



Foliar absorption coefficient derived from reflectance spectra: A gauge of the efficiency of *in situ* light-capture by different pigment groups

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ARTICLE INFO

Keywords:

Anthocyanins

Carotenoids

Chlorophylls

Absorption coefficient

Photosynthetically active radiation

ABSTRACT

The absorption of Photosynthetically Active Radiation (PAR) by different foliar pigments defines the amount of energy available for photosynthesis and also the need for photoprotection. Both characteristics reveal essential information about productivity, development, and stress acclimation of plants. Here we present an approach for the estimation of the efficiency by three foliar pigment groups (chlorophylls, carotenoids, and anthocyanins) at capturing light, via the absorption coefficient derived from leaf reflectance spectra. The absorption coefficient (and hence light capture efficiency) of the pigment is quantitatively related to the ratio of light absorbed by each pigment group over the total amount of light absorbed by the leaf. The proposed approach allows discerning the contribution of pigment groups to the overall light absorption, despite the strong interference by other pigments with overlapping absorption spectra. For photosynthetic pigments, like chlorophylls, this is indicative of the energy captured for photosynthesis and hence of potential plant productivity. For photoprotective pigments, like anthocyanins or secondary carotenoids, it gives information about the spectral ranges where their optical screening works best and their screening capacity. In addition, the approach allows the selection of optimal spectral bands where different pigments operate. Such information improves our understanding of the phenological, physiological and photosynthetic dynamics of plants over space and through time, useful for developing better monitoring and management strategies.

1. Introduction

Absorption of photosynthetically active radiation (PAR) is a fundamental plant function at the core of almost all ecosystem processes supporting life on our planet. Since most of the land surface on Earth is covered by vegetation, absorption of solar radiation by terrestrial plants plays a fundamental role in global biogeochemical cycles, provides energy for trophic networks, and supports the world's economy, including the use of fossil fuels which are the product of photosynthetic activity millions of years ago.

Foliar pigments are the basis of the absorption of PAR by plants, contributing not only to photosynthesis but also to photoprotection and adaptation to short-, intermediate- and long-term environmental

changes. Thus, the dynamics of foliar pigments (i.e., changes in their absolute and relative amounts) are not only influenced by, but also influence carbon, nutrient, and water cycles, as well as the energy flows through trophic networks (Asner et al., 2004; Féret et al., 2017; Shipley et al., 2006). Chlorophylls (Chl), carotenoids (Car), and flavonoids are the three main foliar pigment groups occurring in terrestrial plants. Chl are involved in light-harvesting for photosynthesis, while Car are accessory pigments that also contribute to light-harvesting but also to photoprotection. Flavonoids contribute not only to photoprotection, but as is the case of anthocyanins (AnC), are also associated with plant resistance to environmental stresses, including nutrient deficiencies and herbivory (Gould, 2004). Thus, assessing contents and composition of foliar pigments in terrestrial ecosystems at multiple scales (from

Abbreviations: AnC, anthocyanins; Car, carotenoids; Chl, chlorophylls; K-M, Kubelka-Munk; NIR, near infra-red; PAR, photosynthetically active radiation.

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<https://doi.org/10.1016/j.jplph.2020.153277>

Received 22 June 2020; Received in revised form 16 August 2020; Accepted 3 September 2020

Available online 17 September 2020

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individual leaves to the entire land surface of the Earth) and their light absorption characteristics is crucial for studying and understanding the spatio-temporal dynamics of vegetation and the processes causing them. This is fundamental not only from the scientific perspective, but also for the development of monitoring and management applications that contribute to the sustainability of terrestrial ecosystems, including man-made agroecosystems.

The absorption coefficient is a rate of decrease of the intensity of radiation passing through a given substance. When expressed on a per wavelength basis, it reveals an absorption coefficient spectrum. This spectrum exhibits a large variability depending on the amount and composition of absorbers in the optical medium, on the specific absorption coefficient of each absorber (expressed as absorption per pigment content), and on the nature of the medium itself. Thus, absorption coefficient spectra are useful not only for understanding the absorption properties of an optical medium, but also for assessing the amount and composition of the absorbers present and their individual contributions to the overall absorption spectrum of the optical medium.

In the case of terrestrial plants and in the PAR spectral region, foliar absorption coefficient spectra contain information on the amount and composition of pigments, hence carry a plethora of information on photosynthetic activity, stress acclimation, and developmental progression (Féret et al., 2008, 2017; Gitelson and Solovchenko, 2018), among many others. Yet, isolating the absorption coefficient spectra of key pigment groups (e.g. Chl, Car, AnC) *in vivo* is quite challenging not only because these pigment groups exhibit overlapping absorptions at particular wavelengths, but also because absorption of radiation in the PAR spectral region is also influenced by leaf structure.

Here we describe a novel procedure for retrieving the absorption coefficient spectra of the main foliar pigment groups from leaf reflectance spectra based on previous insights into relationships between leaf reflectance and foliar pigment content (see e.g., Gitelson et al., 2001, 2002, 2006, 2015; Gitelson and Solovchenko, 2017, 2018 and references therein). Based on a model previously developed to obtain the absorption coefficient of Chl from reflectance spectra at canopy scales (Gitelson et al., 2019), this procedure allows assessing the individual contribution of each foliar pigment to the overall leaf absorption coefficient spectra, even in spectral regions where these pigments exhibit overlapping absorptions. The procedure was tested using leaves of different plant species, with diverse pigment compositions and contents, and with different leaf structures.

