concentration and Chl a/b values between slight and moderate discoloration classes were not statistically significant.

Carotenoid concentration correlated significantly with discoloration classes ($r = -0.777, P < 0.01$), but its range of variation was small and it allowed identification of only two discoloration classes. There was no correlation between Chl/Car and discoloration.

Figure 2. Relative deviations of the investigated biochemical variables (A) and chlorophyll fluorescence (CF) variables (B) from the reference values calculated for each stand.

Figure 3. Discoloration classes plotted versus chlorophyll (Chl) concentration, carotenoid (Car) concentration, Chl a/b and performance index (PI).
Among the CF variables, only PI was significantly correlated with discoloration ($r = -0.638$, $P < 0.05$); however, the correlation was poor and PI did not discriminate among the classes of discoloration because PI is characterized by a faster dynamic that responds to physiological processes. Because CF values showed high variation within a stand and even within a tree, differences among stands were rarely statistically significant.

Correlations between canopy discoloration classes and quantitative leaf variables may be lowered because of the sampling strategy: quantitative variables were measured on leaves sampled from the top of the canopy, whereas visual assessment was conducted on the part of the canopy visible from below. This is more critical when the canopy is dense and closed. Moreover, the range typical of natural variables is inadequately expressed by discrete categories commonly used in forest condition assessment, whereas it is better expressed by continuous variables such as the leaf and canopy quantitative variables investigated.

Among the canopy structural properties, LAI showed significant correlations with both stand basimetric area ($r^2 = 0.679$, $P < 0.05$) and defoliation ($r^2 = -0.884$, $P < 0.001$). Assessment of crown defoliation was based on a visual comparison between the assessed tree and the reference tree. We believe that the defoliation scores assigned are biased because they reflect the tree development rather than the deviation of the tree foliage amount from the reference tree. We contend that the main problem was the definition of the reference tree in our study stands because of their heterogeneity in abiotic and ecosystem variables.

**Estimation of foliar variables from optical properties**

The SPAD values ranged from 11.40 to 53.34 with a mean value of 39.84, whereas Chl concentrations had a wider range of variation (i.e., $5.07–69.83 \mu g \text{ cm}^{-2}$, mean = $48.60 \mu g \text{ cm}^{-2}$). The empirical relationship obtained between Chl concentrations and SPAD values is represented by the nonlinear expression ($r^2 = 0.861$, $r^2_{cv} = 0.859$; Figure 5; Chl = $0.103 \times \text{SPAD}^{1.662}$). This nonlinearity reflects saturation of the SPAD measurements at high Chl concentrations and confirms the low sensitivity of SPAD to high Chl concentrations.

Vegetation indices calculated from leaf optical properties were related to foliar pigment concentrations and CF variables (Table 4). All regressions were statistically significant ($P < 0.001$). Chlorophyll concentration showed high correlations with all the indices tested. Correlation with MT-CI was stronger than correlation with $\lambda$RE (i.e., $r^2 = 0.851$, $r^2_{cv} = 0.846$ and $r^2 = 0.661$, $r^2_{cv} = 0.653$, respectively). Carotenoid concentration was best correlated with the optical index proposed by Datt (1998) $R_{672}/(R_{550} \times R_{708})$ ($r^2 = 0.421$, $r^2_{cv} = 0.399$).

There was a positive relationship between PRI and both $F_o/F_m$ and PI, but with poor coefficients of determination ($r^2 = 0.524$ and $r^2 = 0.311$, respectively) and weak coefficients of determination in cross validation ($r^2_{cv} = 0.443$ and $r^2_{cv} = 0.290$, respectively). The parallel changes in PRI, PI and $F_o/F_m$ were expected because the energy dissipation processes are comple-

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**Table 4. Leaf pigment concentrations and chlorophyll fluorescence (CF) variables tested for significant differences among foliar discoloration classes. Statistically different ($P < 0.05$) means are designated with different letters.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Discoloration class</th>
<th>0 = none</th>
<th>1 = slight</th>
<th>2 = moderate</th>
<th>3 = severe</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td>SD</td>
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<tr>
<td>Pigments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl ($\mu g \text{ cm}^{-2}$)</td>
<td>59.65 a</td>
<td>7.17</td>
<td>51.31 b</td>
<td>7.28</td>
<td>51.16 b</td>
<td>6.51</td>
</tr>
<tr>
<td>Car ($\mu g \text{ cm}^{-2}$)</td>
<td>10.09 a</td>
<td>1.64</td>
<td>9.13 b</td>
<td>1.21</td>
<td>9.13 b</td>
<td>1.14</td>
</tr>
<tr>
<td>Chl a/b</td>
<td>2.86 c</td>
<td>0.50</td>
<td>3.40 b</td>
<td>0.52</td>
<td>3.38 b</td>
<td>0.39</td>
</tr>
<tr>
<td>Chl/Car</td>
<td>5.95 a</td>
<td>0.43</td>
<td>5.65 ab</td>
<td>0.63</td>
<td>5.63 ab</td>
<td>0.50</td>
</tr>
<tr>
<td>CF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_o$</td>
<td>422.16 b</td>
<td>23.45</td>
<td>441.07 a</td>
<td>31.68</td>
<td>431.95 ab</td>
<td>24.94</td>
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<td>$F_m$</td>
<td>2470.5 a</td>
<td>167.9</td>
<td>2283.2 bc</td>
<td>273.1</td>
<td>2320.5 ab</td>
<td>226.9</td>
</tr>
<tr>
<td>$F_o/F_m$</td>
<td>0.810 a</td>
<td>0.017</td>
<td>0.782 b</td>
<td>0.032</td>
<td>0.789 b</td>
<td>0.022</td>
</tr>
<tr>
<td>PI</td>
<td>46.06 a</td>
<td>11.71</td>
<td>28.43 b</td>
<td>12.95</td>
<td>28.16 b</td>
<td>9.43</td>
</tr>
</tbody>
</table>
mentary. However, they were measured in different ways, which explains the scatter in the relationships among PRI, \( F_v/F_m \) and PI.

