RAMIS: A NEW PORTABLE FIELD RADIOMETER TO ESTIMATE LEAF BIOCHEMICAL CONTENT

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ABSTRACT

The capability for *in situ* measurements of leaf biochemistry is very useful for applications in agriculture, forestry or ecology. A portable field radiometer, RAMIS (*RAdiomètre portatif de Mesure In Situ*) has been developed by the University of Paris 7 – Denis Diderot for this purpose. Unlike the SPAD-502 (Minolta) and CCM-200 (Opti-Sciences) devices which were only designed to determine total chlorophyll concentration, RAMIS should additionally estimate the leaf equivalent water thickness and the leaf mass per area (or specific leaf area) by measuring leaf transmittance at five wavelengths in the VIS-NIR-MIR and inversion of the PROSPECT leaf optical properties model.

In order to validate it, a collaborative field campaign organized in June 2003 in the INRA of Angers (France) led us to build a database, gathering 324 leaf samples: 222 maple (*Acer pseudoplatanus* L.) leaves coming from trees grown under glass in variable environmental conditions (light intensity, nitrogen content, water supply, etc.) and 102 leaves of about forty different species collected outdoors. The chlorophyll concentration, equivalent water thickness and leaf mass area were determined for each sample by classical methods. In parallel, radiometric measurements have been performed using RAMIS, SPAD-502, and a portable field spectrophotometer, FieldSpec-FR (Analytical Spectral Devices).

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A database gathering all the data was used to validate RAMIS. We first compared the transmittance signal measured by the three instruments to check their consistency. The leaf biochemistry was then related to the transmittance measured by RAMIS at five wavelengths: empirically, by using neural networks, and by inversion of the PROSPECT model.

Keywords: photometry, leaf, chlorophyll, water, dry matter content, leaf optical properties model, PROSPECT

INTRODUCTION

In many applications like agriculture, forestry or global ecology, it is now crucial to determine the biochemical content of plant leaves, i.e. mainly chlorophyll *a* and *b*, water, and leaf mass per area (Ustin et al., 2004). Chlorophyll content, which can be roughly related to nitrogen content, recently became one of the key variables for measuring plant canopies in precision farming. That farming concept takes into account intra-field variability and ultimately aims at applying the appropriate amounts of inputs, fertilizers for instance, in various locations within a field to achieve an economically profitable yield while preserving the environment (Moran et al., 2004). The monitoring of water content is also subject to special attention in agriculture because this variable limits productivity of crop plants exposed to water stress. In natural vegetation, it is essential for drought assessment and prediction of wildfire susceptibility (Ceccato et al., 2001). The leaf mass per area (or dry matter content) is equivalent to specific leaf area, a widely used key variable in plant ecology which is correlated with plant growth, light interception, gas exchange, and photosynthesis (Meziane and Shipley, 2001; Wright et al., 2004).

The last generation of spaceborne sensors dedicated to monitor crops and to better understand ecosytem functioning measures the radiance of targets in continuous spectral bands. They provide an opportunity to map variation in canopy biochemistry, for the moment chlorophyll and water content. In order to validate these new remote sensing products, several international field campaigns have been started (Justice et al., 2000), which require the determination of the leaf biochemical content among other variables.

While the determination of water and dry matter "only" requires a precision balance and an oven, which can be brought into the field, the extraction and measurement of chlorophyll pigments involve much more complicated and expensive wet chemistry methods. Moreover, these processes are almost always destructive and difficult to implement when the experimental field is far distant from the laboratory. As soon as cut, the leaf tends to deshydrate and to loose pigments by degradation. All these factors limit sampling capacity, thus the representativeness of such measurements.