2. Background

The Kubelka-Munk (K-M) theory (Kortum, 1969; Kubelka and Munk, 1931; Wendlandt and Hecht, 1966) describes the foundations of reflectance spectroscopy in terms of the remission function, which relates the infinite reflectance (ρ_{inf} , in which further increases in medium thickness do not result in noticeable changes of its reflectance) of an optically dense medium with its inherent optical properties: absorption (α) and scattering (β) coefficients.

The measured reflectance of a finite optical medium is defined as ρ_0 , where the index 0 is used to designate an ideal black background. ρ_{inf} may be retrieved from ρ_0 using the equation (Kortum, 1969):

$$f(\rho_{inf}) = (1 + \rho_{inf}^2)/2\rho_{inf} = (1 + \rho_0^2 - T_0^2)/2\rho_0 - 1 \quad (1)$$

where ρ_0 and T_0 are the measured reflectance and transmittance of the medium, respectively.

Due to the strong absorption of radiation by leaves in the PAR spectral region, leaves are considered an optically dense medium within this region, therefore suitable to be analyzed through the K-M theory. The numerator of Eq. 1, $(1 + \rho_0^2 - T_0^2)$, in leaves is close to unity, thus $f(\rho_{inf})$ constitutes a hyperbolic function of $(\rho_0)^{-1}$ (Gitelson et al., 2019, 2003). For leaves studied with ρ_0 ranging from 0 to 50 % (Chl content ranges from 0.66 to 675 mg m⁻²) the relationship between $f(\rho_{inf})$ and ρ_0^{-1}

was found to be linear and with $R^2 > 0.99$. This laid a solid foundation for the retrieval of absorption coefficient from reflectance spectra in the form (Gitelson et al., 2003):

$$(\rho_\lambda)^{-1} = (\alpha_{pig} + \alpha_0)/\beta \quad (2)$$

where $(\rho_\lambda)^{-1}$ is reciprocal reflectance, α_{pig} is the absorption coefficient of the pigment of interest, α_0 is the absorption coefficient of other absorbers, and β is the scattering coefficient of the medium.

One of the features of $(\rho_\lambda)^{-1}$ spectra is having positive $(\rho_\lambda)^{-1}$ values in the near infra-red (NIR) spectral region beyond 760 nm where absorption of healthy leaves is negligible (Merzlyak et al., 2002; Gitelson et al., 2003). The reasons for these non-zero $(\rho_\lambda)^{-1}$ values in the NIR is the fraction of light transmitted but not accounted by $(\rho_\lambda)^{-1}$ and also the absorption by pigments other than Chl e.g., ‘browning’ pigments produced upon the oxidation of polyphenols during senescence (Merzlyak et al., 2004). Subtraction of NIR reciprocal reflectance ρ_{NIR}^{-1} allowed to significantly decrease the effect of α_0 on the accuracy of absorption coefficient retrieval (Gitelson et al., 2003). The final step in model development was decreasing the effects of leaf scattering coefficient β variability on α_{pig} . NIR reflectance, which is closely related to leaf scattering, was introduced in Eq. 2 and it decreased, if not eliminate, the effect of β on α_{pig} retrieval. Thus, the absorption coefficient (α_λ) of leaves and canopies at wavelengths (λ) throughout the visible and red edge regions was suggested in the form (Gitelson et al., 2003):

$$\alpha_\lambda \propto [(\rho_\lambda)^{-1} - (\rho_{NIR})^{-1}] \times \rho_{NIR} = (\rho_{NIR}/\rho_\lambda) - 1 \quad (3)$$

This allowed the development of simple, accurate and generic (i.e., applicable across species with contrasting leaf pigment contents and compositions, leaf structures, developmental stages, and under different stress levels) procedures for the quantification of Chl, Car, and AnC from leaf reflectance spectra (Gitelson and Solovchenko, 2017, 2018; Gitelson et al., 2003, 2006; Gitelson et al., 2001, 2002).

While Eq. 3 is useful for obtaining the overall foliar absorption coefficient in spectral region 400–750 nm, it is challenging to use it for isolating individual pigment effects, given that the absorption of two or more pigments or pigment groups overlap. For instance, the spectra of specific absorption coefficients of Chl (α_{chl}^*), Car (α_{car}^*), and AnC (α_{AnC}^*) obtained using PROSPECT-D, a leaf optical properties model (Féret et al., 2017), shows that Chl absorb throughout the PAR region with two wide peaks centered at 430 nm and 670 nm (Fig. 1A). AnC absorb between 400 nm and 650 nm, with a wide peak centered at 540–550 nm, thus overlapping with those of Chl (Fig. 1A). Car absorb between 400 and 550 nm, therefore their absorption overlaps those by Chl and AnC, although exhibiting significantly higher specific absorption coefficients (Fig. 1A). Furthermore, absorption coefficient spectra (α_λ) of maple leaves calculated by Eq. 3 using leaf reflectance, exhibiting a wide variability of pigment contents and compositions (Table 1S, Fig. 1B), show the challenge of isolating individual pigment effects from the overall foliar absorption coefficient in the 400–650 nm spectral region where the absorption of two or more pigment groups overlap.

