AN ADVANCED LEAF OPTICAL PROPERTIES MODEL INCLUDING PHOTOSYNTHETIC PIGMENTS

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ABSTRACT:

PROSPECT, a widely-used leaf directional-hemispherical reflectance and transmittance model, currently treats the behaviour of chlorophylls *a*, *b*, carotenoids, and anthocyanins uniformly, leading to errors. A finer discrimination among these pigments in light-absorbing plant tissues should improve the model. In this paper, a new calibration and validation phase of PROSPECT is performed using two comprehensive databases containing hundreds of leaves collected in temperate latitudes in Angers, France, and in a tropical environment in Hawaii, USA. Leaf biochemical (chlorophylls *a*, *b*, carotenoids, water, and dry matter) and optical properties (directional-hemispherical reflectance and transmittance measured from 400 nm to 2500 nm) were measured and used in the model development steps. The first step consists in providing distinct *in vivo* specific absorption coefficients for the leaf pigments using the Angers database. The model is then inverted to predict the biochemical content of intact leaves from both data sets. The main result of this preliminary study is that the new chlorophyll and carotenoid specific absorption coefficients are in good agreement with available *in vitro* absorption spectra, that the chlorophyll predictions are improved, and that the carotenoids are reasonably retrieved.

RÉSUMÉ:

PROSPECT, le modèle de réflectance et de transmittance directionnelle-hémisphérique des feuilles aujourd'hui le plus utilisé par la communauté scientifique, suppose que l'absorption de la lumière par les chlorophylles *a*, *b*, caroténoïdes, et anthocyanes est uniquement due aux chlorophylles, ce qui conduit à des erreurs. Une discrimination plus fine de ces pigments dans les tissus végétaux absorbant la lumière devrait permettre d'étendre le domaine d'application du modèle. Cet article présente une nouvelle phase d'étalonnage et de validation de PROSPECT utilisant deux bases de données rassemblant plusieurs centaines de feuilles récoltées dans une région tempérée à Angers, France, et dans une forêt tropicale à Hawaï, USA. La composition biochimique des feuilles (chlorophylles *a*, *b*, caroténoïdes, eau, et matière sèche) et leurs propriétés optiques (réflectance et transmittance directionnelle-hémisphérique mesurées entre 400 nm et 2500 nm) ont été mesurées et ont servi à améliorer le modèle. La première étape consiste à séparer les coefficients spécifiques d'absorption *in vivo* des pigments foliaires en utilisant la base de données d'Angers. Le modèle est alors validé en inversion en déterminant la composition biochimique de feuilles intactes issues des deux jeux de données. Le principal résultat de cette étude préliminaire est que les nouveaux coefficients spécifiques d'absorption des chlorophylles et des caroténoïdes sont en bon accord avec les spectres d'absorption *in vitro*, que les estimations du contenu en chlorophylle sont améliorées, et que les caroténoïdes sont déterminées avec une précision raisonnable.

1. INTRODUCTION

Quantification of vegetation canopy physiological status can be achieved by better measurement and knowledge of leaf pigments. Because chlorophyll is directly linked to photosynthetic potential and primary production, the detection and quantification of individual foliar pigments (mostly chlorophylls *a* and *b*, carotenes and xanthophylls, anthocyanins, etc.) by remote sensing techniques is essential to improve our understanding of plant functioning. There are many applications in precision farming (nitrogen management), environmental studies (geobotany), plant physiology (photosynthesis), or ecosystem studies (global change) that would directly benefit from a more detailed knowledge of multiple plant pigments.

The continuing improvement in the spectral resolution of optical sensors could provide new opportunities for large-scale studies of plants and ecosystems. However, physical, chemical, and biological processes in ecosystems are highly complex, and thus remote sensing approaches require accurate quantitative methods such as radiative transfer models that exploit all types of information in the optical signal. PROSPECT is a widely-used leaf directional-hemispherical reflectance/transmittance model (Jacquemoud and Baret, 1990). To date, its spectral resolution was restricted to 5 nm and only total chlorophyll, water, and dry matter content were incorporated into the model, thus retrievable. Pigment discrimination or solar-induced chlorophyll *a* fluorescence measurement using the next generation of hyperspectral sensors necessitates much finer and more accurate spectral resolutions. The availability of new datasets at 1 nm sampling provides an opportunity to upgrade and refine the model.

