ELSEVIER

Estimating Leaf Biochemistry Using the PROSPECT Leaf Optical Properties Model

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The biophysical, biochemical, and optical properties of 63 fresh leaves and 58 dry leaves were measured to investigate the potential of remote sensing to estimate the leaf biochemistry from space. Almost 2000 hemispherical reflectance and transmittance spectra were acquired from 400 nm to 2500 nm using a laboratory spectrophotometer. The amount of chlorophyll, water, protein, cellulose, hemicellulose, lignin, and starch was determined on these leaves using standard wet chemistry techniques. These experimental data were used to improve the PROSPECT model, a simple but effective radiative transfer model that calculates the leaf optical properties with a limited number of input parameters: a structure parameter and the leaf biochemistry. The new model construction mainly consisted in providing specific absorption coefficients for the biochemical constituents; the comparison with absorption spectra of pure materials derived from the literature showed good agreement. In the inversion, however, it was necessary to group some leaf components in order to estimate leaf biochemistry with reasonable accuracy. Predictive power varied with the chemistry variable, wavelengths used in analysis, and whether leaves were fresh or dry. r^2 ranged from 0.39 to 0.88 for predictions on dry leaves; on fresh leaves, water and chlorophyll had high r^2 values, 0.95 and 0.68 respectively, carbon based compounds reasonable r^2, from 0.50 to 0.88, while the estimation of protein is still at issue.

INTRODUCTION

The remote estimation of leaf biochemical content from spaceborne platforms has been the subject of many studies aimed at better understanding of terrestrial ecosystem functioning. The major ecological processes involved in exchange of matter and energy, like photosynthesis, evapotranspiration, respiration, primary production, and decomposition, are related to nutritional status and growth conditions, for example, chlorophyll, water, protein, cellulose, and lignin contents (Peterson and Hubbard, 1992). As leaves are the most important plant surfaces interacting with solar energy, it is critical to understand physiological processes that relate foliar optical properties to biophysical characteristics. In particular, a top priority has been to relate light absorption and scattering to biochemical constituents. Two different approaches have been considered. First, statistical correlations between the leaf reflectance (or transmittance) and biochemical content (Jacquemoud et al., 1995), and second, physically based models of photon transport inside leaves, developed using the laws of optics. Recently reviewed by Verdebout et al. (1994), the development of such models has resulted in better understanding of the interaction of light with plant leaves.

Among these models, radiative transfer models have been successfully used in the foreward mode to calculate leaf reflectance and transmittance, and in inversion to estimate leaf biophysical properties. Up to the present, these models have been mainly used to estimate chlorophyll and / or water contents as input parameters (Jacquemoud and Baret, 1990; Fukshansky et al., 1991; Yamada and Fujimura, 1991; Martinez v. Remisowsky et al., 1992). The influence of protein, cellulose, lignin, and starch on leaf reflectance has been recently introduced by Conel et al. (1993), who proposed a two-stream Kubelka–Munk model, but in fact, the estimation of leaf biochemistry from remote sensing remains an open question. In order to clarify it, a laboratory experiment associating visible / infrared spectra of plant leaves both with physical measurements and biochemical analyses was conducted at the Joint Research Centre in Italy during the summer of 1993. Thousands of measure-

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Received 25 July 1995; revised 18 October 1995.

REMOTE SENS. ENVIRON. 56:194–202 (1996)

655 Avenue of the Americas, New York, NY 10010

0034-4257 / 96 / $15.00
SSDI 0034-4257(95)00238-3

ments were collected in a unique data set partially used to upgrade the PROSPECT model (Jacquemoud and Baret, 1990) by including leaf biochemistry. In an earlier article, Fourty et al. (1995) analyzed the optical properties of the dry leaves and decomposed the absorption spectra of dry vegetation into six specific absorption coefficients having the characteristics of protein, cellulose, hemicellulose, lignin, starch, and sugar. However, in inversion, the estimation of the biochemical constituents was poor.

In this article, we develop a general model applicable both to fresh and dry leaves that include a wide range of internal cellular structures and biochemical compositions. In the first part we describe the experiment and the correlation relationships between the biochemicals. The construction of PROSPECT is only described where improvements were made in the original model; otherwise the reader is referred to Jacquemoud and Baret (1990). The validation, that is, the comparison between the measured and estimated leaf biophysical and biochemical properties, is a key section of this article, which justifies the hypotheses of the model. Finally we perform a sensitivity analysis, as a last step before applying the model at the canopy level in the future.

THE EXPERIMENT

The LOPEX93 (*L*eaf *O*ptical *P*roperties *Ex*periment) is described in detail in Hosgood et al. (1995). It consists of about 70 leaf samples representing 50 woody and herbaceous species that were obtained from trees and crops near the Joint Research Centre in Ispra, Italy. A wide range of variation in leaf internal structure, pigments, water, and biochemistry contents was available, leading to a wide range of variation in leaf optical properties. Although many biophysical and spectrophotometric measurements were performed on leaf samples, only a part of the data set was used in this study. For instance, needles which do not meet the model requirements were removed from the analysis. The hemispherical reflectance (R) and transmittance (T) of fresh and artificially dried leaves were acquired over the 400–2500 nm region in an integrating sphere coated with BaSO4 attached to a Perkin Elmer Lambda 19 spectrophotometer. Spectra were originally scanned in 1-nm steps, but the wavelength interval was averaged over 5 nm to reduce the noise, the number of data, and consequently the calculation time. Each species spectrum is the average of five spectra measured on different leaves of the same plant or from the same branch on larger perennials.