The *in situ* measurement of these biochemical constituents can be performed by non-destructive techniques, generally optical ones for pigments (chlorophyll meters) or water, athough other techniques exist for the latter. Studies on leaf optical properties performed over the last fifty years have shown that they vary as a function of two main factors: the anatomical structure and the biochemical content. The visible radiation (400-700 nm) and the middle-infrared radiation (1200-2500 nm) are mainly absorbed by photosynthetic pigments and water (and by cell walls to a lesser extent), respectively, so that they provide information about cellular contents, while the near-infrared radiation (700-1200 nm) is insensitive to the biochemistry but is informative about the internal leaf structure.

Laboratory or field spectroradiometers, equipped with an integrating sphere, can measure leaf optical properties in several contiguous wavebands to determine the chlorophyll concentration by using spectral indices (see Le Maire et al., 2003 for a review). However, such instruments are expensive and often difficult to use in the field. Portable photometers sensitive to chlorophyll have been designed for this purpose (Hardwick and Baker, 1973; Wallihan, 1973; Macnicol et al., 1976; Hardacre et al., 1984 for instance). The most popular is the SPAD-502 (*Soil Plant Analysis Development*, Minolta) which has given rise to an abundant literature during the last two decades (Yadava, 1986; Schaper and Chacko, 1991; Markwell et al., 1995; Manetas et al., 1998; Richardson et al., 2002, among others). The principle on which it works is as follows: the leaf surface is illuminated by two LEDs (Light Emitting Diodes) at 650 nm and 950 nm and the transmitted fraction of light is measured by a Silicon photodiode. The two transmittances are then combined to produce a spectral index which is related to the chlorophyll concentration using a calibration relationship provided by the manufacturer. Although largely distributed, the SPAD-502 does not totally satisfy users: first, the calibration depends on the leaf species due to the wide range of anatomical structures in nature (Richardson et al., 2002). The blade structure is known to change the leaf optical properties not only in the near-infrared where absorption is weak but also on the whole spectrum. It means a lower accuracy when various species are analyzed by the chlorophyll meter. Moreover, measurements cannot be performed in many mature dicot leaves which present high chlorophyll concentration, particularly persistent leaves. Finally, the SPAD-502 is restricted to chlorophyll and is sold at a prohibitive price for many users. The CCM-200 (*Chlorophyll Content Meter*, Opti-Sciences) is based on the same principle so that its readings are highly correlated to those of the SPAD-502 (Richardson et al., 2002). Finally, digital analysis of video images (Spomer et al., 1988) or color photographs (Andrieu et al., 1992) have been also used for quantifying leaf chlorophyll concentration.

As for water, techniques based upon the observation of leaf temperature kinetics following changes in leaf energy balance (Buriol et al., 1984; de Parceveaux et al., 1995) or upon the transmission measurement of terahertz radiation (Hadjiloucas et al., 1999) are available to measure leaf water state. As with chlorophyll, water can be also determined using spectral indices in the near- and middle-infrared or using radiative transfer models (Ceccato et al., 2001), but to date there is no commercial portable device devoted to its measurement.

A new portable field radiometer, RAMIS (*RAdiomètre portatif de Mesure In Situ*) has been developed at the University of Paris 7 – Denis Diderot to determine total chlorophyll concentration, leaf equivalent water thickness and leaf mass per area by measuring the leaf transmittance at five wavelengths in the VIS-NIR-MIR and inverting a neural network or the PROSPECT leaf optical properties model.

The RAMIS measurements are compared to the SPAD-502 outputs and the FieldSpec-FR portable field spectrophotometer spectra.