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Individually, leaf pigments express specific absorption features that should facilitate analysis based on reflectance and transmittance measurements. This assertion is tempered by two issues: first, *in vitro* absorption spectra are available, but it is a well-known fact that some spectral shifts occur depending on the solvent used to extract them from foliage, and because the membrane-bound protein complex is removed during extraction. Therefore, the *in vivo* configuration of plant pigment absorption coefficients remains uncertain. Second, the overlapping wavelengths of these absorption coefficients make their identification in leaf reflectance or transmittance spectra difficult to predict. In this context, it is particularly challenging to develop a method to estimate the pigment content directly from an intact leaf *via* spectral measurements and modelling.

In this paper, we first refine the core of PROSPECT with the computation of a new refractive index and the setting of a new leaf surface roughness parameter. Then, an analysis of the behaviour of the model is used to develop an optimized calibration stage, the most important improvement of which is the discrimination of relevant specific absorption coefficients for different pigments. Finally, we evaluate inversion methods on independent data sets to predict biochemical concentration from intact leaves by testing the prediction against measured biochemical composition.

2. MATERIAL AND METHOD

2.1 Available datasets

Two databases encompassing hundreds of leaves were used to improve PROSPECT. The first one called ANGERS was collected in 2003 on temperate plants at INRA Angers (France) and the second one called HAWAII was collected in 2007 on tropical Hawaiian plants (USA). Both datasets contain leaf directional-hemispherical reflectance and transmittance spectra measured at 1 nm resolution from 400 nm to 2400 nm using ASD FieldSpec instruments equipped with integrating spheres. Chlorophyll *a* and *b* (C_{ab}), total carotenoids (C_{cx}), water (C_w also named equivalent water thickness) and dry matter (C_m also named leaf mass per area) content are available for each sample. Table 1 summarizes the main characteristics of this database.

	ANGERS 2003	HAWAII 2007
Number of leaf samples	276	41
Number of species	49	41
Mean(Chlorophyll *a*)	25 µg cm^{-2}	37 µg cm^{-2}
Mean(Chlorophyll *b*)	9 µg cm^{-2}	13 µg cm^{-2}
Mean(Carotenoids)	9 µg cm^{-2}	12 µg cm^{-2}
Mean(Water)	0.0116 cm	0.0275 cm
Mean(Dry matter)	0.0052 g cm^{-2}	0.0125 g cm^{-2}

Table 1. Characteristics of the databases

In ANGERS, pigments were passively extracted in ethanol 95% in a test tube using fresh material. In HAWAII, frozen leaf discs were ground in 100% acetone with a small amount of quartz sand and $MgCO_3$ to prevent acidification in a chilled mortar. Following centrifugation, the absorbance of the supernatant was measured in both experiments using dual beam scanning UV-VIS spectrophotometers. Chlorophyll *a*, *b* and total carotenoid content was determined using a multi-wavelength analysis at 470, 648.6 and 664.2 nm in ANGERS (Lichtenthaler, 1987) and at 470, 645, 662 and 710 nm in HAWAII (Lichtenthaler and Buschmann, 2001). The relative distribution of pigments in Figure 1 shows that the two datasets are consistent, however with slightly higher distribution of concentrations in HAWAII. The cause of this difference may be explained by:

- A different solvent extraction efficiency which may lead to different amounts of pigments extracted from a leaf according to the protocol carried out.
- Datasets collected in two different ecosystems, under two different climates. This ecological factor may influence the distribution of pigments in vegetation.

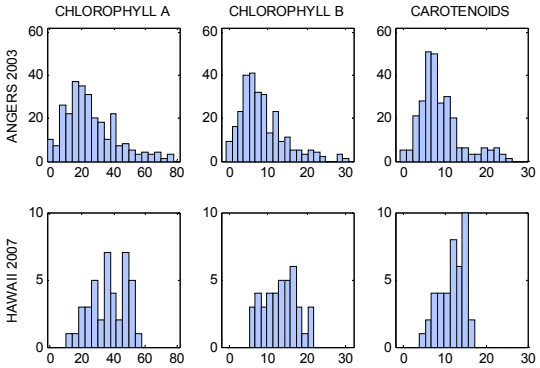

Figure 1. Pigment distribution in the two databases

A preliminary analysis of the data highlights the difficulties to come when trying to discriminate pigment contents. As shown in Figure 2, pigment concentrations are strongly correlated.