Among the physical and biological measurements performed in the frame of LOPEX93, the blade thickness, the specific leaf area (1 / SLA = dry weight per unit leaf area), the equivalent water thickness (EWT = water mass per unit leaf area), the photosynthetic pigments (chlorophyll a, b and total carotenoids expressed in μm cm^{-2}), some biochemical components (protein, cellulose, hemicellulose, lignin, and starch), and finally the elementary composition (C, H, O, N) were available. In near-infrared reflectance spectroscopy (NIRS) studies, the leaf biochemistry is typically given as a percentage of dry weight. We believe that this unit is not suitable for our study because fractions do not represent the amount of matter interacting with light. This assertion needs some explanation. First, consider the composition of plant foliage. Water represents on average 66.4% of the fresh weight (Fig. 1a). The remaining part is dry matter composed of cellulose, hemicellulose, lignin, protein, starch, and minerals (Fig. 1c). All these constituents explain 85.8% of the dry mass of monocotyledons and 67.8% of the dry mass of dicotyledons. The missing matter may be attributed to lipids, soluble sugars, aminoacids, and other primary and secondary metabolites not measured in this study (Fourty et al., 1995). It is not surprising that terrestrial plants have such similar chemistries since they share basic metabolic pathways. On the other hand, the basis for differences between the two groups of flowering plants is unclear, although it may reflect their ecological differentiation and the more herbaceous nature of the monocotyledons chosen for this study. Details of the decomposition can be found in Table 1. Although the concentration of the carbon based constituents may vary, their global fractions are remarkably stable in accordance with the very stable concentration of carbon in plant leaves which averages 47 g g^{-1} of dry matter. That kind of low variance information is not very useful! Consequently, as for water and pigments, we expressed the other concentrations in mass per unit leaf area using the SLA. Figures 1b and 1e illustrate the increased variability expressed when concentration units are in g cm^{-2}. On an area basis, the biochemical variation increases by a factor between 1 and 10.

Several correlative relationships among biochemicals were also established, including leaf thickness and EWT, protein and SLA or total chlorophyll. For instance, we showed that 1 / SLA varied inversely with the weight-based measure of leaf protein, consistent with values in the literature (Field and Mooney, 1986; Dijkstra, 1989). The strongest relationships were obtained between total nitrogen (N) and protein, and between total carbon (C) and cellulose + lignin when expressed in g cm^{-2} (Fig. 2). This equivalence is very important because it suggests that the C / N ratio which drives the decomposition rates of forest litter, affecting nutrient cycling and trace gas fluxes, could be replaced by the indirect measure of the ratio of cellulose + lignin to protein. Thus, spectral measures of water, photosynthetic pigments, total C, and total N could provide significant information related to canopy nutrient status,

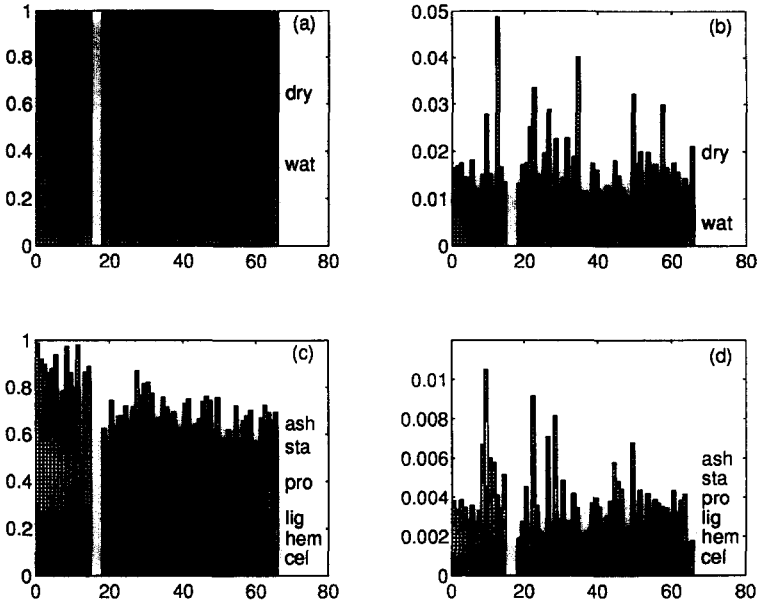

Figure 1. Distribution of leaf biochemistry expressed in g g^{-1} of fresh matter (1a), g g^{-1} of dry matter (1c), and g cm^{-2} (1b and 1d). Wat, dry, cel, hem, lig, pro, sta, and ash respectively stand for water, dry matter, cellulose, hemicellulose, lignin, protein, starch, and minerals.

physiological state, and allocation of photosynthate to aboveground canopy components.

CONSTRUCTION OF THE MODEL

PROSPECT is a radiative transfer model which calculates the leaf hemispherical reflectance and transmittance from 400 nm to 2500 nm. Scattering is described by the refractive index of leaf materials (n) and by a parameter characterizing the leaf mesophyll structure (N). In the original version, absorption was modeled using pigment concentration (C_{ab}), water depth ($C_w \approx$ EWT), and the corresponding specific absorption coefficients K_{ab} and K_w. The introduction of leaf chemistry does not change radically the structure of the model; in a sense, it simplifies it. For instance, the absorption of fresh leaves in the near-infrared (NIR) plateau (780–920 nm) is now both a function of the N parameter and the leaf chemistry. The literature is very unclear about the origin of this absorption; most of the time it is ignored, and the articles presenting negative values are received in silence! Nonetheless, this NIR absorptance typically increases with biochemical concentration.