To quantify the absorption coefficient spectra of individual pigment groups and to assess the contribution of each group to the total leaf absorption coefficient, one needs to find the response of α_λ to variation in the content of the corresponding pigment group. Similar to a sensitivity metric developed to assess the signal-to-noise ratio (Viña, 2004; Viña and Gitelson, 2005), here we use a response function, $R(P)$, defined as the ratio of the first derivative of the best-fit function relating α_λ to the content of the pigment of interest $[P]$ and the normalized root mean square error (NRMSE) of this relationship (Gitelson and Solovchenko, 2018):

$$R(P) = \{d\alpha_\lambda/d[P]\}/NRMSE\{\alpha_\lambda \text{ vs. } [P]\} \quad (4)$$

The magnitude of $R(P)$ shows the sensitivity of α_λ to changes in $[P]$. This function also reveals the efficiency of absorbing light *in situ* by the pigment of interest, and how close the α_λ vs. $[P]$ relationship is to

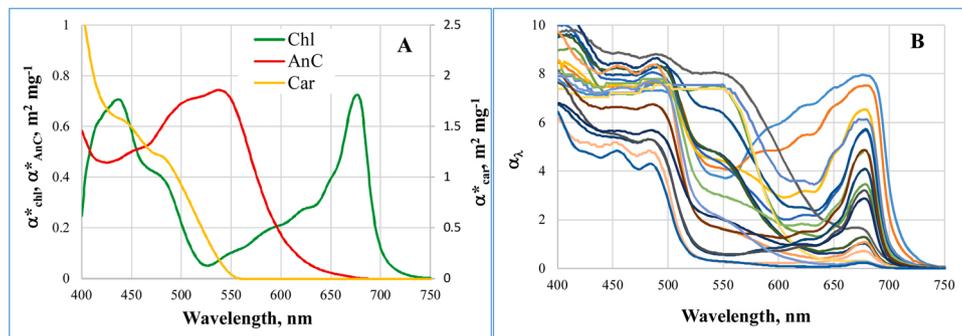


Fig. 1. (A) Specific absorption coefficient of chlorophylls (α_{chl}^*), carotenoids (α_{car}^*) and anthocyanins (α_{AnC}^*) modeled using PROSPECT-D (Féret et al., 2017). (B) Absorption coefficient spectra of maple leaves with varying contents of Chl, Car, and AnC.

linearity. Notice that the higher the response function, the closer α_λ is to [P] (i.e., higher $d\alpha_\lambda/d[P]$ and/or smaller NRMSE).

3. Materials and methods

3.1. Plant material and pigment analysis

This study used previously collected datasets (Gitelson et al., 1998, 2003; Gitelson and Merzlyak, 1998; Gitelson et al., 2001, 2002; Solovchenko et al., 2017). Briefly, these datasets contained reflectance spectra and corresponding pigment contents of juvenile, mature, and senescent AnC-free and AnC-containing leaves of Norway maple (*Acer platanoides* L.), Siberian dogwood (*Cornus alba* L. (*Swida alba* (L.) Opiz.), and horse chestnut (*Aesculus hippocastanum* L.), as well as of second-flush beech (*Fagus sylvatica* L.) leaves. Measured leaves were visually selected according to color differences and absence of damage. A more detailed description of these datasets is presented in the Supplementary Material (Tables S1–S5). Leaf pigment content was determined spectrophotometrically following procedures described in earlier studies (Gitelson et al., 2001; Merzlyak et al., 2008; Steele et al., 2009).

3.2. Measurements of leaf reflectance and transmittance

Hemispherical adaxial leaf reflectance spectra were recorded using spectrophotometers [a 150–20 Hitachi, Japan for measurements of maple, dogwood, and chestnut leaves (Gitelson et al., 2001; Merzlyak et al., 2008), and a 2101 PC, Shimadzu, Japan for measurements of beech leaves (Gitelson and Merzlyak, 1998)], equipped with integrating spheres of the corresponding manufacturer. Leaf reflectance spectra were recorded against barium sulphate as a standard and using black velvet as a background. The data were sampled at 2 nm intervals in the 400–800 nm spectral range.

4. Results

4.1. Responses of the absorption coefficients of chlorophylls, carotenoids and anthocyanins to pigment content

Fig. 2 shows the spectral responses of chlorophylls, R(Chl) and anthocyanins, R(AnC) in two species, maple and dogwood, with different pigment contents and compositions (Tables S1 and S2). Maple leaves have almost twice as high median chlorophyll contents [Chl], and smaller carotenoid [Car] and anthocyanin [AnC] contents. An example of the effects of different Chl contents can be seen in the main spectral features of R(Chl) in maple and dogwood leaves (Fig. 2). In maple leaves, (a) two peaks (around 630 nm and 700 nm) both located 30–40 nm away from the red peak of the specific absorption coefficient of Chl *in situ*, α_{chl}^* (see Fig. 1A and Feret et al. 2017), and (b) minimal R(Chl) values in the red range around 670 nm and in the blue range around 400–500 nm where α_{chl}^* peaks (Fig. 1A). In contrast, in dogwood leaves R(Chl) peaks in the range around 670 nm which is closer to the range of maximal α_{chl}^* values (Fig. 2B). Due to the absorption of AnC in the green and blue spectral ranges, R(Chl) in both species decreases at wavelengths below 600 nm; in this range R(Chl) depends significantly on pigment composition (e.g., on the ratio [AnC]/[Chl]). Noteworthy, even in AnC-rich dogwood leaves (average [AnC] = 87 mg m⁻²) the magnitude of R(Chl) at 570 nm reaches 15 % that of R(AnC); thus Chl contribution to total absorption remains significant.