EXPERIMENT

Plant material

Deciduous and persistent leaves were chosen to cover a wide range of anatomy structure, thickness, surface roughness, and biochemical content (biomass, water and chlorophyll content). A field campaign performed in June 2003 in the INRA Agricultural Experiment Station of Angers (France) allowed us to build a database containing 324 leaf samples divided into 222 ornamental maple leaves (*Acer Pseudoplatanus* L.) picked from trees grown under glass or outside in variable environmental conditions (light intensity, nitrogen fertilization, water supply, etc.) and 102 leaves of about forty different species collected outdoors: boxelder (*Acer negundo* L.), sycamore maple (*Acer pseudoplatanus* L.), European alder (*Alnus glutinosa* L.), beauty berry (*Callicarpa bodinieri*), European chestnut (*Castanea sativa* P. Mill.), Judas tree (*Cercis silicastrum*), dogwood (*Cornus alba* L.), giant filbert (*Corylus maxima* Mill.), European smoketree (*Cotinius coggygria* Scop.), cider gum (*Eucalyptus gunnii* Hook.f.), winter creeper (*Euonymus fortunei*), European beech (*Fagus sylvatica* L.), geranium (*Geranium pratense* L.), English ivy (*Hedera helix* L.), French hydrangea (*Hydrangea macrophylla*), English holly (*Ilex aquifolium* L.), English walnut (*Juglans regia* L.), European privet (*Ligustrum vulgare* L.), sweetgum (*Liquidambar styraciflua* L.), holly osmanthus (*Osmanthus heterophyllus*), Boston ivy (*Parthenocissus tricuspidata* Planch.), white poplar (*Populus alba* L.), cherry laurel (*Prunus laurocerasus* L.), Portugal laurel (*Prunus lusitanica* L.), holly oak (*Quercus ilex* L.), pin oak (*Quercus palustris* Muenchh.), rhododendron (*Rhododendron calophytum*), black locust (*Robinia pseudoacacia* L.), grey willow (*Salix atrocinerea*), umbrella tree (*Schefflera arboricola* Merr.), staghorn sumac (*Rhus tiphyna* L.), common lilac (*Syringa vulgaris* L.), silver linden (*Tilia tomentosa* Moench**),** tuliptree (*Liriodendron tulipifera* L.), Japanese snowball (*Viburnum plicatum* Thunb.), leatherleaf arrowwood (*Viburnum rhytidophyllum* Hemsl.), laurustinus (*Viburnum tinus* L.), wine grape (*Vitis vinifera* L.), weigela (*Weigelia florida*).

The maples trees are genetically similar siblings: their leaf biochemical content and mesophyl structure have been artificially modified under controlled conditions to produce a wide range of leaf transmittances. Various chlorophyll concentrations have been obtained by applying two levels of nitrogen fertilization (N^+ and N^-). Four levels of PAR (photosynthetic active radiation) created by shading the seedlings with neutral semitransparent nets, causing variance in the internal leaf structure: PAR1 $\rightarrow$ solar radiation (no shadow), PAR2 $\rightarrow$ incident light divided by two, PAR3 $\rightarrow$ incident light divided by three, and PAR4 $\rightarrow$ incident light divided by four. Finally, water content was changed by stressing the plants.

Thirty or so leaves (mostly maple) of 324 were reddish. The purple color which affects both the adaxial and/or abaxial side of the leaves, more or less markedly, is due to anthocyanins and is known to appear during leaf development

(Hoch et al., 2001) or following a stress (Lee and Gould, 2002). These leaves will be useful to analyse the influence of those pigments on chlorophyll content prediction.

Leaf optical properties

Optical measurements have been alternately performed on half the blade of each leaf sample with three instruments: the SPAD-502 above-mentioned, the FieldSpec-FR portable spectrophotometer coupled with an integrating sphere coated with $BaSO_4$, to acquire full reflectance and transmittance spectra between 350 and 2500 nm, and RAMIS.

Fig. 1. Use of RAMIS in glasshouse.

RAMIS is a hand-held light transmittance meter for use in measuring leaf chlorophyll content, water content and mass per area. The leaf to be measured is subjected to the irradiation of light from five LEDs covering the optical domain. The level of light transmitted through the blade is measured by a photodiode. The device is controlled and the acquisition of the output signal is achieved using a data acquisition card (Labview, National Instrument) connected to a laptop. The calibration of RAMIS is performed with the help of three Lambertian diffusers (SphereOptics) of thickness 1 mm, 250 μm, and 100 μm, spectrally calibrated by the manufacturer and corresponding to average transmittances of 0.08, 0.25, and 0.50, respectively.