**Conclusions**

We compared quantitative leaf and canopy variables with visual indices that are generally employed in forest assessment protocols. Traditional visual indices provide valuable information, but they can be subjective and do not allow coverage of extended areas. To overcome these drawbacks, we investigated several variables that can be quantitatively estimated by remote observations over extended areas and therefore, may integrate traditional forest assessment protocols.

Leaf biochemical variables (i.e., Chl, Car, Chl a/b and Chl/Car) and CF variables (i.e., \( F_o \), \( F_m \), \( F_v/F_m \) and PI) were selected for study because their use in stress detection is well documented. Among the biochemical variables, Chl concentration and Chl a/b were the quantitative variables that correlated highly to discoloration and both variables were able to distinguish three discoloration classes out of the four visually evaluated.

Leaf area index was correlated with both stand development and defoliation scores, as assigned by the field crew. We contend that the field estimate of defoliation is biased. Because abiotic and ecosystem variables are heterogeneous in our stands, the defoliation scores reflect the tree development rather than the deviation of the tree foliage amount from the reference tree. Thus, we conclude that in our study area, LAI is not a reliable index of forest condition.

Our research focused on the quantitative estimation of foliar pigment concentrations and fluorescence variables based on the values of leaf optical properties acquired by field spectroradiometry. Statistical relationships between the measured variables and leaf optical properties were obtained by RMA regression models. Vegetation indices calculated from the red edge resulted in high correlations with Chl concentration, whereas weak correlations were found with the CF variables.

Overall, Chl concentration was the most reliable variable to assess and map forest condition in forests characterized by a high degree of heterogeneity such as those of the Ticino Regional Park forested area. Besides being highly correlated with

### Table 5. Optical indices most closely related to leaf biochemical and physiological variables (chlorophyll (Chl), carotene (Car), photosystem II photochemical efficiency in the dark (\( F_v/F_m \)) and performance index (PI)).

<table>
<thead>
<tr>
<th>Index</th>
<th>Reference</th>
<th>( r^2 )</th>
<th>RMSE</th>
<th>RMSE(_{cv} )</th>
<th>( r^2_{cv} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Related to Chl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTCI</td>
<td>Dash and Curran 2004</td>
<td>0.851</td>
<td>3.158</td>
<td>3.205</td>
<td>0.846</td>
</tr>
<tr>
<td>( R_{750}/R_{710} )</td>
<td>Zarco-Tejada et al. 2001</td>
<td>0.840</td>
<td>3.270</td>
<td>3.318</td>
<td>0.836</td>
</tr>
<tr>
<td>( (R_{750} - R_{645})/(R_{705} - R_{445}) )</td>
<td>Sims and Gamon 2002</td>
<td>0.822</td>
<td>3.454</td>
<td>3.505</td>
<td>0.816</td>
</tr>
<tr>
<td>( (R_{750} - R_{720}) - (R_{705} - R_{672}) )</td>
<td>Le Maire et al. 2004</td>
<td>0.837</td>
<td>3.302</td>
<td>3.369</td>
<td>0.830</td>
</tr>
<tr>
<td>( \lambda_{RE} )</td>
<td>Curran et al. 1990</td>
<td>0.661</td>
<td>4.762</td>
<td>4.821</td>
<td>0.653</td>
</tr>
<tr>
<td><strong>Related to Car</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>( R_{672}/(R_{550} \times R_{708}) )</td>
<td>Datt 1998</td>
<td>0.421</td>
<td>1.017</td>
<td>1.036</td>
<td>0.399</td>
</tr>
<tr>
<td>( R_{750}/R_{555} )</td>
<td>Gitelson and Merzlyak 1994</td>
<td>0.379</td>
<td>1.054</td>
<td>1.073</td>
<td>0.355</td>
</tr>
<tr>
<td>( (R_{750} - R_{705})/(R_{750} + R_{705}) )</td>
<td>Gitelson and Merzlyak 1994</td>
<td>0.336</td>
<td>1.089</td>
<td>1.110</td>
<td>0.311</td>
</tr>
<tr>
<td>( (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445}) )</td>
<td>Sims and Gamon 2002</td>
<td>0.357</td>
<td>1.072</td>
<td>1.092</td>
<td>0.332</td>
</tr>
<tr>
<td>( (R_{750} - R_{445})/(R_{705} - R_{445}) )</td>
<td>Sims and Gamon 2002</td>
<td>0.379</td>
<td>1.053</td>
<td>1.070</td>
<td>0.359</td>
</tr>
<tr>
<td><strong>Related to ( F_v/F_m )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRI</td>
<td>Gamon et al. 1992</td>
<td>0.524</td>
<td>0.032</td>
<td>0.034</td>
<td>0.443</td>
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<tr>
<td><strong>Related to PI</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>PRI</td>
<td>Gamon et al. 1992</td>
<td>0.311</td>
<td>10.880</td>
<td>11.085</td>
<td>0.290</td>
</tr>
</tbody>
</table>

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**Figure 5.** Empirical relationship between SPAD chlorophyll meter values (SPAD unit) and chlorophyll (Chl) concentration. Solid line represents the best regression fitted (\( P < 0.001, r^2 = 0.861, r^2_{cv} = 0.859 \)).
foliage discoloration, Chl concentration was highly correlated to leaf spectral properties, suggesting that it can be quantitatively estimated at the canopy and landscape scales from remote observations.

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References