To test how closely α relates to pigment content, we compared α_{chl} to destructively measured [Chl], [Car] and [AnC]. Among the pigment groups evaluated, Chl is the only absorber functioning in the spectral range above 630 nm (Fig. 1A). The NRMSE of the α_{chl} vs. [Chl] relationship in maple leaves was below 35 % in the 620–720 nm spectral range (Fig. 3A). In dogwood leaves, α_{chl} in the range 630–700 nm related closer to [Chl], with a NRMSE below 20 % (Fig. 3B). In maple leaves featuring a higher [Chl] than dogwood (57 vs. 32 mg m⁻²), NRMSE in the red absorption band of Chl (around 675 nm) increased significantly.

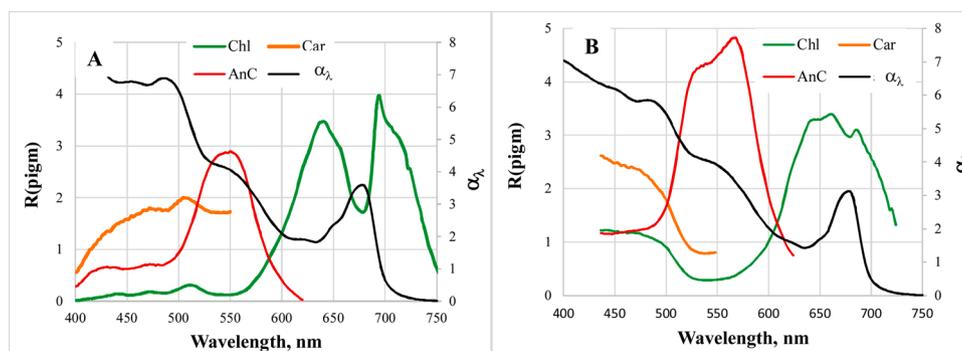


Fig. 2. (A) Spectral responses of absorption coefficient α_λ to chlorophyll, R(Chl), anthocyanin, R(AnC), and carotenoid contents R(Car) in maple (A) and dogwood (B) leaves. Absorption coefficients α_λ for each data set plotted as black solid lines.

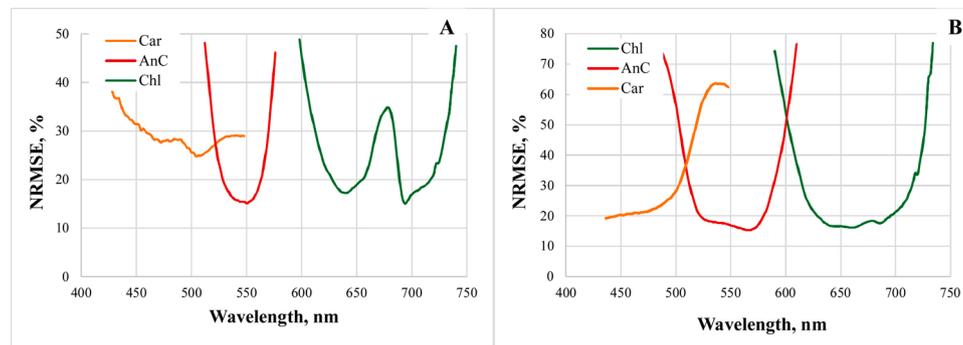


Fig. 3. Normalized root mean square error (NRMSE) indicative of the degree of deviation from linearity of the relationship between foliar pigment absorption coefficient and pigment content in maple (A) and dogwood (B) leaves. The absorption coefficients were retrieved from reflectance spectra (α_{Chl} and α_{Car}) were calculated using Eq. 3, while $\alpha_{\text{AnC}} = \alpha_{\lambda} - \alpha_{700}$; [Chl], [Car], and [AnC] were measured destructively.

Thus, the relationship between α_{chl} and [Chl] significantly depends on the pigment content even in spectral regions where only Chl absorb.

Both α_{AnC}^* and $R(\text{AnC})$ exhibit a peak in the green spectral region around 550 nm (Figs. 1 and 2, respectively). The NRMSE spectra of the α_{AnC} vs. [AnC] relationship (Fig. 3) corresponds to the spectra of $R(\text{AnC})$ (Fig. 2). In both maple and dogwood leaves the α_{AnC} vs. [AnC] relationship was significant (i.e., minimal NRMSE) in the green spectral region around 550 nm where maximal values of $R(\text{AnC})$ were found. However, in the green spectral region leaf absorption is impacted by Chl (Buschmann and Nagel, 1993); thus $R(\text{Chl})$ and $R(\text{AnC})$ depend on the fraction of [Chl] to total pigments. The effects of Chl and AnC to the overall leaf absorption is especially conspicuous at early stages of foliar senescence or stress, when $[\text{AnC}] \leq [\text{Chl}]$. Thus, the main challenge for an accurate retrieval of α_{AnC} is to quantify α_{chl} in the green region, which can then be subtracted to assign the remainder to α_{AnC} .

For quantification of the contribution of Chl to light absorption in the green spectral region, a fundamental spectral feature of foliar reflectance was used: reciprocal reflectance at 550 and 700 nm are closely related to [Chl] in AnC-free leaves, while also exhibit similar magnitudes (i.e., $\rho^{-1}_{550} \approx \rho^{-1}_{700}$) (Gitelson et al., 2003, 2001). We tested whether this feature is equally valid for the reflectance-derived absorption coefficient and found close linear relationships α_{550} vs. α_{700} , α_{550} vs. [Chl] and α_{700} vs. [Chl] (Figs. S1A and S1B). We also found that in both AnC-free and AnC-containing leaves, the α_{700} vs. [Chl] relationship was linear and with low dispersion from the regression line (determination coefficient $R^2 > 0.99$; Fig. S1C). While α_{700} depends solely on [Chl], α_{550} is affected by both [Chl] and [AnC]. Therefore, α_{550} represents the superposition of the absorption coefficients of Chl and AnC. Thus, the subtraction of α_{700} from α_{550} allows an accurate retrieval of α_{AnC} , with NRMSE below 20% (Fig. 3).