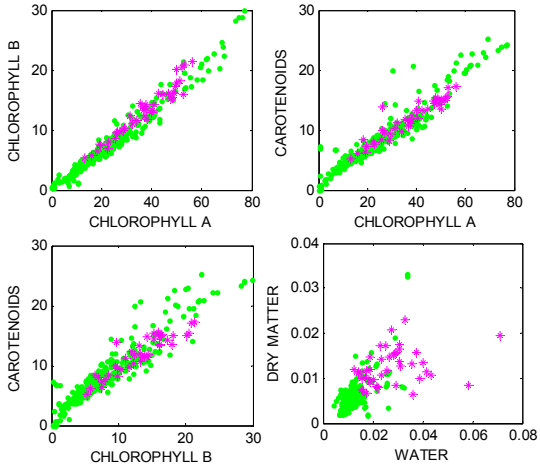

Figure 2. Pigment, water and dry matter correlations
(• ANGERS and * HAWAII)

A particularly interesting point to notice is that the ratios chlorophyll *a* over chlorophyll *b* and total carotenoids over chlorophyll *a* or chlorophyll *b* are similar and particularly stable, although the two datasets have been collected from different ecosystems and different field campaigns. This systematic high correlation of pigments may complicate the calibration. The gathering of new databases including a wider range of pigments and other contents characteristics (by collecting for example leaves in various physiological states) may help us to understand the conditions for stability or variation of these ratios. Since ANGERS is the largest database with a wider range of chlorophyll concentrations, it is used for the calibration stage.

2.2 Why an improved version of PROSPECT?

Unlike canopy reflectance models, PROSPECT requires a calibration phase where some physical and optical constants like the leaf surface roughness parameter σ, the refractive index of leaf material $n(\lambda)$, and the specific absorption coefficients of the leaf absorbers $K_{spe}(\lambda)$ must be set up using experimental data. These values are assumed to be invariable from one species to the other, which is true for $k_{spe}(\lambda)$ but not totally true for σ and $n(\lambda)$ because of the changing nature of leaf surfaces and wax types (Pfündel et al., 2006). Surprisingly, σ and $n(\lambda)$ have not been updated since Jacquemoud and Baret (1990). Their accuracy is however crucial to the remaining work and, as PROSPECT is based on physical laws, it is fundamental to develop a model coherent with our knowledge of these constants (le Maire et al., 2004).

2.2.1 Model's weakness: Simulations of the reflectance of a compact (leaf structure parameter $N=1$) and totally absorbing (transmission of the elementary layer $\phi=0$) leaf using the former version of PROSPECT demonstrate its inability to reach the lowest experimental values of several datasets, specially in the blue part of the visible region where absorption by pigments is the highest (Figure 3). This disagreement leads to numerical instabilities and underestimation of the specific absorption coefficients in the calibration stage of the model. Moreover, the procedure followed so far, which splits this crucial stage into several steps, raises saturation problems when absorption exceeds a certain level. A new approach which combines updated physical and optical constants and a more efficient algorithm may likely resolve these problems.

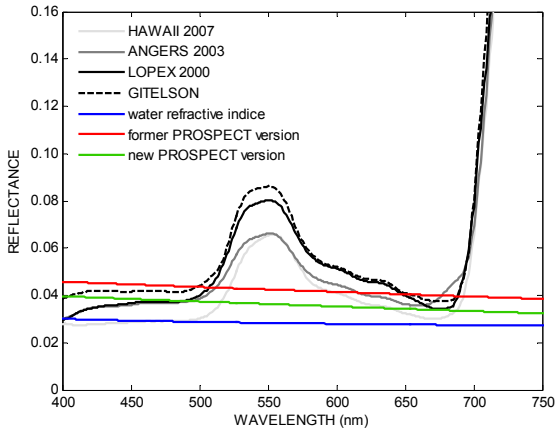

Figure 3. Minimum reflectance of different databases (grey curves) and reflectance of a totally absorbing compact leaf with three indices of refraction (colour curves)

2.2.2 Reassessment of physical and optical parameters: In PROSPECT, the incident radiation is assumed to light up a horizontal plane at all angles between 0° and α to mimic the leaf surface roughness on an intuitive base. Up to now, α has been set at 60° but this seems to be overestimated. Recent work on leaf BRDF modelling links the probability density function of facet orientations to this angle on a physical base (Bousquet et al., 2005) where α is the maximum angle between the leaf normal, i.e. the direction of the incident light, and the facet normal. Actual values of the surface roughness parameter $\sigma < 0.5$ correspond to $\alpha < 40°$, which is a more realistic value. Moreover, since the average transmissivity at the interface varies very slowly between 0° and 40°, the latter value was fixed. This permits a decrease in the minimum reflectance and an improvement in PROSPECT accuracy at high absorption wavelengths.

