Visible and near-infrared reflectance techniques for diagnosing plant physiological status

When light strikes a leaf, part of the light spectrum is reflected towards the observer. Plant reflectance is governed by leaf surface properties and internal structure, as well as by the concentration and distribution of biochemical components, and thus remote analysis of reflected light can be used to assess both the biomass and the physiological status of a plant. Such assessment is of great value for applications such as determining the extent to which vegetation is stressed or at risk from fire.

Characteristics of leaf and canopy reflectance

Leaf reflectance can be detected using narrow-bandwidth spectroradiometers that measure in the visible and near-infrared part of the spectrum. In the visible spectrum (400–700 nm), leaf reflectance is low because of absorption by photosynthetic pigments (mainly chlorophylls and carotenoids) (Fig. 1). In the near-infrared domain (700–1300 nm), where there are no strong absorption features (Fig. 1), the magnitude of reflectance is governed by structural discontinuities encountered in the leaf. The middle infrared region (1300–3000 nm) presents variable reflectance values mainly linked to the absorption characteristics of water and other compounds.

At the canopy level, spectral reflectance is a combination of soil and vegetation reflectance, and the weighting for either of these two factors depends on external parameters such as illumination (e.g. the position of the sun or angle of view) or canopy structure. In addition to reflectances at particular wavelengths, many ‘high spectral resolution reflectance vegetation indices’ (which partly ‘remove’ disturbances caused by external factors) have been proposed, with the aim of monitoring biomass, phenology (periodic change) and physiological conditions of plants and canopies. Here, those indices that are used in field spectroradiometry (i.e. leaf, plant and canopy level) for diagnosing the physiological status of the plant are discussed (Table 1).

Assessing reflectance of plant biomass

The sharp contrast between soil and leaf reflectance in the near-infrared and visible bands has been exploited in the construction of ‘reflectance spectral indices’. The ‘near infrared : red (NIR : R) reflectance ratio’ and related indices, especially the ‘normalized difference vegetation index’ (NDVI) (NIR – R / NIR + R), are the most widely used and well known in remote sensing, not only at ground
Assessing the reflectance of photosynthetic pigments

Photosynthetic pigment composition can be used as an indicator of the physiological status of a plant. However, the standard methods for the measurement of pigments are slow and labour intensive. Reflectance might provide a rapid and easy alternative means of assessing pigment composition to determine nutrient status (by chlorophyll assessment); phenology and general stress (by assessment of carotenoids and chlorophyll $a$); or photosynthetic efficiency (by xanthophyll assessment).

Chlorophyll concentration can be derived using reflectances at 675 nm and at 550 nm. For very low concentrations, the reflectance sensitivity is higher at the maximum absorption located around 675 nm and, for medium-to-high chlorophyll concentrations, reflectance sensitivity is higher at 550 nm (Ref. 2). More recently, chlorophyll-concentration indices have been developed that include several of these waveband reflectances. Moreover, derivative techniques have been incorporated to minimize variations as a result of soil reflectance. Thus, the "red edge" – the wavelength of maximum slope in the increase of reflectance from red to near infrared (Fig. 1) – has been found to be a good indicator of chlorophyll content at the leaf level, and also (more controversially) at the canopy level.

There is a close correlation between leaf chlorophyll concentration and nitrogen availability, and thus the reflectance assessment of chlorophyll content can also be used to characterize the nitrogen status of vegetation and crops. Currently, simple computerized imaging systems based on these observations are being developed to maximize economic profit, to avoid excess nitrogen in the environment and to eliminate field heterogeneity.

Increased in the relative concentration of carotenoids with respect to chlorophyll are level, but also at airborne and satellite levels. They are closely related, with a range of highly intercorrelated biomass-related biological variables such as green biomass, leaf area index, leaf cover, fraction of radiation intercepted, or chlorophyll per unit ground area. More recently, it has been shown that NDVI principally assesses green biomass.