Modeling absorption processes first implies that the effects of mesophyll structure are well accounted for by the model. These effects influence the whole spectrum but are maximum in the NIR where the absorption features of chlorophyll and water are minimal if not negligible. On fresh leaves, this low absorptance is materialized by a plateau of constant reflectance and transmittance values at about 10% incident light. This plateau is somewhat disturbed in artificially dried leaves due to the appearance of brown pigments or denatured proteins that absorb light shorter than 1100 nm. In the original version of PROSPECT, the leaf optical properties in the NIR were only driven by the parameter N, the number of stacked elementary layers; the absorption by one of these layers was small and assumed to

Table 1. Leaf Biophysical Measurements[a]

	Unit	*Range*	*Mean*	*Std.*	*Unit*	*Range*	*Mean*	*Std.*
Thickness	μm	86.4–780.0	194.5	114.9				
SLA	$cm^2\ g^{-1}$	73.9–535.3	224.6	93.4				
Water	% FM	44.9–92.39	66.4	11.0	g cm^{-2}	0.0046–0.0405	0.0115	0.0067
Chlorophyll a	μg cm^{-2}	12.8–64.2	36.9	11.4				
Chlorophyll b	μg cm^{-2}	3.7–21.3	11.7	3.8				
Carotenes	μg cm^{-2}	3.7–19.4	10.5	3.6				
Cellulose	% DM	9.1–37.2	19.7	6.4	g cm^{-2}	0.00031–0.00545	0.00108	0.00072
Hemicellulose	% DM	0.3–38.8	15.2	10.0	g cm^{-2}	0.00002–0.00332	0.00080	0.00064
Lignin	% DM	1.1–27.5	10.2	6.4	g cm^{-2}	0.00003–0.00305	0.00060	0.00057
Protein	% DM	7.4–36.8	20.0	7.0	g cm^{-2}	0.00048–0.00172	0.00096	0.00029
Starch	% DM	0.0–10.0	2.0	2.1	g cm^{-2}	0.0–0.00098	0.00011	0.00015
C	% DM	38.5–52.3	47.4	2.9	g cm^{-2}	0.00079–0.00665	0.00253	0.00120
N	% DM	1.2–5.9	3.4	1.1	g cm^{-2}	0.00009–0.00033	0.00016	0.00005

[a] The leaf thickness, pigment content and water content are provided for fresh leaves.

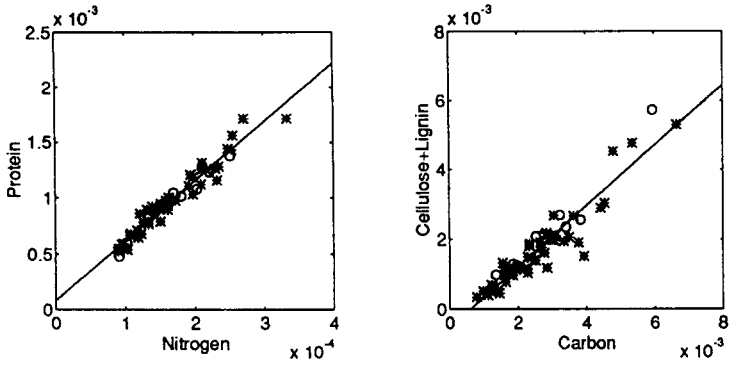

Figure 2. Comparison between a) nitrogen and protein concentrations b) carbon and cellulose + lignin concentrations (g cm^{-2}). Circles indicate Monocotyledons and stars Dicotyledons.

be constant. Although the origin of this absorption is uncertain, it cannot be attributed to either chlorophyll or water. Likewise, no major leaf-soluble cell fractions have absorptions across this wavelength interval. So we have hypothesized that the absorption was due to some component in the cell walls. Since the amount of dry matter per unit area varies from sample to sample, the absorption was also allowed to vary. In consequence, leaf optical properties in the NIR are now modeled by the N parameter and by the absorption coefficient $k(\lambda)$ of this elementary layer. To determine N, we defined three wavelengths corresponding to the maximum reflectance (λ_r), the maximum transmittance (λ_t), and the minimum absorptance (λ_a). For fresh leaves, these three wavelengths are located in the NIR plateau and may be confounded; for dry leaves, they are shifted towards longer wavelengths (Fig. 3). The structure parameter of each leaf was adjusted at the same time as the three absorption coefficients by minimizing:

$$\sum_{\lambda}[R_{mes}(\lambda) - R(\lambda,N,k_\lambda)]^2 + [T_{mes}(\lambda) - T(\lambda,N,k_\lambda)]^2, \quad (1)$$

where $R_{mes}(\lambda)$ and $T_{mes}(\lambda)$ are respectively the three reflectances and three transmittances measured at λ_r, λ_t, and λ_a. For the same species, N estimated on dry leaves is higher than N estimated on fresh leaves. This is due to an increase of the multiple scattering resulting from the loss of water in dry leaves. Jacquemoud and Baret (1990) reported that the structure parameter of leaves grown on plants in a greenhouse ranged from 1 to 1.5 for monocotyledons with a compact mesophyll structure, and from 1.5 to 2.5 for dicotyledons with a differentiated mesophyll structure. This distinction was not found in this study which used plants grown outside under natural conditions.

