2006

Three-Band Model for Noninvasive Estimation of Chlorophyll Carotenoids and Anthocyanin Contents in Higher Plant Leaves

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Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves

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Received 7 April 2006; revised 26 April 2006; accepted 1 May 2006; published 10 June 2006.

[1] Leaf pigment content and composition provide important information about plant physiological status. Reflectance measurements offer a rapid, nondestructive technique to estimate pigment content. This paper describes a recently developed three-band conceptual model capable of remotely estimating total of chlorophylls, carotenoids and anthocyanins contents in leaves from many tree and crop species. We tuned the spectral regions used in the model in accord with pigment of interest and the optical characteristics of the leaves studied, and showed that the developed technique allowed accurate estimation of total chlorophylls, carotenoids and anthocyanins, explaining more than 91%, 70% and 93% of pigment variation, respectively. This new technique shows a great potential for noninvasive tracking of the physiological status of vegetation and the impact of environmental changes. **Citation:** Gitelson, A. A., G. P. Keydan, and M. N. Merzlyak (2006), Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves, *Geophys. Res. Lett.*, *33*, L11402, doi:10.1029/2006GL026457.

1. Introduction

[2] Pigment content and composition are related to the leaf physiological status. Chlorophylls (Chl) absorb solar light energy and provide mechanisms for its utilization in photosynthetic reactions. Carotenoids (Car) contribute to light-harvesting and also play a photo-protective role, preventing damage to the photosynthetic systems [e.g., *Chappelle et al.*, 1992; *Dawson et al.*, 1998; *Gitelson et al.*, 2002, 2003; *Merzlyak et al.*, 2003]. The red pigments, anthocyanins (Anth), protect leaves from excess light [*Gitelson et al.*, 2002; *Merzlyak and Chivkunova*, 2000].

[3] Traditional methods of wet chemical pigment analysis are time consuming and expensive. They require destruction of the measured leaves and thus do not permit measurement of changes in pigments over time in a single leaf. In contrast, spectral reflectance measurements provide a non-invasive, rapid technique that can be used at different spatial scales. Despite development of good theoretical models relating Chl, water content, and structure with leaf reflectance [e.g., *Jacquemoud and Baret*, 1990; *Dawson et al.*, 1998], needed information about leaf structure may not be available. To the best of our knowledge, there is no model that includes anthocyanin and carotenoid contents thus preventing prediction of content for these pigments.

[4] To date, relationships between leaf reflectance and pigment content have been derived empirically. While the many models relating Chl content to reflectance [e.g., *Gitelson and Merzlyak*, 1994a, 1994b, 1997; *Sims and Gamon*, 2002; *Richardson et al.*, 2002; *Gitelson et al.*, 2003; *le Maire et al.*, 2004, and references therein] are quite robust in Chl prediction, only few models support anthocyanin and carotenoids content retrieval [e.g., *Chappelle et al.*, 1992, *Gitelson et al.*, 2001, 2002; *Sims and Gamon*, 2002].

[5] Recently, a conceptual three-band model has been developed and successfully used to relate reflectance with Chl content in leaves [*Gitelson et al.*, 2003]. In this study we investigated the applicability of this model to noninvasive quantitative estimation of content for various pigments (total chlorophyll, carotenoid and anthocyanin) in the leaves of different tree and crop species.

2. Methods

[6] For calibration of the Chl and Car models, anthocyanin-free juvenile, mature and senescent leaves collected from 1992 to 2005 were used; Norway maple and horse chestnut leaves were from a park at Moscow State University (Russia), beech leaves from the University of Karlsruhe campus (Germany), maize, soybean and dogwood leaves were collected at Mead Nebraska (USA). For calibration of the Anth model, Anth-containing leaves from Norway maple and dogwood were used. The leaf total Chl, Car and Anth content was determined analytically from the same leaf samples used for reflectance measurement [see *Gitelson et al.*, 2001, 2002, 2003]. Anth content was determined after extract acidification with concentrated HCl [see *Gitelson et al.*, 2002]. Pigment content was expressed on a leaf area basis.

[7] Adaxial reflectance (R) spectra of leaves were taken in a spectral range between 400 and 800 nm with (a) a Hitachi 150−20 spectrophotometer (maple and chestnut), (b) a Shimadzu 2101 PC spectrophotometer (beech), and (c) a clip with a 2.3-mm diameter bifurcated fiber-optic attached to both an Ocean Optics USB2000 radiometer and to an Ocean Optics LS-1 light source (dogwood, soybean, and maize). Leaf reflectance spectra were recorded against $BaSO_4$ as a standard. The reflectance spectrum was calculated as a ratio of leaf radiance to standard radiance at wavelength λ.