A new refractive index was determined by inversion of the plate model (Allen et al., 1969) using an albino corn (*Zea mays*) leaf grown under glass. Such a leaf is analogous to a single pigment-free layer: The reflectance and transmittance levels in the near infrared plateau are consistent with a compact (leaf structure parameter $N=1$) and non-absorbing (transmission of the elementary layer $\phi=1$) leaf. The plateau also continues in the visible before falling below 450 nm (Figure 4). The refractive index $n \approx 1.45$ in the visible and $n=1.42$ at 800 nm agrees well with the literature (Brown, 1920; Woolley, 1975). The whole spectrum which does not express any particular features due to absorption validates our approach. It was fitted by a third degree polynomial. Figure 5 presents its new spectral variations compared to the refractive index of water (Segelstein, 1981). Although it probably varies somewhat from leaf to leaf, *n* is not supposed to change.

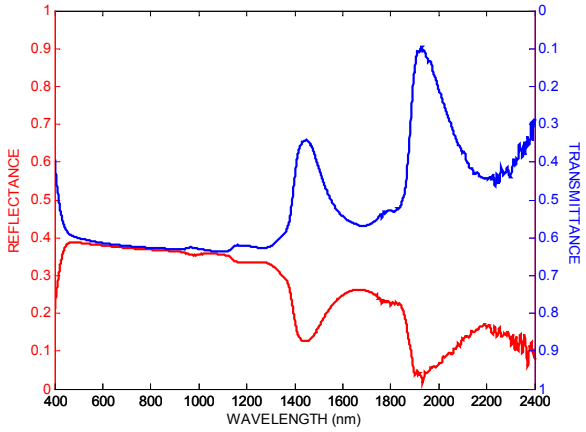

Figure 4. Optical properties of a corn albino leaf

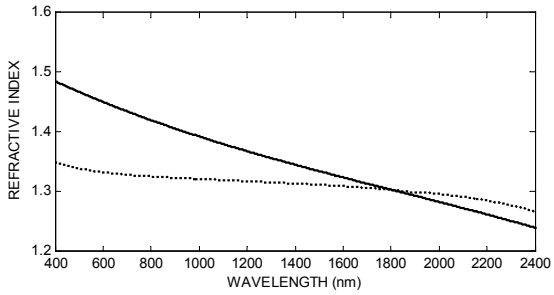

Figure 5. Refractive index of the corn albino leaf (line) and water (dots)

2.2.3 A new algorithm for calibration: In addition to the new physical and optical parameters, the algorithm for the calibration phase was simplified to avoid intermediate steps which multiply the sources of errors, leading to inaccuracies. A diagram of this calibration is presented in Figure 6.

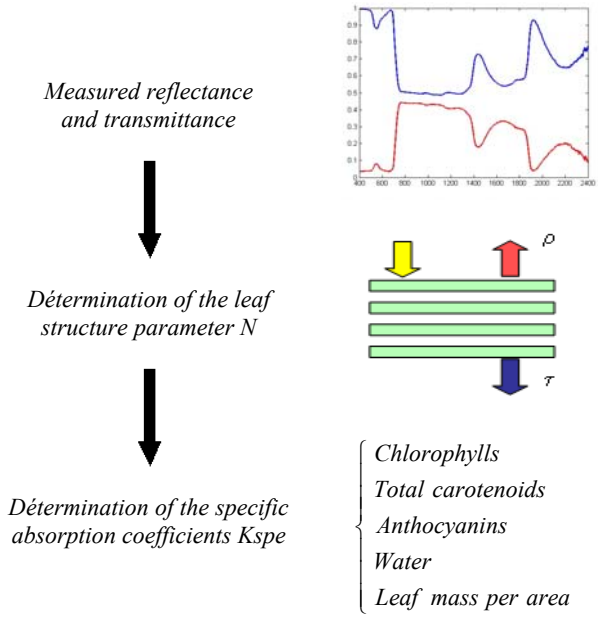

Figure 6. Diagram of the calibration stage of PROSPECT

The structure parameter N of each leaf, which is assumed to be independent of wavelength, is first determined by inverting PROSPECT on the reflectance and transmittance measured at λ_1, λ_2 and λ_3, the wavelengths of the maximum reflectance and transmittance and minimum absorptance, respectively. For fresh leaves, these three wavelengths are located in the near infrared plateau and may be confounded. The three absorption coefficients are determined at the same time but not used. Second, the specific absorption coefficients of the leaf absorbers are determined by inverting PROSPECT on all the leaves, wavelength by wavelength. The leaf structure parameters fitted in the first step, as well as the pigment, water and dry matter contents measured in the laboratory constrain the inversion.