The wavelength independent mesophyll structure parameter N allows the inversion of the Stokes equations: using measured reflectance $R(\lambda)$ and transmittance $T(\lambda)$, the optical properties of the compact layer ($N = 1$) are easily calculated for each leaf, permitting the determination of a spectral absorption coefficient $k_0(\lambda)$. If the assumption is made that the leaf is a homogeneous mixture of biochemical components, this coefficient can

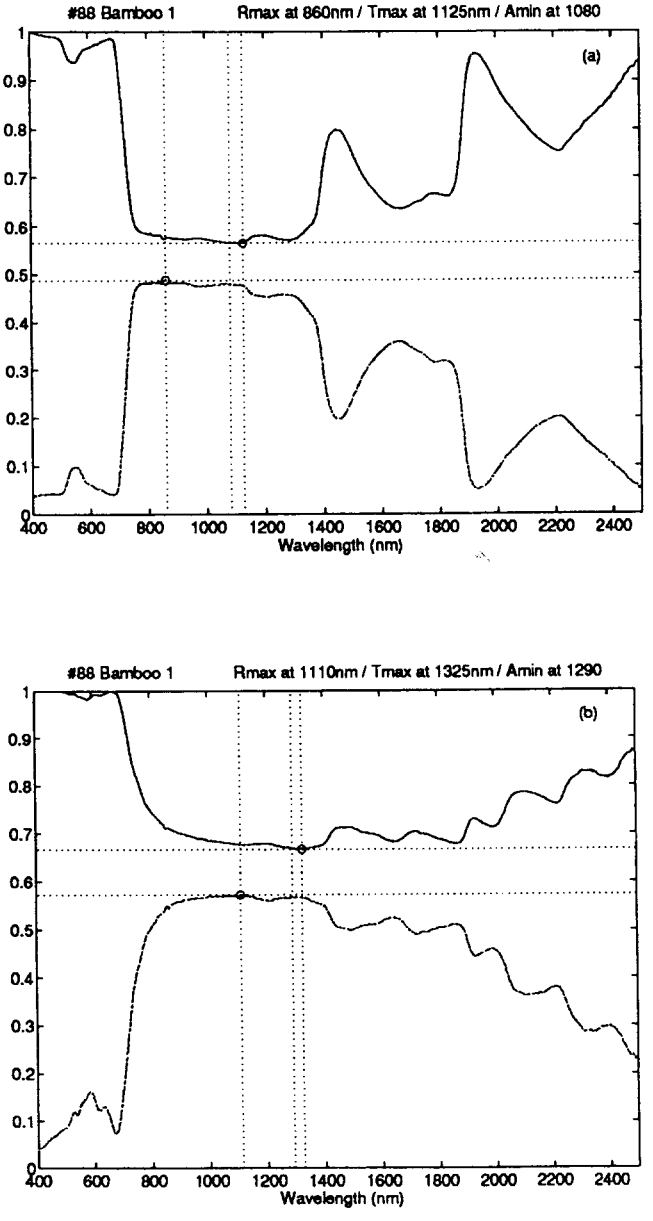

Figure 3. Leaf optical properties of a fresh (a) and dry (b) bamboo leaf. The positions of the reflectance maximum, transmittance maximum, and absorptance minimum are located on the spectra.

be written as

$$k_0(\lambda) = k_e(\lambda) + \sum_i \frac{C_i \cdot K_i(\lambda)}{N}, \quad (2)$$

where λ is the wavelength, C_i the concentration of the constituent i, and $K_i(\lambda)$ the corresponding specific absorption coefficients. $k_e(\lambda)$ explains the nonzero absorption of an albino leaf under 500 nm. At this point, two strategies may be considered.

The first strategy consists in predicting the constituent concentrations and, then, in comparing predictions with measured values. This approach presumes that the specific absorption coefficients are known, for example, deduced from optical measurements performed on pure substances. Thus, the spectral specific infrared absorption coefficients for distilled water have been carefully measured by Curcio and Petty (1951). The data for chlorophylls and, to a lesser extent, for accessory pigments (carotenoids, xantophylls) are also available from the literature (Lichtenthaler, 1987). In this case, though, the use of these absorption coefficients presents a problem: The absorption spectrum obtained from extracts of chlorophyll in a solvent does not correspond closely to the *in vivo* measurement; spectral shifts of the order of 10 nm are observed (Buschmann and Nagel, 1991; Chappelle et al., 1992), which are attributed both to the influence of the solvent and to the fact that the chlorophylls inside leaf tissues are complexed with other pigments and proteins. The complex three-dimensional macromolecular structure of chlorophyll has been associated with various distortions that contribute to wavelength spreading and shifting of the *in vivo* optical properties of pigments. For different reasons, the spectral signatures of the other biochemicals are also complex: even if some substances are composed of well characterized repeating units (e.g., starch, sugar), molecular weights can vary, while others are families of biochemical substances which cannot be precisely defined or even isolated with the molecular structure intact (e.g., protein, cellulose, lignin). Moreover, these large classes of macromolecules contain many chemical bonds in common (C—H, N—H, C—O, O—H, etc.) which occur in various proportions, inducing an overlapping variation in absorption features (Barton et al., 1992).

In order to bypass these difficulties, another strategy was adopted: Using the absorption coefficients $k_0(\lambda)$ and the measured concentrations, we deduced the specific absorption coefficients of leaf biochemical components $k_i(\lambda)$. Different combinations of leaf biochemical composition and leaf water status have been tested. For instance, in Eq. (2) we decomposed the absorption into chlorophyll a + b, water, protein, and structural biochemicals like cellulose, hemicellulose, or lignin. We also investigated coefficients determined on fresh leaves, dry intact leaves, and fresh + dry leaves. It allowed us to address a delicate problem experienced by those using NIRS techniques to estimate leaf biochemistry. Generally, a regression equation is established between the leaf biochemistry and the optical properties of entire blades or dry vegetation powders. For a given component, Jacquemoud et al. (1959) showed that the wavelengths selected by multiple stepwise regression analysis depended on whether the basis for comparisons was reflectance or transmittance values on fresh / dry single leaves, or leaf stacks. This discrepancy contradicts the idea that a specific biochemical should produce a consistent effect due to its absorption of light at specific allowable energy states. Nonetheless, many statistical analyses have resulted in selections of significantly different wavelengths and the need for taxon specific relationships to particular biochemicals. Let us consider the biochemical coefficients derived in this analysis. The coefficients for water, protein, and cellulose + lignin over the 800–2500 nm range are shown in Figure 4. One can see that protein and cellulose + lignin coefficients occur at the same wavelength position whatever the leaf water status. Even where water tends to mask the absorption peaks of these constituents, as expected in fresh leaves, it does not fundamentally shift them to other wavelengths in our analysis. This important result allows us to build a very general model suitable for many kind of flowering plant leaves.