[8] Nine data sets containing 306 leaves (beech, chestnut, dogwood, maple, maize and soybean) were used for Chl model calibration (Table 1). Six data sets containing 234

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0094-8276/06/2006GL026457$05.00

Table 1. Slopes (m) and Intercepts (n) of the Linear Relationships Between Green (Equation (3)) and Red Edge (Equation (4)) Models Versus Total Chl Content With Corresponding Root Mean Square Error of Chl Estimation (RMSE, in mg/m^2) Coefficient of Determination (r^2), Mean Total Chlorophyll Content (Chl_{mean}) and Number of Samples (N) For Each Species Studied[a]

			Green				Red Edge			
Species	N	Chl_{mean}	*m*	*n*	RMSE	r^2	*m*	*n*	RMSE	r^2
Beech 1996	38	375	0.76	8.22	40	0.95	0.62	−27.70	40	0.95
Beech 2000	28	194	0.64	2.27	25	0.94	0.50	−8.12	25	0.95
Chestnut 96–97	20	117	0.84	21.4	25	0.95	0.65	−0.35	25	0.95
Chestnut 2000	22	142	0.76	18.3	35	0.93	0.60	−3.41	35	0.94
Maple 1992–99	66	200	0.63	13.6	40	0.95	0.51	2.29	35	0.95
Maple 2000	30	179	0.68	21.6	30	0.94	0.54	−1.23	30	0.94
Maize	30	364	0.30	29.7	75	0.92	0.30	−22.20	50	0.95
Soybean	20	408	0.51	25.3	70	0.91	0.52	−17.40	60	0.92
Maple[b]	48	83					0.49	−6.16	8	0.92
Dogwood[b]	52	266					0.44	−5.25	10	0.91

[a]For each species, the linear relationship was found to be the best fit function.
[b]Anthocyanin-containing leaves; Chl was retrieved using red edge model (equation (4)).

leaves (beech, chestnut, and maple) were used for Car model calibration (Table 2). Three data sets containing 100 leaves (dogwood and maple) were used for Anth model calibration.

3. Results and Discussion

3.1. Model for Pigment Retrieval

[9] The infinite reflectance of a leaf, R_∞, in which further increase in thickness resulted in no noticeable differences in reflectance, was found to be closely related to the reciprocal of reflectance, R^{-1} [*Gitelson et al.*, 2003]:

$$R^{-1} \propto R_\infty = a/b_b \quad (1)$$

where a and b_b are the absorption and backscattering coefficients, respectively. a is a sum of absorption coefficients for the pigment of interest (a_p) and other pigments (a_0).

[10] To isolate a_p, the conceptual model [*Gitelson et al.*, 2003] uses reflectances at three spectral bands. Reflectance in the first band $R_{\lambda 1}$ is maximally sensitive to absorption by the pigment of interest a_p. But reflectance is also affected by the absorption of other pigments a_0 and by the variability in backscattering among samples b_b. To remove the effect of absorption by other pigments one needs to find a spectral band λ_2 where absorption by the pigment of interest is much lower than at λ_1, $a_p(\lambda_2) \ll a_p(\lambda_1)$, and absorption by other pigments and the effect of backscattering are quite close to that at λ_1 (i.e., $a_0(\lambda_2) \sim a_0(\lambda_1)$ and $b_b(\lambda_2) \sim b_b(\lambda_1)$). If $R_{\lambda 2}^{-1}$ is subtracted from $R_{\lambda 1}^{-1}$, that gives $(R_{\lambda 1}^{-1} - R_{\lambda 2}^{-1}) \propto a_p(\lambda_1)/b_b$. To remove b_b, a third spectral band λ_3 should be used where backscattering controls reflectance (i.e., $R_{\lambda 3} \propto b_b$). Multiplying the difference $(R_{\lambda 1}^{-1} - R_{\lambda 2}^{-1})$ by $R_{\lambda 3}$, we have the model that may isolate a_p:

$$\left[R(\lambda_1)^{-1} - R(\lambda_2)^{-1}\right] \times R(\lambda_3) \propto a_p \quad (2)$$

To find the optimal spectral bands λ_1, λ_2, and λ_3 in the model, we used a stepwise technique based on linear regression of the model vs. content of the pigment of interest.