3. RESULTS AND DISCUSSION

In this section, we present the results of the calibration of the model performed with the ANGERS database, and its validation on both the ANGERS and HAWAII databases.

3.1 Computation of the specific absorption coefficients

The most critical stage of this work is the calibration where the *in vivo* specific absorption coefficients are computed. These link the optical and biochemical properties together and are actually known for most pigments but only *in vitro* for purified molecules dissolved in an organic solvent. The main absorption peaks are known nevertheless to shift with the polarity of the solvent so that they cannot be used in PROSPECT. Thus, we must determine these coefficients directly from the experimental data. In the model, the absorption coefficient of a compact layer $k(\lambda)$ is written as a linear combination of the absorption of each biochemical constituent i:

$$k(\lambda) = \sum_i K_{spe,i}(\lambda) \times \frac{C_i}{N} \qquad (1)$$

with λ the wavelength, C_i the constituent concentration in the leaf, $K_{spe,i}$ the corresponding specific absorption coefficient, and N the leaf structure parameter, i.e. the number of compact layers. The separation of the $K_{spe,i}$ in the inversion process is another difficulty to overcome because of overlapping wavelengths of their main absorption peaks and because of the high correlations between their concentrations, as seen earlier.

We first treated the behaviour of all the pigments uniformly, as previously but following the updated method, for computing the specific absorption coefficient of total ²chlorophyll. We call this version of the model PROSPECT-4. Higher contrasts between non-absorptive (550 nm) and absorptive wavelengths (450 nm and 680 nm), as well as between these two peaks, are noticeable in Figure 7. The new shape, in contrast to the flatness of the former version, is more realistic and corresponds with the literature. The *in vitro* spectrum displayed in Figure 7 is the sum of Gaussian functions calculated after Maier (2000) considering the ratio chlorophyll *a*/*b* of Figure 2. Furthermore, one can suppose that pigment discrimination will decrease the contrast between the two peaks because of distinct absorption effect of chlorophylls and carotenoids in the blue, which remain unaccounted for.

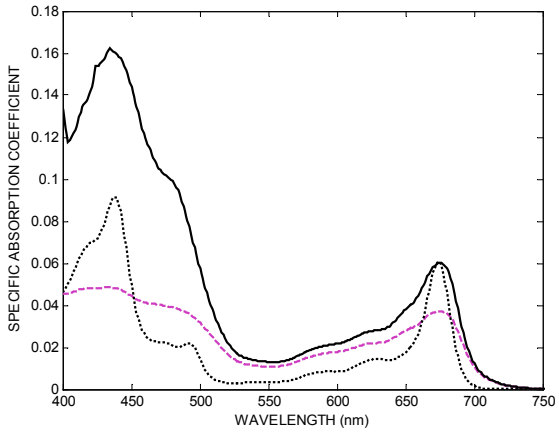

Figure 7. Chlorophyll a and b specific absorption coefficient (expressed in $cm^2\ \mu g^{-1}$) (line: new version, dashed: former version, dots: *in vitro*)

We now call PROSPECT-5 the version of the model which introduces carotenoids in addition to the chlorophylls. The first attempt to discriminate pigments is presented in Figure 8.