We calculated the specific absorption coefficients of leaf biochemicals for the following three combinations: chlorophyll, water, protein, cellulose + hemicellulose, and lignin [C1], chlorophyll, water, protein, and cellulose + hemicellulose + lignin [C2], and chlorophyll, water, protein, and cellulose + lignin [C3]. K_{ab} was determined on fresh leaves in the 400–800 nm region, K_w on fresh + dry leaves in the 800–2500 nm region, and the other coefficients on dry leaves in the 800–2500 nm region. To describe these results, lets examine the case for C3: $K_{ab}(\lambda)$ displays classical features of photosynthetic pigments with spectral shifts toward longer wavelengths of the principal absorption peaks of chlorophyll compared to *in vitro* observations as discussed earlier (Fig. 5a). $K_w(\lambda)$ shows good agreement with the fundamental constants published for pure liquid water (Fig. 5b). Results are surprisingly also very convincing for both protein (K_p) and cellulose + lignin (K_{cl}): except in a few cases, the absorption peaks are well represented (Figs. 5c and 5d). C1 and C2 produced very similar results. The specific absorption coefficients of chlorophyll a + b and water equal zero, respectively, after and before 800 nm; due to the appearance of brown pigments or denatured protein during the drying of the leaves, those of the other constituents have been fixed to the value calculated at 1100 nm. This assumption is reasonable since the optical properties of an albino leaf devoid of pigments are constant along the visible and the NIR wavelength region.

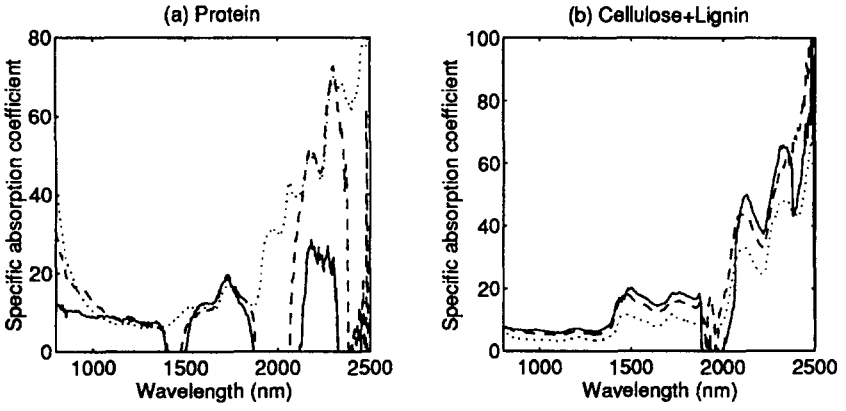

Figure 4. Specific absorption coefficients of (a) protein and (b) cellulose + lignin determined on 63 fresh leaves (——), 58 dry leaves (· · ·), and 121 fresh + dry leaves (- - -).

VALIDATION

Before a model can be used with confidence, it must be validated. We first tested our model in direct mode, by simulating the reflectance and transmission spectra of 63 fresh leaves from 400 nm to 2500 nm and of 58 dry leaves from 1100 nm to 2500 nm. For that, we used the measured concentrations of pigments, water, protein, cellulose, hemicellulose, lignin, and the estimated values of the mesophyll structure parameter N. The three combinations of biochemicals, C1, C2, and C3 were tested. The spectral rmse's are generally less than 0.03, indicating good spectrum reconstruction both for fresh and dry leaves, and both for reflectance and transmittance. This latter rmse is a little surprising since transmittance is known to be more sensitive to the model parameters than is reflectance.

The validation was carried out with the same data sets. It consisted in estimating the model parameters symbolized by the vector θ by minimizing the following merit function:

$$\chi^2(\theta) = \sum_{\lambda 1}^{\lambda 2} \{R_{mes}(\lambda) - R_{mod}(\lambda,\theta)\}^2 + \{T_{mes}(\lambda) - T_{mod}(\lambda,\theta)\}^2, \quad (3)$$

where λ is the wavelength, R_{mes} and R_{mod} are respectively the measured and modeled reflectances. Inversions were performed using the routine CONSTR.M, a bound-constrained optimization package in Matlab. In Table 2 we show the coefficients of determination for each biochemical. On dry leaves, each combination provides a good estimate of leaf biochemistry. Water, which is present in very small amounts in dry leaves, is despite everything estimated with reasonable accuracy ($r^2 = 0.54$). On fresh leaves, the retrieval of water whose effects predominate in the infrared is excellent with an r^2 equal to 0.95; as a consequence of the masking of minor absorptions by water in the infrared, the coefficients of determination of the other constituents fall sharply. The leaf optical properties of fresh leaves seem to be definitely insensitive to protein. This result contrasts with most of the work which, in recent years, concluded that nitrogen (protein here is used as a surrogate for N) was the leaf constituent which could be estimated with the greatest accuracy. We are not very surprised by this result since on average, protein only represents $0.2 \times 0.33 = 0.066$ g g^{-1} of fresh leaf matter, and nitrogen $0.034 \times 0.33 = 0.012$ g g^{-1}. The 1–5% leaf mass attributable to nitrogen and nitrogen compounds is a very small amount and perhaps the positive correlations in NIRS studies have been due to covariance with compounds found in greater abundance (e.g., water or pigments). The combination cellulose + lignin (nearly equivalent to total carbon) provided the best results with an r^2 of 0.5 over the infrared region. If the inversion covers the whole spectrum from 400 nm to 2500 nm, chlorophyll a + b can be also estimated with reasonable but disappointing accuracy ($r^2 = 0.68$). Carotenoids, which represent about 25% of the total photosynthetic pigments (Demmig-