<table>
<thead>
<tr>
<th>Reflectance index</th>
<th>Acronym</th>
<th>Formulation*</th>
<th>Estimated biological parameter</th>
<th>Refs</th>
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</thead>
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<td>Simple ratio</td>
<td>SR</td>
<td>NIR/R</td>
<td>Green biomass</td>
<td>1</td>
</tr>
<tr>
<td>Normalized difference vegetation index</td>
<td>NDVI</td>
<td>NIR - R/NIR + R</td>
<td>Green biomass</td>
<td>1</td>
</tr>
<tr>
<td>Red edge</td>
<td>$\lambda_{RE}$</td>
<td>Wavelength of maximum slope in the increase of reflectance from red to near infrared</td>
<td>Chlorophyll</td>
<td>4</td>
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<tr>
<td>Structural independent pigment index</td>
<td>SIFI</td>
<td>R800 - R445/R800 - R680</td>
<td>Carotenoids : chlorophyll $a$ ratio</td>
<td>20</td>
</tr>
<tr>
<td>Photochemical reflectance index</td>
<td>PRI</td>
<td>R531 - R570/R531 + R570</td>
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<td>9, 10</td>
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<tr>
<td>Water index</td>
<td>WI</td>
<td>R900/R970</td>
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<td>12</td>
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<tr>
<td>Normalized phaeophytinization index</td>
<td>NQPI</td>
<td>R415 - R435/R415 + R435</td>
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*NIR, near infrared; R, red; Rx, reflectance at x nm.
Box 1: Empirical and semi-empirical approaches to the formulation of reflectance indices for estimating the carotenoids: chlorophyll ratio

Previously used reflectance indices, such as the normalized difference pigment index (NDPI), were empirically derived and might suffer from confounding effects introduced by leaf surface and structure. The NDPI is given by the following equation:

$$\text{NDPI} = \frac{R_{660} - R_{680}}{R_{660} + R_{680}}$$

Leaf reflectance, $R$, can be approximated by the following semi-empirical model$^5$:

$$R = R_i + S \exp(-5kC_i)$$

($R_i$ is the reflectance for a very high absorption coefficient; $S$ is the structural effect on reflectance; and $C_i$ is the concentration of absorbing compounds).

In order to minimize the specular component dominant in the $R_i$ term and the structural component dominant in $S$, a structure-independent pigment index (SIPi) that uses a near-infrared waveband ($800$ nm) as a subtracted and ratioed reference was recently defined in order to remove any additive and multiplicative factors$^6$.

$$\text{SIPi} = \frac{R_{680} - R_{640}}{R_{660} - R_{680}}$$

It provided a very good semi-empirical estimation of the carotenoids: chlorophyll $a$ ratio for data from several species$^6$.

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often observed when plants are subjected to stress and in senescing leaves$^2$. Ratios of reflectances in the blue domain (where carotenoids and chlorophylls absorb) and the red domain (where only chlorophylls absorb) have been found to be highly correlated with this pigment ratio in different plant species, both at the leaf and canopy levels$^7$ (Box 1).

Part of the light energy absorbed by chlorophyll for photosynthesis is lost as heat or fluorescence, and changes in the photosynthetic rate cause complementary changes in fluorescence emission or heat dissipation. Thus, measurement of these two de-excitation processes provides an indirect assessment of photosynthetic efficiency. Fluorescence is already widely used as a diagnostic tool for plant stress and photosynthetic performance$^7$, but heat dissipation has only recently been proposed for this indirect assessment.