Leaf biochemical constituents extraction

Immediately after the optical measurements, fragments of leaf tissue were sampled to determine their biochemical content (Figure 2): photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total carotenoids expressed in μg cm^{-2}) were extracted in ethanol 95% (v/v) from 5 discs of 5 mm in ∅ following Lichtenthaler (1987). Water content (expressed in g $cm^{-2} \equiv cm$) and dry matter content (expressed in g cm^{-2}) were calculated by weighing three discs of 10 mm in ∅ of fresh and dry samples of leaf material, and averaging. Table 1 shows measured ranges in leaf biochemical contents.

Table 1. Range values on leaf biochemistry.

	Chlorophyll *a+b* (μg cm^{-2})	Carotenoids (μg cm^{-2})	Water (cm)	Leaf mass per area (g cm^{-2})
Min	1.9	0.0	0.00439	0.00166
Max	173.0	25.3	0.03400	0.03310
Mean	52.4	8.6	0.01146	0.00504
Std	33.9	5.2	0.00461	0.00347

Figure 2 presents their biochemical relationships. As expected, there is a close linear relationship between chlorophylls *a* and *b,* as well as between total carotenoids and chlorophylls. Water and dry matter content do not show any marked trend.

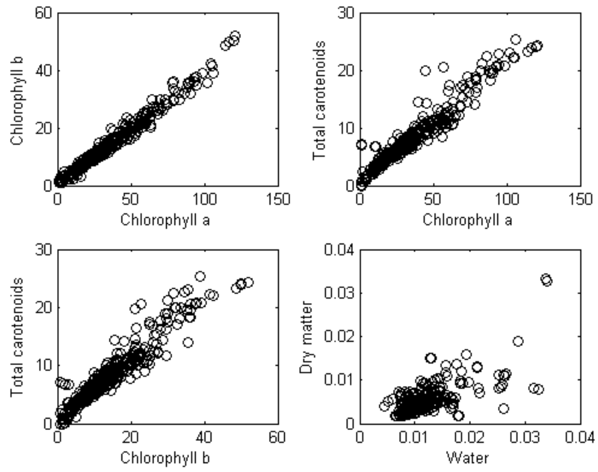

Fig. 2. Biochemical measurements performed during Angers'03.

THE PROSPECT MODEL

PROSPECT (Jacquemoud and Baret, 1990) is a leaf optical properties model now widely used in the remote sensing community. It is based on Allen et al. (1969, 1970) representation of the leaf as one or several absorbing plates with rough surfaces giving rise to isotropic scattering of light. Its four input parameters are the structure parameter N (number of compact layers specifying the average number of air/cell walls interfaces within the mesophyll), the chlorophyll *a+b* content C_{ab} (μg cm^{-2}), the water content or equivalent water thickness C_w (g cm^{-2} $\equiv$ cm), and the dry matter content or leaf mass per area C_m (g cm^{-2}). The outputs of the model are the hemispherical reflectance and transmittance of various plant leaves (monocots, dicots or senescent leaves) over the solar spectrum (from 400 to 2500 nm). The last calibration performed some years ago (Jacquemoud et al., 2000) was upgraded using the Angers'03 database described above. Models are very useful to understand how electromagnetic radiation interacts with the leaf and to relate remote sensing observables to fundamental biophysical attributes (see Ustin et al., 2004). In direct mode, sensitivity analyses allow to quantify the contribution of each of the input parameters to the model outputs, as well as their interactions. In this study, the Design Of Experiments for Simulation (DOES) method has been applied to selection of the five RAMIS wavebands (Figure 3). In inverse mode, PROSPECT turned out to generally perform well both in terms of spectrum reconstruction and leaf biochemistry estimation.