[11] Pigment content in leaves varied widely. In anthocyanin-free leaves (Anth <3 mg/m^2), Chl ranged between 1 and 860 mg/m^2, and Car between 14 and 166 mg/m^2. In Anth-containing leaves, Anth was between 5 and 102 mg/m^2, Chl ranged between 83 and 440 mg/m^2 and Car between 30 and 190 mg/m^2.

3.2. Model Tuning for Chlorophyll Content Retrieval

[12] As the first step in model tuning we found the optimal position of λ_2 using an initial $\lambda_1^0 = 670$ nm (red Chl absorption maximum) and $\lambda_3^0 = 760$ nm ($a_{Chl}(\lambda_3) \sim 0$ and b_b controls reflectance). RMSE of Chl estimation by the model $(R_{675}^{-1} - R_{\lambda 2}^{-1}) \times R_{800}$ had minimal values at $\lambda_2 >$ 760 nm for all species (Figure 1 for beech); we selected $\lambda_2^1 =$ 790 nm. In the second step we found the optimal position of

Table 2. Slopes (m) and Intercepts (n) of the Linear Relationships Between Red Edge Model (Equation (6)) Versus Total Car Content With Corresponding Root Mean Square Error of Car Estimation (Car RMSE, in mg/m^2), Coefficient of Determination (r^2), Minimal (Car_{min}), Maximal, (Car_{max}) and Mean (Car_{mean}) Car Contents, Coefficient of Variation (CV = Car RMSE/Car_{mean}), and Number of Samples (N) for Each Species Studied[a]

	N	Car_{min}	Car_{max}	Car_{mean}	r^2	Car RMSE	CV, %	*m*	*n*	Chl RMSE
Beech 1996	38	28	138	90	0.83	11.9	13	2.78	0.68	15.6
Beech 2000	28	18	80	50	0.91	4.0	8	2.88	−9.28	7.6
Chestnut 1996–97	20	29	94	52	0.71	7.7	15	1.81	−2.46	11.9
Chestnut 1999	22	52	166	96	0.70	17.2	18	1.52	−29.52	23.0
Maple 1992–1999	66	29	165	84	0.70	15.7	19	1.49	−16.35	23.8
Maple 2000	30	16	124	72	0.71	11.8	16	1.55	2.73	15.3

[a]Chl RMSE is RMSE of Car estimation by Chl model (equation (4)). For each species, the linear relationship was found to be the best fit function.

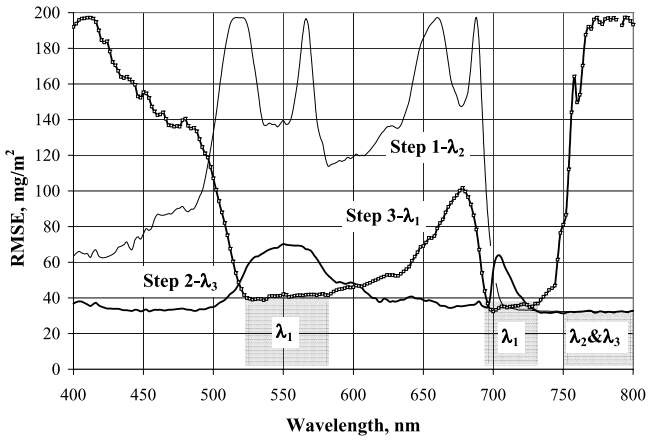

Figure 1. Three steps of model tuning for Chl retrieval from reflectance spectra of 38 beech leaves with Chl_{mean} = 375 mg/m^2. RMSE was calculated for linear regression of the $[R(\lambda_1)^{-1}-R(\lambda_2)^{-1}] \times R(\lambda_3)$ model versus total Chl content.

λ_3 in the model $(R_{670}^{-1}-R_{790}^{-1}) \times R_{\lambda 3}$. Minimal RMSE was in the NIR range where $R_{\lambda 3}$ relates closely to b_b. In the third step we found the optimal position of λ_1 in the model $(R_{\lambda 1}^{-1}-R_{790}^{-1}) \times R_{790}$. RMSE had two distinct minima: in the green (around 550 nm) and in the red edge (690–725 nm) ranges (Figure 1). Therefore, two models can be used for Chl estimation in anthocyanin-free leaves if NIR is set beyond 760 nm:

$$Chl_{green} \propto \left[R_{540-560}^{-1} - R_{NIR}^{-1}\right] \times R_{NIR} = \left(R_{NIR}/R_{green}\right) - 1 \quad (3)$$

$$Chl_{red\ edge} \propto \left[R_{690-725}^{-1} - R_{NIR}^{-1}\right] \times R_{NIR} = \left(R_{NIR}/R_{red\ edge}\right) - 1 \quad (4)$$

For each species, the linear relationship between the Chl content and the models (equations (3) and (4)) was found to be the best fit function (Table 1).