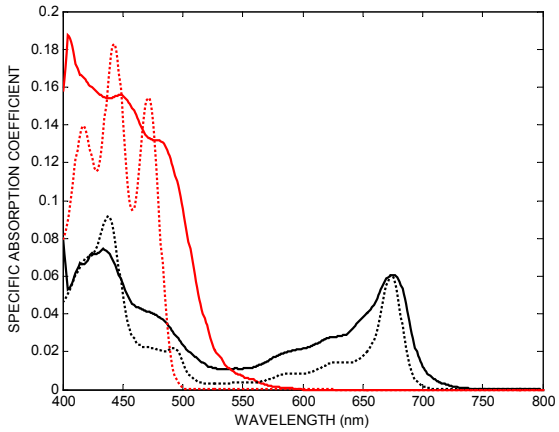

Figure 8. Chlorophylls a and b (black) and total carotenoids (red) specific absorption coefficient (expressed in cm2 μg^{-1}) (line: new version, dots: *in vitro*)

In vivo absorption peak for chlorophyll at 680 nm matches *in vitro* data well, and the shape is globally correct. As expected, the differential between the two main absorption peaks decreases and becomes closer to the observed *in vitro* ratio. The specific absorption coefficient of carotenoids, which contributes to this decrease, is also in good agreement with the literature (Maier, 2000). Slight spectral shifts, a possible effect of *in vivo* absorption, are noticeable for carotenoids, whereas they are less obvious for chlorophylls. Because absorption tends to saturate when pigment content is high, one should mention that it is of major importance to determine accurate absorption coefficients for leaves with low concentrations.

Unfortunately, attempts to separate chlorophyll *a* and *b* with the same method failed, although we surprisingly still obtained good results with carotenoids. A classical numerical algorithm to separate highly correlated data such as leaf photosynthetic pigments is not efficient and may require that we explore new methods, for instance by using leaves with unusual combinations of pigments, or *a priori* constraints, to compute the specific absorption coefficients.

3.2 Biochemical content retrieval

The next step is the retrieval of the leaf biochemical constituents, for each sample, by inversion of PROSPECT both on reflectance and transmittance spectra. Although this paper is focussed on leaf pigments, the near infrared and shortwave infrared are also investigated and the water and dry matter content determined at the same time. In practice, the inversion consists of finding the parameter set symbolized by the vector θ which minimizes the following merit function:

$$\chi^2(\theta) = \sum_{\lambda\min}^{\lambda\max} \left(R^*(\lambda) - R_{\mathrm{mod}}(\lambda,\theta)\right)^2 + \left(T^*(\lambda) - T_{\mathrm{mod}}(\lambda,\theta)\right)^2 \tag{2}$$

where $R^*(\lambda)$ and $T^*(\lambda)$ are the measured reflectance and transmittance, and R_{mod} and T_{mod} the modeled ones. The optimization is processed by running a constrained Powell search method for a minimum (Press et al., 1992). Figure 9 confirms the ability of PROSPECT-4 to retrieve the total chlorophyll, water and dry matter contents. As seen in Table 2, the root mean square errors (RMSE) are lower on ANGERS used for the calibration, but still good for HAWAII. As expected, the results obtained with C_m are not as good as with C_w because water largely hides the absorption features of dry matter in the shortwave infrared. Unfortunately, the specific absorption coefficient of dry matter could not been re-computed following this new calibration method because only fresh leaves were available in this study. A reassessment of the LOPEX database (Hosgood et al., 1994) which includes such samples will be considered soon.

	PROSPECT-4		PROSPECT-5	
	ANGERS	HAWAII	ANGERS	HAWAII
C_{ab} (µg cm^{-2})	6.75	12.38	6.39	12.88
C_{cx} (µg cm^{-2})	×	×	4.75	2.94
C_w (cm)	0.00180	0.00571	0.00180	0.00570
C_m (g cm^{-2})	0.00255	0.00530	0.00256	0.00531

Table 2. Root Mean Square Errors (RMSE) calculated on all the retrieved parameters

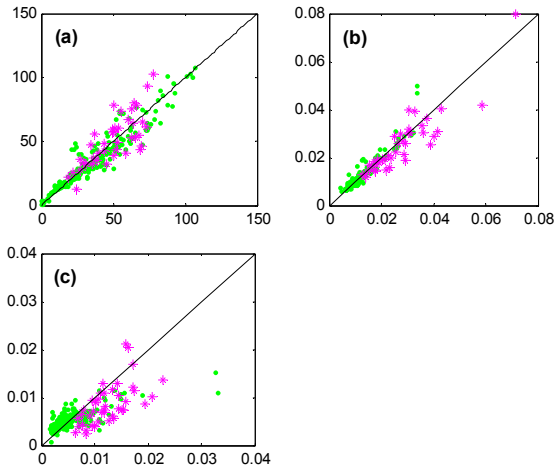

Figure 9. Measured vs. estimated (a) total chlorophyll content, (b) water content, and (c) dry matter content while inverting PROSPECT-4 (• ANGERS and * HAWAII)