It is important to underline that the close values of α_{chl} at 550 nm and 700 nm in AnC-free leaves (Fig. S1A), together with the close linear relationship between α_{700} and [Chl] in both AnC-free and AnC-containing leaves (Fig. S1C) allow an accurate quantification of α_{chl} at 550 nm using the corresponding α_{chl} value at 700 nm. Thus, in addition to α_{chl} retrieval in the spectral range above 600 nm, we have found a procedure to accurately estimate the fraction of α_{chl} at 550 nm where both AnC and Chl absorb. While the fraction of α_{AnC} is a measure of the light-blocking efficiency of AnC, the fraction of α_{chl} in this spectral region characterizes the potential energy available for photosynthesis in AnC-containing leaves.

Regarding carotenoids, $R(\text{Car})$ overlaps with $R(\text{Chl})$ and $R(\text{AnC})$ at wavelengths shorter than 550 nm (Fig. 2), but $R(\text{Chl})$ is conspicuously smaller than $R(\text{Car})$ and $R(\text{AnC})$, which are then the pigment groups exerting a dominant contribution to foliar light absorption in the 480–550 nm spectral range. $R(\text{AnC}) \approx R(\text{Car})$ in the 500–520 nm spectral range (Fig. 2). The spectral position of the intersection point between $R(\text{AnC})$ and $R(\text{Car})$ depends on [AnC]; e.g., it shifts to shorter wavelengths in red leaves, as in dogwood leaves with higher [AnC]

(Fig. 3B). Thus, in this spectral range the effects of all three pigment groups remain significant. In addition, although it is outside the scope of this study, the potential effects of UV-absorbing phenolic pigments in this spectral region should also be considered, given that their absorption may significantly tail into the blue and blue-violet regions of the electromagnetic spectrum (Gitelson and Solovchenko, 2018).

The complexity of three-pigment interaction is one of the main reasons for the high uncertainties in the estimation of [Car] using non-destructive techniques, including radiative transfer (Féret et al., 2011; Hernández-Clemente et al., 2012), semi-analytical (Gitelson et al., 2006, 2002), and empirical (Fassnacht et al., 2015; Kira et al., 2015) models. However, the spectral responses of these three pigment groups (i.e., Chl, AnC, Car) in the green edge spectral region (where their absorptions overlap) brings useful information about their interactions. Our results obtained in two taxonomically and ecologically distant plant species show that $R(\text{AnC})$ and $R(\text{Car})$ exhibit similar values within a narrow range around 500–520 nm (Fig. 2A and 2B). At wavelengths shorter than 500 nm, $R(\text{Car})$ is higher than $R(\text{AnC})$ and $R(\text{Chl})$. Thus, α_{Car} plays the main role in leaf absorption in the range 480–500 nm. However, even in this spectral region, the absorption by Car is not the only factor controlling the overall leaf absorption, since responses of $R(\text{Chl})$ and $R(\text{AnC})$ are non-negligible reaching 30% of $R(\text{Car})$ (Fig. 3). Subtraction of the effects of AnC and Chl in this range is challenging, since they are species-specific and depend not only on the absorption coefficient fractions of Car, Chl, and AnC, but also on [Car].

Despite these challenges, the approach based on spectral responses of α_{λ} to each pigment group, made possible a breakthrough in α_{Car} retrieval, with NRMSE below 28% in maple and 24% in dogwood leaves (Fig. 3), and also estimating [Car] in AnC-containing leaves (Fig. S2B), a feat that had not been previously obtained.

4.2. Responses of the absorption coefficients of chlorophylls and carotenoids to pigment content in anthocyanin-free leaves

Three datasets obtained from AnC-free leaves (beech, maple and chestnut) with contrasting pigment contents and compositions exhibited spectral response features to [Chl] as those shown for maple leaves in Fig. 4A. The $R(\text{Chl})$ has two distinct peaks in the green and the red edge spectral regions, as well as a trough centered at around 675 nm (Fig. 4A). Notably, $R(\text{Chl})$ peaks at wavelengths located far from spectral regions of maximal Chl absorption and have minimal values in the blue and red absorption bands of Chl (see α_{λ}^* presented in Fig. 1A).

To test the linearity of α_{chl} vs. [Chl] relationships, we compared α_{chl} to destructively measured [Chl]. Our results show that in the broad spectral range between 500 nm and 750 nm α_{chl} was reasonably close to [Chl], while in the green (520–650 nm) and red edge (690–750 nm) spectral ranges α_{chl} was closely related to [Chl], with NRMSE well below 20% (Fig. 4B).