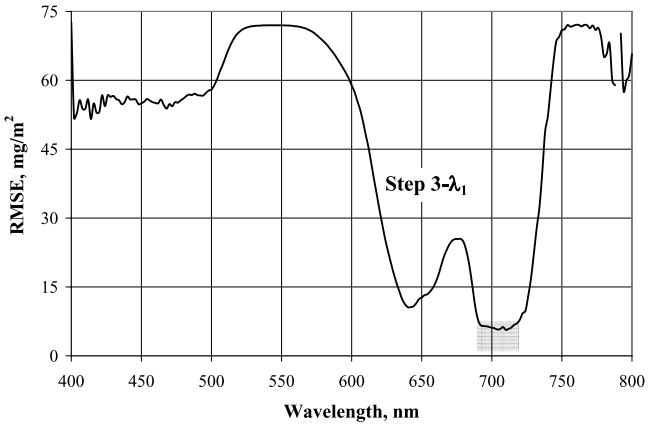

Figure 2. The third step of model tuning for 25 dogwood Anth-containing leaves with Chl_{mean} = 266 mg/m^2. RMSE was calculated for linear regression of the $[R(\lambda_1)^{-1}-R_{790}{}^{-1}] \times R_{790}$ model vs. total Chl content.

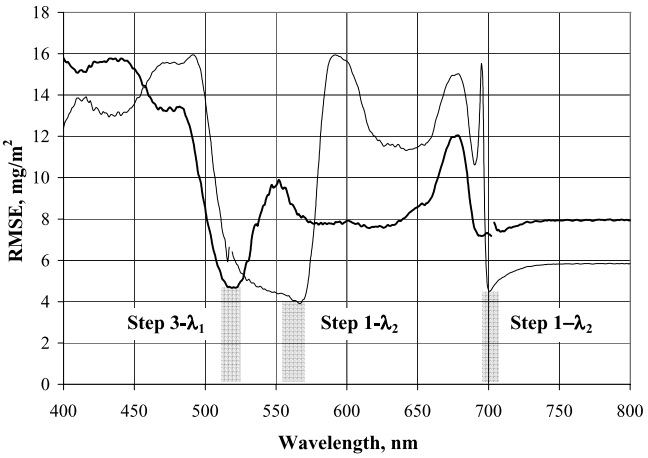

Figure 3. The first (λ_2) and third (λ_1) steps of model tuning for carotenoid content retrieval from reflectance spectra of 28 beech leaves with Car_{mean} = 50 mg/m^2. RMSE was calculated for linear regression of the $[R(\lambda_1)^{-1}-R(\lambda_2)^{-1}] \times R(\lambda_3)$ model versus total carotenoid content.

[13] In Anth-containing leaves, the first and second steps of tuning gave the same results as for Anth-free leaves: $\lambda_2^1 = \lambda_3^1$ =790 nm (not shown). However, in the third step (Figure 2) minimal RMSE was in the red edge range only (690 to 725 nm). In the green range RMSE was maximal due to Anth absorption [*Gitelson et al.*, 2001]. Thus, for Chl retrieval from Anth-containing leaves the equation (4) model should be used (Table 1, bottom lines: maple and dogwood).

3.3. Model Tuning for Carotenoids Content Retrieval

[14] Carotenoids content in crops and dogwood was related very closely ($r^2 > 0.97$) with total Chl content, therefore Car content cannot be treated as an independent variable. However, in tree species (beech, chestnut and maple), it was possible to estimate Car content separately from Chl content despite the quite close correlation between Chl and Car (r^2 was for beech: 0.78 in 1996 and 0.86 in 2000, for chestnut: 0.69 in 96–97 and 0.72 in 2000, for maple: 0.65 in 92–99 and 0.75 in 2000).