Heat dissipation is linked to the xanthophyll-depoxidation cycle$^8$, and this has been found to correlate with reflectance at $531$ nm

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Fig. 2. (a) Kinetic plot showing nearly parallel changes in photochemical reflectance index (PRI) (open circles) and the fluorescence parameter $\Delta F/Fm'$ (filled circles), which is an indicator of photosystem II photochemical efficiency, for a bean leaf exposed to a ‘dark–light–dark’ transition in a leaf gas-exchange chamber under steady-state conditions. The steady-state net assimilation rates were $-1.9 \mu$mol $\text{CO}_2 \text{m}^{-2} \text{s}^{-1}$ for ‘dark’ conditions and $12 \mu$mol $\text{CO}_2 \text{m}^{-2} \text{s}^{-1}$ for ‘light’ conditions$^9$. (b) Photosynthetic radiation-use efficiency (mol $\text{CO}_2$ mol$^{-1}$ PPFD) presents similar relationships with $\Delta F/Fm'$ and PRI. These two plots show these relationships for randomly sampled leaves of two species ($\text{Phaseolus vulgaris}$ (filled circles) and $\text{Heteromeles arbutifolia}$ (open squares)) in field conditions under PPFD ranging from shade to full sun. Each point represents a separate leaf$^8$. Significant relationships between PRI, $\Delta F/Fm'$ and photosynthetic radiation-use efficiency have been found across species and functional types (annuals, deciduous and evergreen perennials) and for both sun and shade conditions$^9$. 

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(Refs 9 and 10). Several studies have shown closely related changes in the photochemical reflectance index (calculated as $R531 - R570/R531 + R570$, where $R$ is the reflectance); $\Delta F/Fm^*$, where $F$ is the fluorescence under ambient light, and $Fm^*$ is the fluorescence under a saturating light pulse [a fluorescence index widely used as an optical index of the light-use efficiency of photosystem II (PSII)]; and instantaneous leaf photosynthetic radiation-use efficiency (CO2 assimilation rate/incident photosynthetic flux density)$^9$$^{10}$ (Fig. 2).

The efficiency with which light energy is converted varies significantly between plants, seasons and ecosystems$^1$. By tracking short-term changes in photosynthetic light regulation at PSII, the photochemical reflectance index provides a means of assessing this efficiency. The generally consistent correlation between photochemical reflectance index and other measures of photosynthetic efficiency indicates that this index may be applied to leaf and canopy ground-based measurements of photosynthesis for most species$^{10}$, providing a useful tool for non-destructive, non-contact optical study of photosynthetic function. However, because of the relatively small reflectance signal, application of the photochemical reflectance index at the landscape scale requires careful attention to confounding effects of canopy structure, heterogeneous landscape, atmospheric interference and calibration errors.

**Assessment of drought and fire risks**

Measurement of plant water content is a common practice in ecophysiological studies, and is also useful for drought assessment in natural communities and for defining wildfire risk. The standard lab method for measuring water concentrations involves drying plants in an oven to determine weight loss; this is simple and reliable, but is also slow and time consuming. Again, high-resolution reflectance in the infrared regions of water absorption might provide a method to simplify this process.

For single leaves, the water-absorption bands in the 1300–2500 nm region show the highest sensitivity to leaf water concentration$^{11}$. Nevertheless, the absorption by water is very strong in this region, so the infrared bands are inadequate for measuring the water concentration of whole plants or canopies. Instead, a reflectance trough in the near-infrared region at 950–970 nm, corresponding to a weaker water absorption band (Fig. 3a), has been shown to be effective$^{12}$. The canopy sensitivity of the spectral trough at 970 nm seems to be caused by the ability of radiation at this wavelength to penetrate into the canopy in contrast to other longer absorption wavelengths$^{13}$. This provides reflectance readings that are a better representation of the total plant or canopy moisture content.

When plants are water stressed, the 970 nm trough of the reflectance spectrum tends to disappear and to shift towards lower wavelengths (Fig. 3a). A reflectance water index has been defined as the ratio $R900:R970$, where $R$ is the reflectance$^{12}$. The reflectance at 900 nm is used as a reference because there is no absorption by water at this wavelength, but it is subjected to the same changes in sample structure as the reading at 970 nm. This water index has been found to be highly correlated with plant water content in several species of trees, shrubs, crops and grasses$^{13}$. There is generally a strong correlation between greenness and moisture content, and thus the water index : NDVI ratio has been found to perform better than the water index itself$^{14}$.