Table 2. Coefficient of Determination Obtained for the Estimation of Chlorophyll a + b (chl), Water (wat), Protein (pro), and combinations of Cellulose (cel), Hemicellulose (hem), and Lignin (lig)

		Coefficient of Determination r^2						
Leaf Type	*Spectral Range*	*Chl*	*Wat*	*Pro*	*Cel + Hem + Lig*	*Cel + Lig*	*Cel + Hem*	*Lig*
Dry	1100 nm–2500 nm		0.54	0.49	0.84			
			0.54	0.65		0.84		
			0.54	0.67			0.88	0.39
Fresh	1100 nm–2500 nm		0.96	0.04	0.10			
			0.95	0.02		0.50		
			0.95	0.02			0.07	0.37
	400 nm–2500 nm	0.68	0.95	0.02		0.39		

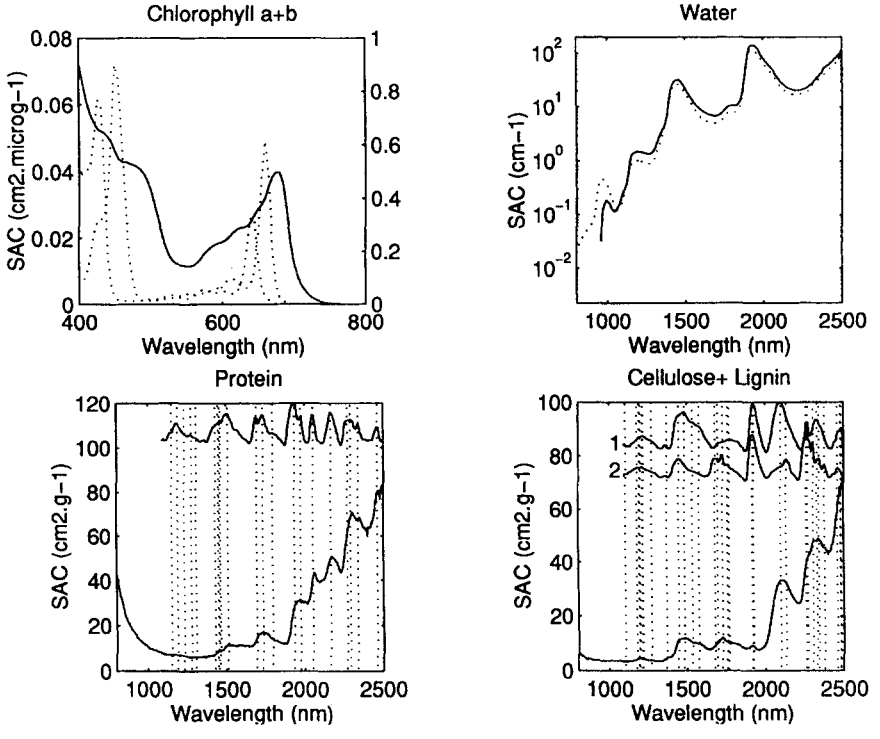

Figure 5. Specific Absorption Coefficients (SAC) predicted for chlorophyll a + b, water, protein, and cellulose + lignin. The dotted points of 5a correspond to measured absorption spectra of chlorophyll a and chlorophyll b (outside and inside curves) in acetone (after Lichtenthaler, 1987), and 5b to pure liquid water (after Curcio and Petty, 1951); The absorption spectra of powdered zein is shown in 5c, cellulose (1) and lignin (2) are shown in 5d (after Barton II et al., 1992). The vertical dotted lines correspond to wavelengths of peaks of maximum absorption in measured spectra.

Adams et al., 1989) and which are ignored in this study, may explain this relative success. Since structural effects on leaf optical properties, not only in the NIR but also in the visible, are explained in the model both by the N parameter and by the cellulose + lignin concentration, the estimation of the latter decreased to 0.39 when considering the whole spectrum. In conclusion, the high correlations for estimating pigments and water show that the inversion procedure is successful in retrieving the major leaf components having major absorption features. Concerning the minor ones, we notice that they are best estimated when water is removed. In fresh leaves, there is no sensitivity for protein, and cellulose + lignin (or other carbon combination) is poorly estimated; in dry leaves, protein and carbon combinations are estimated at higher r^2's. In terms of reflectance and transmittance reconstruction, the very low spectral rmse (< 0.01) obtained in each case demonstrates the capability of this new version of the PROSPECT model to accurately synthesize the whole leaf spectrum for widely different kinds of plant leaves using only five or six parameters.

SENSITIVITY ANALYSIS

We performed simulations to test the influence of each parameter on leaf optical properties. If varying the structure parameter, water, and chlorophyll produces well-known effects (Jacquemoud and Baret, 1990), it is still unclear how other leaf biochemicals modify the *in situ* leaf spectral reflectance and transmittance. Figure 6 illustrates how different simulated mixes of water, protein, and cellulose + lignin affect the reflectance. The protein content produces very little effect on leaf reflectance, in accordance with its very poor concentration estimation (Figs. 6a and 6b). Cellulose + lignin has greater influence, especially in the mid-infrared outside the water absorption peaks, but also on the NIR plateau (Figs. 6c and 6d). We also simulated the behavior of reflectance when water is added to a dry leaf. It can be seen in Figure 6e that a small amount of water rapidly masks the absorption features of protein and cellulose + lignin. These results are very similar to the simulations published by Conel et al. (1993).