[15] The same procedure described above was used for model tuning. In the first step we found the optimal position of λ_2 using an initial λ_1^0 = 500 nm (Car absorption band) [*Zur et al.*, 2000; *Gitelson et al.*, 2002] and λ_3^0 = 760 nm ($a_{car}(\lambda_3) \sim 0$ and b_b controls reflectance). For all species, the RMSE using the $(R_{500}^{-1}-R_{\lambda 2}^{-1}) \times R_{760}$ model showed minimal values at λ_2^1 = 560–570 nm and around 700 nm (Figure 3 for beech). In these spectral bands $a_{Car}(\lambda_2) \ll a_{Chl}(\lambda_1)$ [*Gitelson and Merzlyak*, 1994a, 1994b] and $a_{chl}(\lambda_2) \sim a_{chl}(\lambda_1)$ [*Chappelle et al.*, 1992; *Gitelson and Merzlyak*, 1994a, 1994b] and reciprocal reflectance is governed mainly by Chl content [*Gitelson et al.*, 2003]. Thus, subtraction of either $R_{560-570}^{-1}$ or $R_{690-710}^{-1}$ from $R^{-1}(\lambda_1)$ significantly decreased the RMSE of the Car estimation. For λ_2^1, 560–570 nm or 690–710 nm can be used in the second step of model tuning. The optimal position of λ_3 in the $(R_{500}^{-1}-R_{560}^{-1}) \times R_{\lambda 3}$ and $(R_{500}^{-1}-R_{690-710}^{-1}) \times R_{\lambda 3}$ models was found in the NIR range beyond 760 nm where $a_{car}(\lambda_2) \sim a_{Chl}(\lambda_1) \sim 0$ and

b_b controls reflectance (not shown). For the third step we selected λ_3^1 = 790 nm and found the optimal position of λ_1 in the $(R_{\lambda 1}^{-1}-R_{560-570}^{-1}) \times R_{790}$ and $(R_{\lambda 1}^{-1}-R_{690-710}^{-1}) \times R_{790}$ models at 510–520 nm (Figure 3). Thus, two models can be used for Car estimation in anthocyanin-free leaves with NIR set beyond 760 nm:

$$\mathrm{Car_{green}} \propto \left[R_{510-520}^{-1} - R_{560-570}^{-1}\right] \times R_{NIR} \quad (5)$$

$$\mathrm{Car_{red\ edge}} \propto \left[R_{510-520}^{-1} - R_{690-710}^{-1}\right] \times R_{NIR} \quad (6)$$

[16] For each species, the linear relationship between the Car content and the models (equations (5) and (6)) was found to be the best fit function (Table 2).

[17] Importantly, coefficients of the relationships relating models to Car remained almost the same for the independent data sets of each species (Table 2). Four data sets (maple and chestnut), taken in Russia under the same climatic conditions, had very close model coefficients. This suggests that the models equation (5) and (6) do not require parameterization when one works with the same species with the same origin, but might require parameterization for different species.

[18] As we mentioned above, Chl and Car were interrelated in the leaves studied. Thus it was important to compare the performance of the best Chl (equations (3) and (4)) and Car (equation (5) and (6)) models for Car retrieval. Car models were consistently better than Chl models in predicting Car (compare Car and Chl RMSE in Table 2). This shows that the subtraction of $R^{-1}(\lambda_2)$, which is responsible for Chl absorption, allowed the model to be Car specific even in the case where Car and Chl were quite closely related.

3.4. Model Tuning for Anthocyanin Content Retrieval

[19] In the first step we found the optimal position of λ_2 using an initial λ_1^0 = 530 nm – close to maximum of leaf Anth absorption in acidic alcohols [*Strack and Wray*, 1989] and λ_3^0 = 760 nm. RMSE with the $(R_{530}^{-1}-R_{\lambda 2}^{-1}) \times R_{760}$ model had minimal values for both dogwood and maple at λ_2^1 = 690–700 nm (Figure 4 for dogwood). In this spectral band reciprocal reflectance is governed mainly by Chl content [*Gitelson et al.*, 2003]. The subtraction of $R_{690-700}^{-1}$ from $R^{-1}(\lambda_1)$, caused $R^{-1}(\lambda_1)-R^{-1}(\lambda_2)$ to be closely related to Anth content, however, the difference is also affected by scattering b_b that might vary among samples. The optimal position of λ_3 in the $(R_{530}^{-1}-R_{690-700}^{-1}) \times R_{\lambda 3}$ model was found in the NIR range beyond 760 nm where $a_{Anth}(\lambda_2) \sim a_{Chl}(\lambda_1) \sim 0$ and b_b controls reflectance (not shown). In the third step we found the optimal position of λ_1 in the $(R_{\lambda 1}^{-1}-R_{690-710}^{-1}) \times R_{790}$ model in a wide range around 550 nm. The model for Anth estimation, with NIR range beyond 760 nm, had the form:

$$\mathrm{Anth} \propto \left[R_{530-570}^{-1} - R_{690-710}^{-1}\right] \times R_{NIR} \quad (7)$$

[20] For both species studied, the linear relationship between the Anth content and the model (equation (7)) was found to be the best fit function. The equation (7) model yielded accurate assessment of Anth content, accounting for more than 93% of Anth variation. The coefficients of equation (7) were slightly different for dogwood and maple, thus, the model may require parameterization when applied to various species.