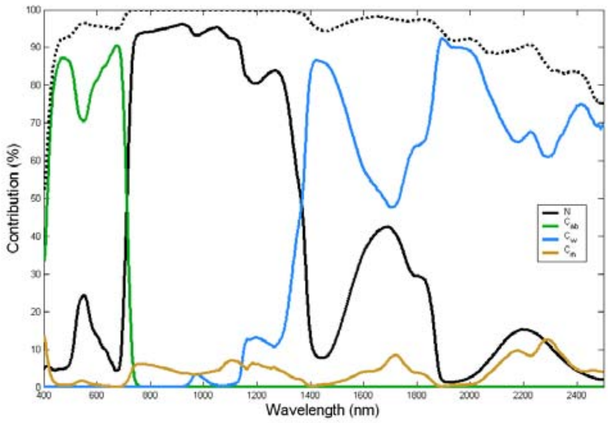

Fig. 3. Contribution of C_{ab} (green), C_w (blue), C_m (brown) and N (black) to leaf transmittance calculated by PROSPECT and the DOES method. The dotted line represents the sum of the contributions (Pavan, unpublished).

RESULTS

Extraction of leaf biochemistry by semi-empirical approaches

This approach consists of relating spectral indices to biochemical characteristics of the leaf. Combinating narrow bands is the most classical method, e.g., application of spectral vegetation indices at the canopy level. All chlorophyll meters are based on this approach. We first decided to compare SPAD, the SPAD-502 reading, with RAM, a similar index calculated as the logarithm of the ratio of the near-infrared transmittance to the red transmittance.

$$RAM = \log\left(\frac{T_{NIR}}{T_{VIS}}\right)$$

The two signals are linearly and highly correlated (R^2 = 0.986). The chlorophyll content C_{ab} has been plotted as a function of both SPAD and RAM. Figure 4 shows a similar trend which has been fitted by second order polynomials.

$$C_{ab} = 1.0919 \times SPAD^2 + 0.0135 \times SPAD$$
$$C_{ab} = 10.394 \times RAM^2 + 5.9888 \times RAM$$

In a second stage, those equations were used to estimate the chlorophyll concentration: C_{ab} is determined with RMSE = 12.73 and 12.24 µg cm^{-2}, R^2 = 0.858 and 0.868, for SPAD and RAM respectively. These RMSE values are higher than those published in the literature for two reasons: first our dataset includes dark green leaves with concentrations higher than 100 µg cm^{-2} and for which the estimation errors become more significant due to saturation effects. Second, it comprises forty species while most published studies are monospecific. For instance, Richardson et al. (2002) who studied paper birch (*Betula papyrifera*) leaves with the SPAD-502 report a RMSE of 2.1 µg cm^{-2} for chlorophyll contents ranging from 0.4 to 45.5 µg cm^{-2}. This value compares with the RMSE of 4.21 µg cm^{-2} that we obtained when we selected leaves with chlorophyll contents less than 50 µg cm^{-2}. The same kind of semi-empirical approach has been tested with water and dry matter by using information of the MIR, unsuccessfully.

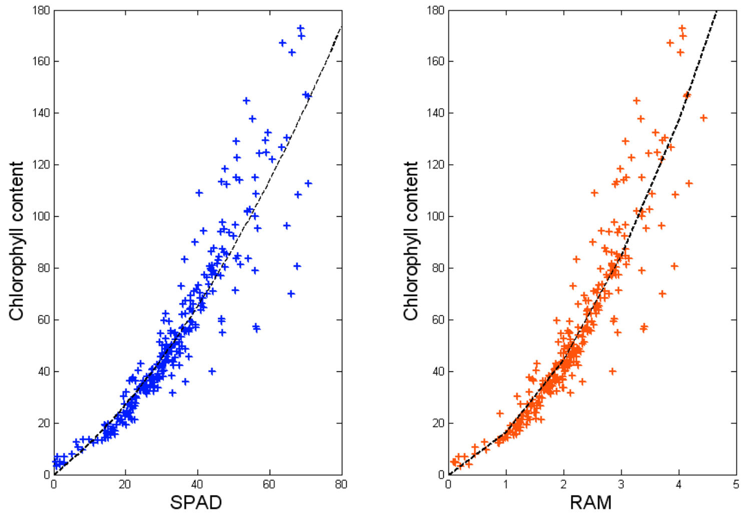

Fig. 4. Correlation of two indices with total chlorophyll content.