**Potential for stress assessment**

Reflectance assessment of different physiological variables can also be developed to assist in the diagnosis of several stress conditions. In some cases, only relatively simple radiometers based on particular narrow wavelength bands are required. For example, for plant water content, a radiometer that measures reflectance specifically at 680, 900 and 970 nm can instantaneously calculate the NDVI and the water index and, by using appropriate calibration functions, give the estimation of plant water content in seconds$^{15}$. A simple radiometer that only measures reflectance at 531 and 570 nm can also calculate the photochemical reflectance index to give photosynthetic efficiency estimates$^5$. Several other examples also illustrate the potential of high spectral resolution reflectance in assessing plant stress.

Soil salinity decreases growth and net photosynthesis, and this in turn reduces biomass and yield. Salt-stressed plants often also show symptoms of water deficit, especially under conditions of high evaporative demand. Thus, a salt-stress response can be characterized by remote sensing of plant biomass and water status. The soil salinity response of ten barley genotypes has been assessed in this way, using the reflectance indices NDVI and water index, or a combination of the two$^{16}$. The symptoms of ozone damage include decreased chlorophyll concentration, resulting in increases in spectral reflectance at

![Image](image-url)
visible wavelengths, a shift of the red edge towards the blue end of the spectrum and increases in the "near-infrared plateau". However, these reflectance responses are similar to those induced by a variety of environmental stress conditions. The availability of high-spectral-resolution spectroradiometers provides a novel opportunity specifically to assess ozone stress. Reflectance changes in the far-red part of the spectrum associated with brown phenolic pigments could provide a more specific assessment of plant ozone stress (Fig. 3b).

Remote sensing techniques could also improve our knowledge of the amount of pest- and pathogen-induced damage, and thus prevent excessive use of chemical controls. Significant changes have been reported in the visible and near-infrared reflectance of plants attacked by pests such as mites or beetles. However, in some cases, such as apple tree field plots subjected to different levels of mite attack, classical reflectance indices such as red edge or NDVI were unable to distinguish between treatments. In those cases, however, other spectral indices based on carotenoid : chlorophyll a ratios and chlorophyll degradation (structural independent pigment index and phaeophytinization index) have been found to be correlated with the level of attack.

The harvest index of many crops has been maximized by past breeding programmes and is approximately constant, so yield is generally proportional to biomass at anthesis. In breeding studies, superior genotypes have recently been identified by biomass determination through destructive sampling, but such sampling is not feasible in large breeding trials. Measurement of ground-based reflectance spectra could provide accurate, non-destructive, and rapid estimates of plant biomass. Some trials of this technique are being conducted in breeding of cereals in the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), an international institute in Mexico.

**Canopy chemistry**

Reflectance in the middle-infrared region acquired not only with ground-based spectroradiometers, but also with airborne sensors such as CASI (compact airborne spectrographic imager) or AVIRIS (airborne visible/infrared imaging spectrometer), has been found to be correlated with field-measured foliage and litter chemical concentration of nitrogen, lignin or starch. Estimates of nitrogen and lignin from these reflectance data have been applied to ecosystem function models that might thus gain spatial and temporal dimensions crucial for understanding ecosystem functioning.

**Future perspectives**

Spectral reflectance provides a variety of means of detecting plant chemical composition and biomass, and therefore plant physiological status and phenology. The application of ground-level spectral reflectance to simple computerized imaging systems will provide spatial and temporally distributed information for plant biology research and crop and vegetation management. Landscape-level application of these techniques for the estimation of the physiological status of vegetation from airborne (Fig. 4) or satellite platforms requires further studies that test whether physiological reflectance signals are stronger than reflectance disturbances introduced by factors such as the position of the sun, heterogeneity of the landscape or atmospheric interference. If this was the case, these optical techniques would allow an impressive step forward in the knowledge of ecosystem functioning at complex landscape level.