Car absorb in the spectral region between 400 and 550 nm,

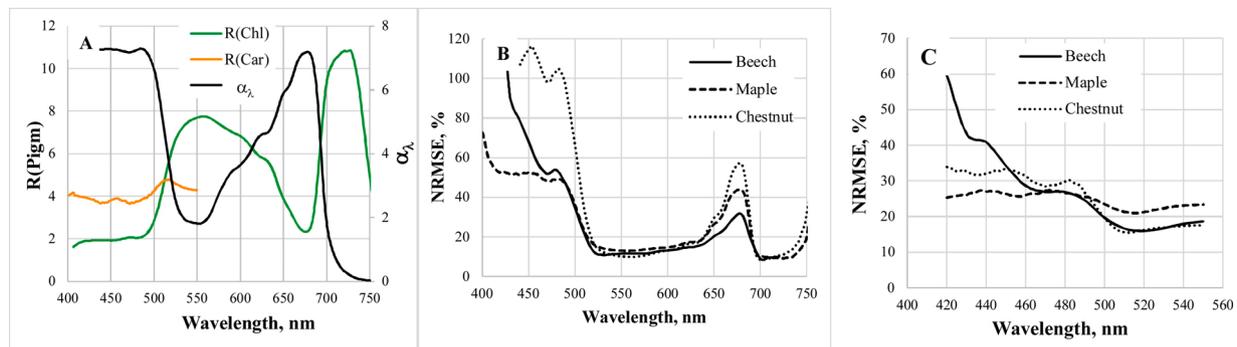


Fig. 4. (A) Response of absorption coefficient to the contents of chlorophylls, R(Chl), and carotenoids, R(Car) in AnC-free maple leaves and spectrum of absorption coefficient α_λ for the maple data set. Normalized root mean square error (NRMSE) of the linear (B) α_{chl} vs. [Chl] and (C) α_{car} vs. [Car] relationships in AnC-free beech, maple, and chestnut leaves. The absorption coefficients of Chl and Car were retrieved from reflectance spectra, while [Chl] and [Car] were obtained destructively.

overlapping with the absorptions of Chl and AnC (Fig. 1A). Similarly, the spectrum of R(Car) overlapped with that of R(Chl). In three taxonomically and ecologically distant species, R(Chl) and R(Car) exhibit similar values within a narrow range around 515 nm (Figs. S3). At wavelengths shorter than 510 nm, R(Car) is higher than R(Chl), though in this spectral region as in AnC-containing leaves, the absorption by Car is not the only factor controlling the overall leaf absorption, since response to Chl is non-negligible reaching 25–30 % of R(Car), Fig. S3. Nevertheless, the spectral responses of α_λ to each pigment content made possible the retrieval of α_{car} with reasonable accuracy. In the range 480–500 nm, the α_{car} relates to [Car] with NRMSE below 24 % in maple and beech, and below 30 % in chestnut (Fig. 4C). Notably, the slopes of the relationship of $\alpha_{480-500}$ vs. [Car] are close in AnC-free and AnC-containing leaves of four species, but beech (Fig. S2).

5. Discussion

The response of α_λ to the changes in pigment content demonstrates the complexity of the leaf structure and the difference in optical properties between a leaf and an ideal K-M system (i.e., infinitely thick layer of a highly reflective medium with uniformly distributed absorbing particles). Leaf structure is more complex, and the concentration of pigments in the chloroplasts as well as the diffusive nature of plant tissues significantly affect the efficiency of light capture (e.g., Terashima et al., 2009; Falcioni et al., 2020; Xiao et al., 2016). In spectral regions exhibiting strong pigment absorption (i.e., high α^*), a leaf in K-M terms constitutes a “strong absorber”, since the incident radiation rapidly gets absorbed in the surface cell layers of the leaf (i.e., within the first layers of the mesophyll below the epidermis), while the inner layers of the mesophyll are shaded. As a result, the condition of independent light absorption by pigment molecules contained in the leaf is not fulfilled. Consequently, the optical properties of the leaf in the spectral range in question depart from those postulated by the K-M theory, giving rise to non-linearity in the α_λ vs. [P] relationship and declining α_λ response. By contrast, in spectral regions with lower α^* , light is less attenuated by the near-surface cell layers and penetrates deeper into the leaf, the so-called sieve effect (Terashima et al., 2009), ensuring a better correspondence with a K-M medium. The diffusive nature of leaf tissues increases the light path length (détour effect) and the probability for light to encounter chloroplasts, leading to an increase in absorption (Vogelmann and Han, 2000).

In this case, the pigment molecules absorb radiation more independently from each other so that the α_λ vs. [P] relationship becomes closer to linearity. In such regions the leaf, in terms of the K-M theory, corresponds to a “weak absorber” that is more efficient in capturing light than a “strong absorber” (Gitelson et al., 2003). The spectral ranges with high response to [P] correspond to an α^* that is low enough to avoid saturation of the α_λ vs. [P] relationship but still high enough to keep the α_λ sensitive to [P].

Equally important, the NRMSE should be, in the context of this analysis, understood as a measure of the departure of the α_λ vs. [P] relationship from linearity and *not* as a metrological error of the [P] assay.

In both AnC-free and AnC-containing leaves, a decline in the α_λ response to [Chl] was observed in the blue and red spectral ranges where *in situ* Chl absorption peaks (Figs. 2A and 4A). This occurred because of a high α^*_{chl} in these spectral regions (Fig. 1A) causing saturation of the relationship between reflectance vs. [Chl] when [Chl] exceeds 100 mg m^{-2} . Such outcome occurs frequently, even in leaves with low [Chl] (Buschmann and Nagel, 1993; Gitelson and Merzlyak, 1994). In contrast, in the green-yellow and red edge spectral regions where α^*_{chl} is smaller (below 15 % that at 450 and 675 nm), the probability of light absorption in the upper layers of the mesophyll decreases, hence increasing the depth of light penetration into the leaf; e.g., α^*_{chl} at 700 nm, corresponding to the maximal response of α_λ to [Chl], is below 13 % of α^*_{675} where the response is minimal (Fig. 1A). Such decrease in α^*_{chl} causes more than a 3.5-fold increase in the response of α_λ to [Chl] and more than a seven-fold increase in the depth of light penetration into the leaf, estimated roughly as $\alpha^*_{670}/\alpha^*_{700}$ (Merzlyak and Gitelson, 1995). Therefore, the reflectance vs. [Chl] relationship becomes close to hyperbolic so that the α_λ vs. [Chl] relationship becomes linear (Gitelson and Merzlyak, 1994).