Extraction of leaf biochemistry by neural networks

Neural networks have been already used at the leaf level to relate leaf biochemistry to their optical properties (de Rosny et al., 1995; Dawson et al., 1998; Le Maire et al., 2004). Inversions were conducted here on the five RAMIS transmittances with a « feedforward » neural network where the neurons are arranged in two layers. The first one is composed of 16 sigmoid neurons and the second of 4 linear neurons. Biochemical and spectral information is equally split into two data sets: the first one is called the training set and contains about 150 leaves, which is used for the training phase of the neural system, the second one contains the remaining leaves and is called the control set, which makes it possible to check the adjustment of the parameters of the neural system.

The neural network was trained for 300,000 epochs. Extractions of the leaf biochemistry on both the training set (RAM_{train}) and the control set (RAM_{valid}) provide very good results as seen in Table 2 and Figure 5. In particular, the estimation errors on chlorophyll *a*+*b* content decreased by 5 µg cm^{-2} when compared to the semi-empirical approach. This is not surprising since the neural networks can take into account spectral variations attributed to the other leaf biochemicals or to leaf structure.

Table 2. Statistics of the inversion on RAMIS signal by neural networks.

	C_{ab}		C_w		C_m	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
RAM_{train}	7.43	0.954	0.00232	0.666	0.00178	0.746
RAM_{valid}	7.30	0.955	0.00289	0.603	0.00251	0.646

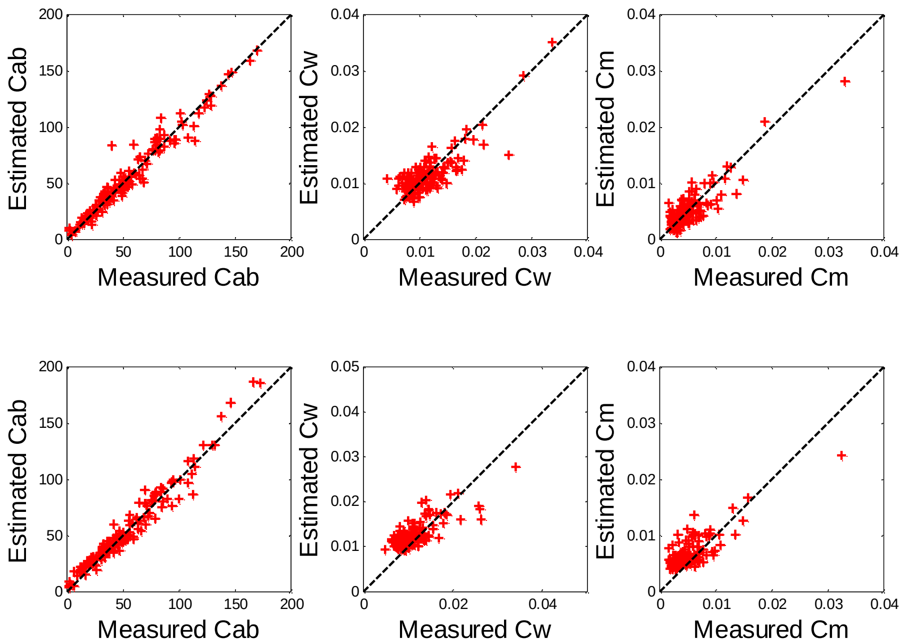

Fig. 5. Measured and estimated values of chlorophyll a+b content (µg cm^{-2}), equivalent water thickness (cm), and dry mater content (g cm^{-2}) by applying neural networks on RAM_{train} (up) and RAM_{valid} (down) transmittances.