Figure 10 and Table 2 present the results obtained with PROSPECT-5. The comparison between Figures 9 and 10 points out that the retrieved C_w and C_m do not vary, which is consistent with the expected result because their influence on leaf optical properties is insignificant in the visible domain. The estimation of C_{ab} is improved in ANGERS but slightly degraded in HAWAII, for no apparent reason. The main advance is obtained in the determination of carotenoids: C_{cx} is well retrieved for light-green leaves with low pigment content whereas the accuracy tends to decrease for dark-green leaves with high pigment content. Since the amount of these pigments is almost five times lower than the amount of total chlorophylls, this result is not surprising.

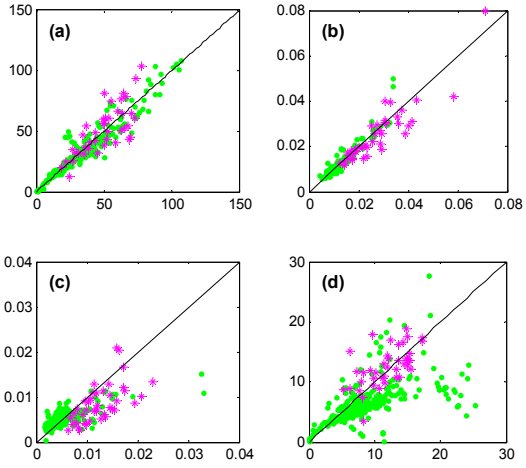

Figure 10. Measured vs. estimated (a) total chlorophyll content, (b) water content, (c) dry matter content while inverting, and (d) total carotenoid content while inverting PROSPECT-5 (• ANGERS and * HAWAII)

3.3 Optical properties assessment

The reflectance and transmittance of a leaf were computed by PROSPECT-4 (Figure 11a) and PROSPECT-5 (Figure 11b) using the retrieved parameters. It is obvious that chlorophylls by themselves cannot explain some specific absorption features

observed in yellowing or light-green leaves which present low chlorophyll content and high carotenoid content. The introduction of carotenoids into the model partly fixed this problem. This result encourages us to go further into pigment distinction.

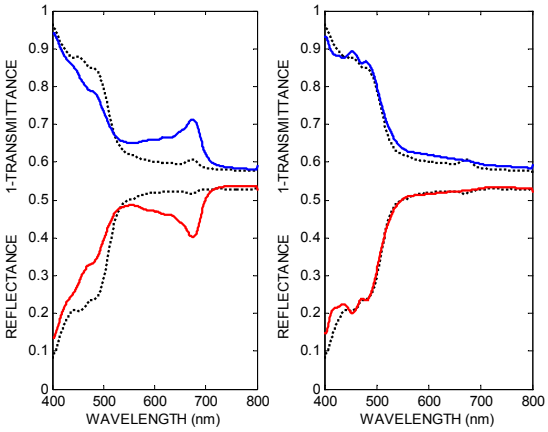

Figure 11. Improvement of the optical properties in the VIS-NIR by assessing carotenoids (dots: measurements, red: modeled reflectance, blue: modeled transmittance)

4. CONCLUSIONS AND FUTURE WORK

PROSPECT is a physically-based model applied to a biological object, the leaf. It consequently appears as a compromise between theoretical physics, applied optics, and biochemical measurements. This paper shows that there is a great potential for further development since the new version of the model provided better simulations of leaf optical properties. Both the optimized calibration stage and the introduction of new pigments improved its accuracy. These results encourage us to continue to refine the calibration method, e.g., to better account for the absorption of dry matter over the whole optical domain. Characterization of *in vivo* specific absorption coefficients for other leaf pigments, e.g. xanthophylls, anthocyanins, and even for chlorophyll *a* and *b* separately could further improve leaf radiative transfer models.

The next critical stage will be to extend the available dataset to the full range of leaf constituents content. A study of unusual leaves (e.g. with unusual ratio between pigments) may help us compute more accurate specific absorption coefficients and thus to resolve the problems due to high correlations between pigments.

PROSPECT is very popular with many current studies on remote sensing of canopies. The model is already included in several applications, which will be able to take advantage of this new version. This modelling work will contribute to develop a more complete understanding of plant pigment function in leaves but also quickly allow an extended field of application for the quantification of photosynthetic processes in ecosystem and remote sensing research by making an advanced use of hyperspectral data.

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