In this sensitivity analysis, the leaf biochemicals were assumed to be physiologically independent, and concentrations were varied independently. In reality, physiological functions limit the range of variation that can be tolerated and still maintain foliar integrity (Field et al., 1992). For instance, protein and chlorophyll are often positively correlated in leaves (Field and Mooney, 1986). This means that an increase in protein content—given some time for metabolic adjustment—should indirectly induce change in leaf optical properties in the

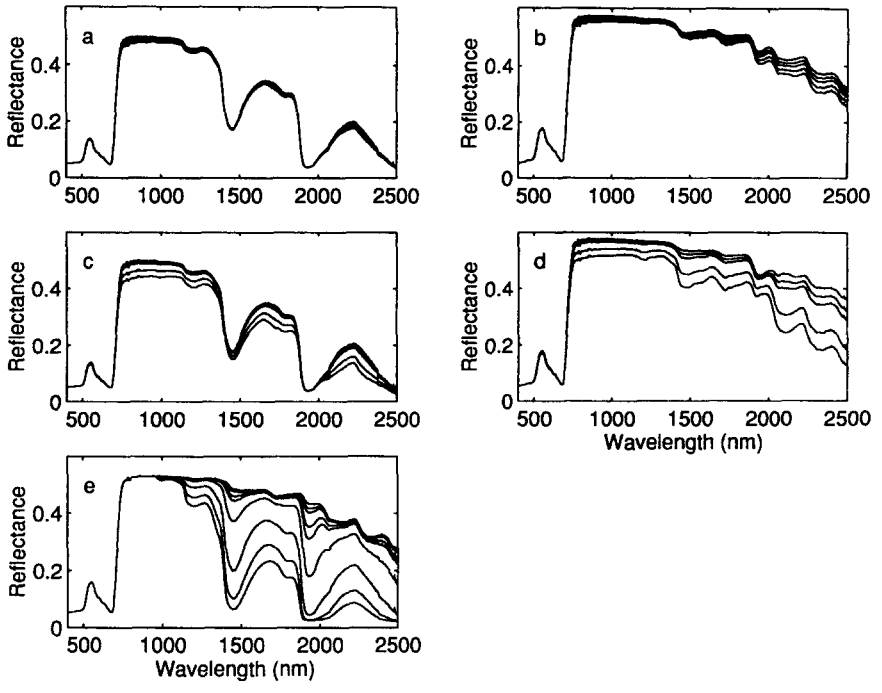

Figure 6. Influence of protein (a-b), cellulose + lignin (c-d), and water (e) content on the reflectance of fresh (left) and dry (right) leaves. The reflectance spectra have been simulated by varying the concentrations of the above constituents around average values: (N; Cab; Cw; Cp; Cc) = (Nf / Nd / Nfd; 48.6; 0.0115; 0.0096; 0.00168) with Nf = 1.7 for fresh leaves (a-c), Nd = 2.3 for dry leaves (b-d), Nfd = 2.0 for fresh to dry leaves (e). The protein content varies from 0.00048 to 0.00172 $g.cm^{-1}$; the cellulose + lignin content varies from 0.00034 to 0.0085 $g.cm^{-1}$; the water content varies from 0.000063 to 0.0405 cm.

visible that results in a greener leaf. Some very high correlations have been established on particular species under controled conditions, for instance, corn (Ercoli et al., 1993); but when a large number of species (e.g., more than fifty in this study) are compared, and when species exhibit a wide range of adaptive growth patterns (evergreen, deciduous, crops, C3, C4, wildland species), these correlations are lower. Another artefact may be produced when varying the water content. As shown by Woolley (1973), a wilting plant leaf tends to lose some water which is replaced by air spaces. In consequence, two different optical effects are combined: a decrease in the absorption (C_w) and an increase in the multiple scattering (N). Simulations of Figure 6e only show a variation of reflectance in the middle infrared due to a variation of C_w while the effect on N was not modeled. One could imagine that other examples of intercorrelations between the model variables could be identified, for instance, between water and pigments. Because of the absence of information about their correct relationships, it is very difficult to take them properly into account.

CONCLUSION

For the first time, a radiative transfer model simulating coherently the reflectance and transmittance both of fresh and dry leaves is presented. The correct simulation of leaf optical properties implies that we were able to correctly estimate the primary leaf biochemical absorption coefficients in the model. The concentration of water was estimated with better than 95% accuracy in fresh leaves and 54% accuracy in dry leaves. The concentration of pigments was estimated at 68% accuracy in fresh leaves. Pigments and water explained most of the absorption features in fresh leaves; it did not surprise us that we could estimate these compounds with good accuracy by inversion of the model. Although the other components together represented only from two-thirds to three-quaters of the dry mass of plant leaves, the model assumed they explained all of the absorption features. This assumption contributed to lowering the fit of C- and N-based compounds. Nonetheless, the coefficient of determination for C and N compounds is higher than 50% based on the infrared spectrum of dry leaves. The specific absorption coefficients of protein, cellulose, hemicellulose, lignin, or combinations of the last three biochemicals, unmixed from our dataset, showed distinctive peaks comparable with experimental data extracted from the literature. Despite this advance in understanding the contributions of foliar biochemicals to leaf reflectance and transmittance, the search for the fundamental properties of the leaf biochemicals is not concluded. It would be interesting to separate the

effects of these constituents and to identify the concentrations of minor components. Moreover, even when their estimation sounds reasonable using dry leaves, it is still rather poor when using fresh leaves. Since most of the time canopy foliage is observable by airborne and satellite sensors in the fresh green state, thus, developing improved models that are more sensitive to changes in fresh leaves is needed. However, this problem may be tricky to fix because it does not really depend on the quality of the specific biochemical absorption spectra but on the presence of water. While water does not absorb all of the signal in the mid-infrared, it is still the main obstacle to ascertaining the concentrations of minor leaf constituents using remote sensing data. Nevertheless, the extension of the PROSPECT model developed here has helped us to better understand the effects of leaf biochemicals on the radiometric signal. In the future, we intend to analyze these effects at the canopy level by coupling PROSPECT with a canopy reflectance model.