Extraction of leaf biochemistry by inversion of PROSPECT

The simplex algorithm, an iterative optimization algorithm, was used to invert the PROSPECT model. Inversions which consist in minimizing a merit function χ^2, difference between the measured reflectance $\rho_{measured}$ (and/or the measured transmittance $\tau_{measured}$) and the model outputs $\rho_{PROSPECT}$ (and/or $\tau_{PROSPECT}$), were performed on about 300 green leaves:

$$\chi^2 = \sum_{\lambda_1}^{\lambda_n} \left(\rho_{\text{measured}}(\lambda) - \rho_{\text{PROSPECT}}(\lambda, N, C_{ab}, C_w, C_m) \right)^2$$

Inversion on ASD spectra: we first decided to invert PROSPECT on full reflectance (ASD_{refl}) and transmittance (ASD_{trans}) spectra measured by the ASD between 400 and 2000 nm with a 1 nm step (Figure 6), as well as a combination of the two ($ASD_{refl+trans}$). This spectral configuration provides the maximum information on leaf biochemistry.

The best estimates are globally obtained with the transmittance as seen in Table 3 and Figure 7. On red maple leaves, model inversions tend to overestimate C_{ab} but are still in a satisfactory range. The reason is that the shift in the absorption peaks in the visible due to anthocyanins (Gitelson et al., 2001) are not accounted for by PROSPECT, which interprets an increase of absorption as a higher chlorophyll content.

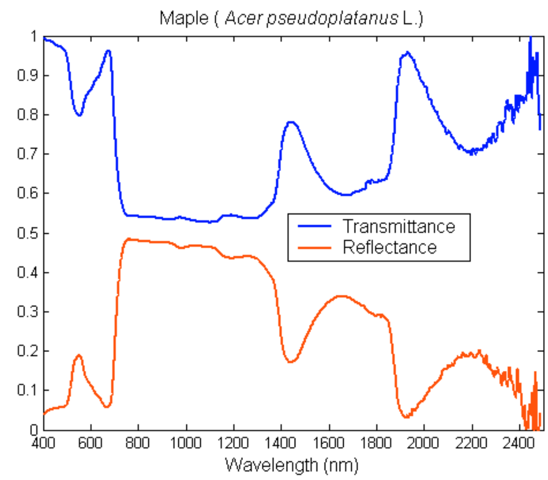

Fig. 6. Reflectance and transmittance spectra of a green maple leaf.

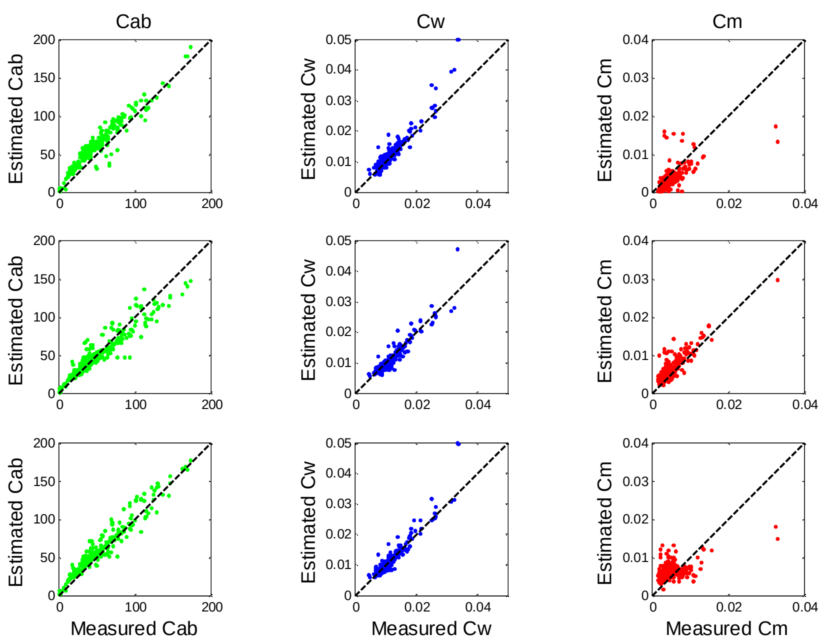

Fig. 7. Measured and estimated values of chlorophyll content (µg cm^{-2}), equivalent water thickness (cm), and dry matter content (g cm^{-2}) by inversion of PROSPECT on leaf spectra measured by the ASD.