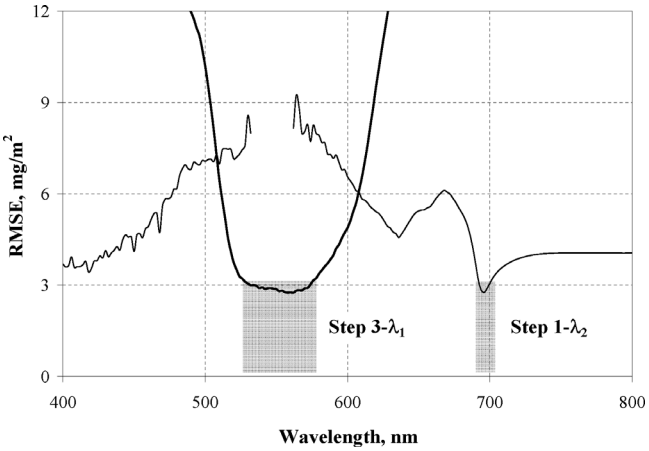

Figure 4. The first (λ_2) and third (λ_1) steps of model tuning for 18 dogwood leaves with $\mathrm{Anth_{mean}}$ = 33 mg/m^2. RMSE was calculated for linear regression of the $[R(\lambda_1)^{-1}-R(\lambda_2)^{-1}] \times R(\lambda_3)$ model versus anthocyanin content.

Table 3. Spectral Bands for Retrieving Pigment Content From Leaf Reflectance Spectra[a]

Pigment	λ_1	λ_2	λ_3
Chlorophylls, Anth-free	540–560	760–800	760–800
Chlorophylls, Anth-free	690–720	760–800	760–800
Chlorophylls, Anth-cont	690–720		760–800
Carotenoids	510–520	540–560	760–800
Carotenoids	510–520	690–710	760–800
Anthocyanins	540–560	690–710	760–800

[a]For chlorophyll content retrieval in Anth-free leaves (Anth < 3 mg/m^2), both the green and the red edge bands can be used as λ_1; in Anth-containing leaves (Anth-cont), only the red edge bands can be used as λ_1. For carotenoids estimation both the green and the red edge bands can be used as λ_2.

4. Conclusions

[21] For the first time one model, using reflectance in three spectral bands has been applied for non-destructive assessment of total chlorophyll, carotenoid and anthocyanin contents in plant leaves. Table 3 summarizes the spectral bands we recommend for each pigment content retrieval. In Anth-free leaves, both the green and the red edge bands can be used as λ_1 for Chl estimation and as λ_2 for Car estimation. Only four spectral bands are required for three pigments retrieval: 510–520 nm (carotenoids), 540–560 nm (anthocyanins), 690–710 nm (total chlorophyll) and 760–800 nm. The same conceptual model has been used for non-destructive pigment retrieval from reflectance spectra of fruit [*Merzlyak et al.*, 2003, 2005], chlorophyll content in crops [*Gitelson et al.*, 2005] as well as chlorophyll-*a* estimation in turbid productive waters [*Dall'Olmo et al.*, 2003; *Dall'Olmo and Gitelson*, 2005] and in hypereutrophic waters [*Zimba and Gitelson*, 2006]. This study

brings additional evidence that the conceptual model may present a unified approach to remote quantification of absorbing constituents in optically deep media.

[22] **Acknowledgments.** We thank Claus Buschmann, Veronica Ciganda, Olga Chivkunova, Mark Steel, Andres Vina and Yoav Zur for data collecting as well as Giorgio Dall'Olmo and Donald Rundquist for fruitful discussions. We acknowledge the support and the use of facilities and equipment provided by the Center for Advanced Land Management Information Technologies (CALMIT), University of Nebraska-Lincoln. A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE, Journal Series 15193. This research was also supported in part by funds provided through the Hatch Act and Russian Fund for Basic Research.

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