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Roots, drugs and transformation

Hairy Roots: Culture and Applications
editor by P.M. Doran
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Hairy roots disease is caused by the soil bacterium Agrobacterium rhizogenes and is characterized by the extensive formation of adventitious 'hairy' roots. The bacteria infect a broad spectrum of plants, and can cause substantial economic losses. However, hairy roots also have favourable characteristics, such as rapid growth, making them attractive as experimental material, and in the 1980s they began to receive a lot of attention for this reason. Now, hairy roots are used in a wide variety of fields, such as plant secondary metabolism and its genetic manipulation, transformation, propagation in agriculture, environmental research, and the development of new engineering technology for large-scale production of plant chemicals. This book presents hairy root studies from a wide range of basic and applied fields: culture and product synthesis; plant propagation and environmental aspects; and bioprocessing. The first chapter also presents useful protocols, covering aspects such as how to establish hairy root cultures, vector construction and gene analysis.

Studies about secondary metabolites and plant chemical production are the most interesting and active fields in hairy root research – natural products from plants are of major pharmaceutical and therapeutic importance. For example, in China, Japan and Korea, crude drugs made from ginseng are commonly used in everyday life (indeed, roots of ginseng have been used as a tonic in Asian countries for millennia). However, ginseng also illustrates one of the common problems with obtaining pharmaceuticals from plants – they are often only present in very small amounts. Recently, saponins were found to be an active component of ginseng roots. Ginseng roots are extremely thin, and the amount of saponin extracted is very small, which can lead to very expensive crude drugs. Other examples of useful natural pharmaceuticals from plants include quinine, which is obtained from the bark of the Cinchona tree and used to alleviate the symptoms of malaria; and Cascara (Rhamnus) bark, which is the source of an effective laxative.

For industry, studies about secondary metabolites and plant chemical production are very attractive because they might make it possible to produce large amounts of crude drugs by plant tissue culture. As with the study of such compounds as hyoscyamine and scopolamine, tropane alkaloid has been extensively studied using hairy roots of various species. In this book, Catharanthus roseus and Cinchona species for metabolite engineering of indole alkaloid biosynthesis, and Nicotiana and Datura for tropane alkaloid biosynthesis, are introduced. Alkaloids constitute a large group of plant secondary metabolites and have particular pharmaceutical importance. Trials continue to be carried out for the production of useful plant products by large-scale culture, and the development of industrial processes to produce secondary metabolites is now under way. The book presents many different kinds of efforts to realize industrialization. In order to increase culture growth rates, bioreactor systems and oxygen supply have been studied. Furthermore, to obtain as much ingredient as possible, studies of operating systems and elicitor treatments are being carried out.

Hairy roots are also promising materials for breeding transgenic plants. The initiation of hairy roots is conveyed by T-DNA genes, which can be transferred to plant chromosomal DNA and stably integrated. Established hairy root cultures are capable of quite rapid growth in vitro on hormone-free medium. In addition, excised root cultures have been used for the production of transgenic plants under hormonal control. Initial interest in using A. rhizogenes as a vector for plant transformation was dented by the finding that plants regenerated from hairy roots often have an altered phenotype, including wrinkled leaves, shortened internodes and a highly branched root system. Despite such disadvantages, transformation by A. rhizogenes does permit relatively easy recovery of transgenic plants, and thus the system has continued to be used for many studies. In New Zealand, field tests of transgenic plants produced using hairy roots are already being conducted in potato and Kapet kale. Altered phenotypes are generally undesirable, but in some cases, in horticulture and transgenic field trials, such alteration can be advantageous. Altered phenotypes are caused by the introduction of rol genes. A recently developed 'hit-and-run cassette system' might remove rol genes after the transformation and eliminate the altered phenotype problem.

Hairy Roots covers a wide field, but manages to be fully representative. The editor’s stated aim – that, in publishing studies from many fields together, she can provide an opportunity for wider appreciation of activity in this field, and for cross-disciplinary exchange of ideas,