In spectral regions with strongly absorbed light (i.e., red or blue), α_λ slightly increases due to the détour effect and significantly decreases by the shading/sieve effect. For green-yellow and red edge light, shading is significantly smaller and the increase in α_λ by the détour effect is large. Therefore, the response to [Chl] in green-yellow and red edge spectral regions is high and significantly smaller in the blue and red.

The response function may also be considered as a measure of self-shading of the pigment molecules within the leaf and, thus, interpreted as a measure of “light-capture efficiency” of the pigments contained in the leaf. In the case of photosynthetic pigments, although both the light absorption and the rate of photosynthesis may be high, only a small fraction of the pigments contained in high amounts actually drives photosynthesis. Following this line of reasoning, one can better understand the widely known weak relationships between foliar [Chl] and photosynthesis rate, particularly in leaves exhibiting high Chl content. This concept is clearly illustrated by a decline in $\alpha_{\text{chl}}/[\text{Chl}]$, and hence in Chl light-capture efficiency along with an increase in [Chl] in beech and maple leaves (Fig. 5). In beech leaves with [Chl] around 600 mg m^{-2} the decrease in $\alpha_{\text{chl}}/[\text{Chl}]$ at 670 nm was more than 7-fold, while in maple leaves with maximal [Chl] = 470 mg m^{-2} it declined more than 3-fold (Fig. S5).

However, other factors affecting light harvesting should not only be taken into account but also compensated for in estimations of pigment efficiency, such as the accumulation of photoprotective pigments intercepting light, so it cannot reach Chl, as well as non-photochemical quenching. As an illustrious example, comparative studies of common

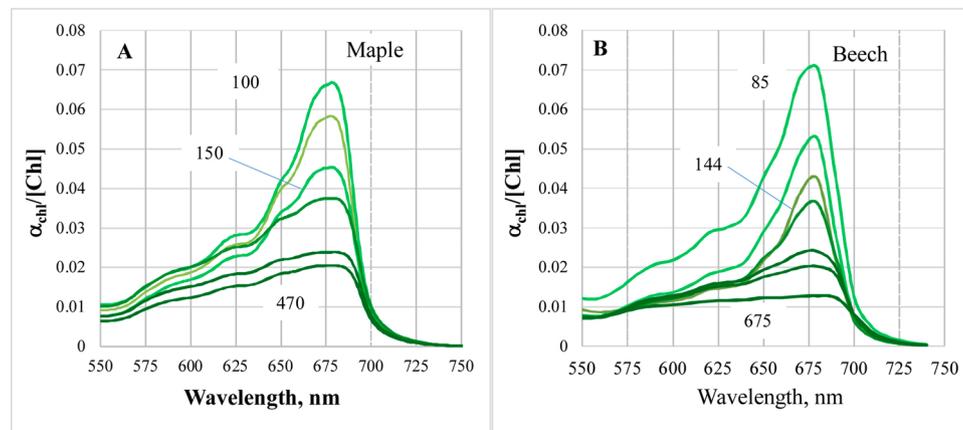


Fig. 5. Spectra of absorption coefficient per unit of Chl content, $\alpha_{chl}/[Chl]$, in AnC-containing maple (A) and AnC-free beech (B) leaves with varying $[Chl]$. With increases in $[Chl]$, $\alpha_{chl}/[Chl]$ sharply decreases especially in the range of high Chl-specific absorption coefficient, α^*_λ . In leaves with $[Chl]$ above 150 mg/m^2 , Chl efficiency is significantly lower than in leaves with $[Chl]$ below 150 mg/m^2 .

hazel juvenile leaves featuring close $[Chl]$ but contrasting in their $[AnC]$ showed a drastic difference in the rate of the linear electron transport (ETR) in the chloroplast electron transport chain (Solovchenko and Chivkunova, 2011). ETR was closely correlated with the $[AnC]$ ($R^2 = 0.87$). In red leaves, the saturation of ETR sensitivity to irradiance was observed at higher values of PAR than in green leaves. There were no differences between red and green leaves in the level of non-photochemical quenching, the content of violaxanthin cycle pigments, a degree of their de-epoxidation under natural illumination and at irradiation with high PAR fluxes unequivocally supporting the photoprotective role of AnC.

Obviously, the assessments of high-light resilience in plants should be done with knowledge of light screening efficiency of the photoprotective pigments in mind, in addition to other possible limitations e. g., CO_2 availability. The approach developed in this study is an appropriate tool for this. Thus, the absorption coefficient deduced from reflectance can serve as a weighing factor utilized for comparison of high light irradiation effects between some experimental treatments. And, likewise, the experimental models for assessing the high light-protective effect of any screening pigment should be ‘equalized’ in terms of light-capture efficiency of photosynthetic pigments, Chl and primary Car.