Table 3. Statistics of the inversion of PROSPECT on full ASD spectra.

	C_{ab}		C_w		C_m	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
ASD_{refl}	13.39	0.915	0.00222	0.910	0.00300	0.435
ASD_{trans}	11.53	0.898	0.00173	0.885	0.00190	0.763
$ASD_{refl+trans}$	9.88	0.936	0.00188	0.899	0.00305	0.282

Inversion on RAMIS signal: we compare here results obtained in two different ways. We first consider an equivalent RAMIS signal (RAM_{ASD}) determined by convolving the ASD transmittance spectra with the optical characteristics of the LEDs and the photodiode, and after that the output signal of RAMIS (RAM_{LED}). Table 4 and Figure 8 summarize the performance of the inversions. It shows that chlorophyll is determined with good accuracy for both RAM_{ASD} and RAM_{LED}, at least the same level as with semi-empirical approaches. The situation is worse for the estimation of water and leaf mass per area which is poor.

Table 4. Statistics of the inversion of PROSPECT on RAMIS signal.

	C_{ab}		C_w		C_m	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
RAM_{ASD}	17.33	0.819	0.00482	0.397	0.00453	0.213
RAM_{LED}	11.91	0.896	0.00619	0.179	0.00649	0.136

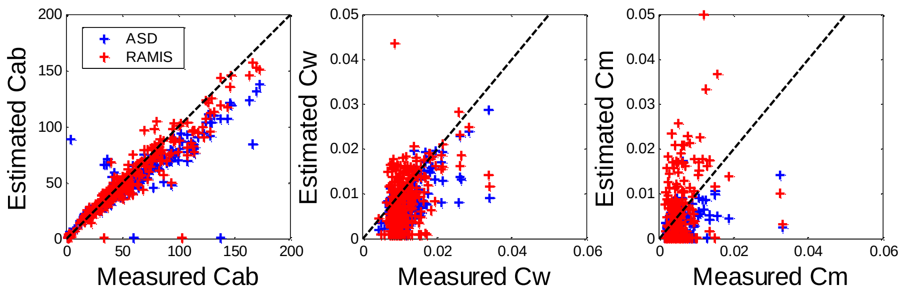

Fig. 8. Measured and estimated values of chlorophyll a+b content (µg cm^{-2}), equivalent water thickness (cm), and dry mater content (g cm^{-2}) by inversion of PROSPECT on RAM_{ASD} and RAM_{LED} transmittances.

CONCLUSION

The originality of this project lies in the fact that it combines two complementary activities: instrumentation and modeling. We demonstrate the ability to collect leaf spectral data in the field to provide accurate and fast non-invasive estimates of leaf biochemical properties using a new instrument and the retrieval of these contents using a modified version of the PROSPECT model.

Contrary to existing instruments which require suitable calibration equations to determine chlorophyll content, generally species dependent, RAMIS has been designed to additionally measure water content and dry matter content on a wide range of leaf species and functional groups. The use first of neural networks techniques, then of a leaf optical properties model, instead of calibration equations, is a key player in that amelioration. The RMSE values obtained with RAMIS by using semi-empirical methods are in the same region as those obtained with the SPAD-502. A neural netwok trained on half of the experimental data set almost decreases these values by two. For the moment, model inversions do not provide satisfactory results in spite of a high potential when applied on reflectance and/or transmittance spectra. They definitely require more work.

The instrument which is still a prototype may also be improved by the addition of new LEDs. The choice of their position in wavelength, as well as their width, is determined by technology and by the variation of leaf transmittance for increasing biochemical contents. Sensitivity analyses of PROSPECT showed that some wavelengths were better suited for extraction of low contents while other ones were more appropriate to high contents.

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