This work was supported by the NASA EOS Program under Grant NAS5 / 31359 and NAS5 / 31714, and the U.S. EPA under Grant R-9821695 provided through the UC Davis EPA Center for Ecological Health Research. We wish to thank the Digital Equipment Corporation for providing the DEC Alpha computers under the Sequoia 2000 Grant Cooperative Research Agreement 1243. We are grateful to A. Pedrini of the JRC / IRSA / AT Unit for his help in the calibration of the spectrophotometer data, and to S. E. Hornig for his assistance during the experiment.

REFERENCES

Barton, F. E., II, Himmelsbach, D. S., Duckworth, J. H., and Smith, M. J. (1992), Two-dimensional vibration spectroscopy: correlation of mid- and near-infrared regions, *Appl. Spectrosc.* 46(3):420–429.

Buschmann, C., and Nagel, E. (1991), Reflection spectra of terrestrial vegetation as influenced by pigment-protein complexes and the internal optics of the leaf tissue, in *Proc. Int. Geosci. Remote Sens. Symp.*, Helsinki, Finland, pp. 1909–1912.

Chappelle, E. W., Kim, M. S., and McMurtrey, J. E., III (1992), Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves, *Remote Sens. Environ.* 39:239–247.

Conel, J. E., van den Bosch, J., and Grove, C. I. (1993), Application of a two-stream radiative transfer model for leaf lignin and cellulose concentrations from spectral reflectance measurements. Parts 1 & 2, in *Proc. 4th Annual JPL Airborne Geoscience Workshop. Vol. 1. AVIRIS Workshop* (R. O. Green, Ed.), 25–29 October, Washington, DC, NASA-JPL Publication 93-26, Pasadena, CA, pp. 39–51.

Curcio, J. A., and Petty, C. C. (1951), The near infrared absorption spectrum of liquid water, *J. Opt. Soc. Am.*, 41(5): 302–304.

Demmig-Adams, B., Winter, K., Krüger, A., and Czygan, F. C. (1989), Light stress and photoprotection related to the carotenoid zeaxanthin in higher plants, in *Photosynthesis* (W. R. Briggs, ed.), Liss, New York, pp. 375–391.

Dijkstra, P. (1989), Cause and effect of differences in specific leaf area, in *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (H. Lambers et al., Eds.) Academic, The Hague, pp. 125–140.

Ercoli, L., Mariotti, M., Masoni, A., and Massantini, F. (1993), Relationship between nitrogen and chlorophyll content and spectral properties in maize leaves, *Eur. J. Agron.* 2(2): 113–117.

Field, C. B., and Mooney, H. A. (1986), The photosynthesis-nitrogen relationship in wild plants, in *On the Economy of Plant Form and Function* (T. J. Givnish, Ed.), Cambridge University Press, Cambridge, pp. 25–55.

Field, C. B., Chapin, F. S., III, Matson, P. A., and Mooney, H. A. (1992), Responses of terrestrail ecosystems to the changing atmosphere: a resource-based approach, *Ann. Rev. Ecol. Syst.* 23:201–236.

Fourty, Th., Baret, F., Jacquemoud, S., Schmuck, G., and Verdebout, J. (1996), Optical properties of dry plant leaves with explicite description of their biochemical composition: direct and inverse problems, *Remote Sens. Environ.* 56: 104–117.

Fukshansky, L., Fukshansky-Kazarinova, N., and Martinez v. Remisowsky, A. (1991), Estimation of optical parameters in a living tissue by solving the inverse problem to the multiflux radiative transfer, *Appl. Opt.* 30(22):3145–3153.

Hosgood, B., Jacquemoud, S., Andreoli, G., Verdebout, J., Pedrini, G., and Schmuck, G. (1995), Leaf Optical Properties EXperiment 93 (LOPEX93) Report EUR-16095-EN, European Commission, Joint Research Centre, Institute for Remote Sensing Applications, Ispra, Italy.

Jacquemoud, S., and Baret, F (1990), PROSPECT: a model of leaf optical properties spectra, *Remote Sens. Environ.* 34:75–91.

Jacquemoud, S., Verdebout, J., Schmuck, G., Andreoli, G., and Hosgood, B. (1995), Investigation of leaf biochemistry by statistics, *Remote Sens. Environ.* 54:180–188.

Lichtenthaler, H. K. (1987), Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymol.* 148:350–382.

Martinez v. Remisowsky, A., McClendon, J. H., and Fukshansky, L. (1992), Estimation of the optical parameters and light gradients in leaves: multi-flux *versus* two-flux treatment, *Photochem. Photobiol.* 55(6):857–865.

Peterson, D. L., and Hubbard, G. S. (1992), Scientific issues and potential remote-sensing requirements for plant biochemical content. *J. Imaging Sci. Technol.* 36(5):446–456.

Verdebout, J., Jacquemoud, S., and Schmuck, G. (1994), Optical properties of leaves: modelling and experimental studies, in *Imaging Spectrometry—a Tool for Environmental Observations* (J. Hill and J. Mégier, Eds.), ECSC, EEC, EAEC, Brussels and Luxembourg, pp. 169–191.

Woolley, J. T. (1973), Change of leaf dimensions and air volume with change in water content, *Plant Physiol.* 41: 815–816.

Yamada, N., and Fujimura, S. (1991), Nondestructive measurement of chlorophyll pigment content in plant leaves from three-color reflectance and transmittance, *Appl. Opt.* 30 (27):3964–3973.