In our view, the interpretation of the interactions of radiation with AnC-containing leaves, specifically the spectral behavior of α_{chl} , can give valuable insights into the photoprotective properties of AnC and the trade-offs between light attenuation for photoprotection and light absorption for photosynthesis. Photoprotection by AnC is often considered costly for plants, since the active production and accumulation of AnC requires biochemical energy, while the anthocyanic light screening cuts down the radiant energy available for photosynthesis.

We compared the fraction of PAR absorbed by Chl (f_{APAR}) in AnC-free and AnC-containing leaves with equal $[Chl]$ using typical composition of AnC and Chl - leaves with rather low $[Chl]$ and high $[AnC]$ (Fig S4A) and high $[Chl]$ and smaller $[AnC]$ (Fig S4B). These two examples clearly show the effect of screening on the fraction of PAR absorbed by Chl. The effect of AnC screening is mostly pronounced in the green range 500–600 nm. In the case of high $[AnC]$ and low $[Chl]$ (Fig. S4A), transmittance of the AnC ‘filter’ in the green range is about 20 % and the fraction of light absorbed by Chl is around 87 % of light absorbed by AnC-free leaf. In the leaf with a high $[Chl]$ and a low $[AnC]$ (Fig. S4B), transmittance of the AnC ‘filter’ is around 50 %, and the fraction of light absorbed by Chl is above 91 % of light captured by the AnC-free leaf. Thus, this analysis suggests that the total potential efficiency of Chl in AnC-containing leaves is around 90 % and commensurate to that in AnC-free leaves. From this, one can draw an important conclusion that the shielding of leaves with AnC does not necessarily entail “energy

starvation” due to insufficient light capture by Chl within the same leaves.

Figs. 2 and 4A illustrate what new information the response spectra bring, in addition to the information obtained from simulated specific absorption coefficients of pigments, α^*_{chl} , α^*_{car} , α^*_{AnC} (Fig. 1A). For instance, high values of α^*_{chl} in the main absorption bands of Chl, the blue and the red, do not mean a high efficiency of Chl absorption (Fig. 5 and S5), since for the examined datasets, α_λ vs. $[Chl]$ relationships in the blue and red spectral regions are essentially non-linear and $R(Chl)$ is small. The high $R(Chl)$ in the green and red edge spectral regions, also indicated high efficiency of Chl in spectral ranges not coinciding with the maximal α^*_{chl} (i.e., the retrieved α_λ relates linearly to $[Chl]$, see also Fig. 4B).

Both pigment content and composition affects α_λ response to pigment content. The spectra of $R(AnC)$ (Fig. 2) and of α^*_{AnC} (Fig. 1) further demonstrate the difference between these two parameters (Fig. S6 shows these two parameters in the same graph). In the range 530–600 nm, the shapes of the spectral curves of the α_λ response and of α^*_{AnC} are quite similar, but the peak of α_λ response is narrower than that of α^*_{AnC} . At shorter wavelengths, while $R(AnC)$ declines four-fold, α^*_{AnC} also decreases but at a lower degree. The sharp decline in $R(AnC)$ is due to interferences from Car and Chl, which also absorb in this spectral range. Thus, in contrast to the specific absorption coefficient, α^* , the spectral response curve shows the contribution of each pigment (exemplified here by AnC) to the total absorption coefficient of leaves.

One may note that the shape of α^*_{AnC} (Fig. S6) resembles the typical spectra of AnC-containing leaves (Féret et al., 2017) with the superimposed tailing contribution of other phenolic pigments (Gitelson and Solovchenko, 2018). On the contrary, the spectral curve of $R(AnC)$ shows that leaf absorption coefficient is governed mainly by AnC in the 520–570 nm spectral region where the contributions of other pigments are minimal. This is also apparent from their corresponding responses (Gitelson and Solovchenko, 2018). The depression of $R(AnC)$ at wavelengths shorter than 500 nm bears a clear spectral signature of the combined absorption by Car, Chl, and AnC (and perhaps also the contribution of the tailing absorption of flavonoids responsible for screening UV light). While α^* gives an idea of the absorption spectra of pigments *in situ*, one should be aware of the difficulties in its interpretation arising from (i) overlap of the absorption spectra of different pigments, and (ii) strong correlation between the contents of different pigments, e.g., cross-correlation between the contents of Chl and Car (Gitelson and Solovchenko, 2018).

To conclude, the response of α_λ to $[P]$ brings new quantitative information about leaf-light interactions in the PAR spectral region and shows the efficiency of *in situ* light capture by different pigment groups. For photosynthetic pigments, it is indicative of the energy captured for

photosynthesis and hence of potential plant productivity. For photo-protective pigments, like AnC and secondary Car, it gives information about (i) spectral ranges where screening works best, and (ii) potential screening efficiency. This information shows (a) how pigments “work” in the leaves under consideration, (b) the spectral ranges where α_r is closely related to the content of the pigments of interest, and (c) the fractions of absorption coefficient of each pigment in the visible to the red-edge spectral range. Such knowledge is invaluable for the interpretation of the variance observed in absorption spectra and thus contributes to the development of better procedures for monitoring the phenological, physiological, and photosynthetic dynamics of plants.

CRediT authorship contribution statement

Anatoly Gitelson: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Alexei Solovchenko:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Andrés Viña:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was partly supported by Israel Institute of Technology, Technion. AS acknowledges the financial support from Russian Foundation for Basic Research (grant #19-016-00016). This work was partially supported by Moscow State University Grant for Leading Scientific Schools "Depository of the Living Systems" in frame of the MSU Development Program.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jplph.2020.153277>.

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