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Sensitivity to Foliar Anthocyanin Content of Vegetation Indices Using Green Reflectance

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***Abstract*—Anthocyanins are nonphotosynthetic water-soluble pigments associated with the resistance of plants to environmental stresses such as drought, low soil nutrients, high radiation, herbivores, and pathogens. Information on the absolute and relative amounts of anthocyanins allows evaluating the physiological conditions of plants and their responses to stress and has the potential for evaluating plant species diversity across broad geographic regions. As anthocyanins absorb radiation in the green region of the electromagnetic spectrum (with a peak of absorption at around 540–560 nm), broadband vegetation indices that use this spectral region in their formulation will exhibit sensitivity to their presence. In this letter, we evaluate the sensitivity of four broadband vegetation indices using reflectance in the green spectral region to foliar anthocyanin content. Among the indices tested, the visible atmospherically resistant vegetation index was found to be closely and linearly related to the relative amount of foliar anthocyanin across five different species (the root-mean-square error of the prediction is *ca.* 0.1, and the relative error is below 20%). While this result was obtained at leaf level, it opens new possibilities for analyzing anthocyanin content and composition across multiple scales by means of spacecraft-mounted broadband sensor systems such as Landsat Thematic Mapper, Landsat Enhanced Thematic Mapper Plus, and Moderate Resolution Imaging Spectroradiometer.**

***Index Terms*—Anthocyanin, chlorophyll, chlorophyll index, foliar pigments, green normalized difference vegetation index (gNDVI), red:green ratio, visible atmospherically resistant vegetation index (VARI).**

I. Introduction

THE AMOUNT and composition of photosynthetic and nonphotosynthetic foliar pigments vary primarily as functions of species [1], [2], developmental and phenological stages [3]–[5], and environmental stresses [6]–[8]. Information on the absolute and relative amounts of these pigments thus provides insights onto the physiological conditions of plants and their responses to stress [9], [10] and has the potential for evaluating species composition and diversity across broad geographic regions [2]. Anthocyanins (Anth), in particular, are water-soluble pigments associated with the resistance of plants to environmental stresses such as drought, low soil nutrients, high radiation, herbivores, and pathogens [7]. Therefore, identifying the presence of Anth as well as their absolute and relative amounts is crucial for many monitoring and management applications in terrestrial ecosystems [8].

While wet chemical methods have been and still are the most used for the determination of foliar pigment content, nondestructive spectroscopic techniques are preferable because they are fast and accurate and can be applied *in situ*. Thus, their use has become more widespread [9]. These techniques have the potential to be scaled up to the entire canopy and possibly to entire geographic regions using data acquired by aircraft- and spacecraft-mounted hyperspectral imaging radiometers [8], [11]. However, the availability of data acquired by such hyperspectral imaging sensors is still limited. In contrast, an enormous wealth of data acquired at multiple spatial, spectral, and temporal scales by a constellation of operational satellites carrying broadband radiometers is amply available. The drawback is that, in most cases, the spectral location and width of the broad spectral bands in these satellite sensor systems are not optimal for detecting changes of pigment contents in general and of Anth content in particular [12]. Therefore, while research on the use of data acquired by proximal and remote (i.e., aircraft- and spacecraft-mounted) hyperspectral sensors is essential (e.g., [2]), it is also imperative to develop and test suitable algorithms for the remote estimation of pigment content and composition to be used with past and current operational broadband satellite sensor systems.

Spectral vegetation indices are mathematical combinations of different spectral bands. Their purpose is to enhance the information contained in spectral reflectance data, such as foliar pigment content, and to minimize confounding effects due to complex scattering patterns induced by the structure of leaves and canopies, as well as other sources of noise, such as background, atmospheric, and sun–target-sensor geometry effects [13]. The most common spectral bands used to develop vegetation indices are located in the red region where chlorophyll (Chl) absorbs (*in vivo* around 670 nm) and in the near-infrared (NIR) region (750–900 nm) where vegetation reflects highly due to leaf cellular structure [14]. Because the reflectance in the red region exhibits saturation under conditions of intermediate-to-high Chl contents, other vegetation indices that use different bands of the electromagnetic spectrum, particularly in the green- and the red-edge regions, have also been developed for the nondestructive estimation of foliar and canopy Chl content [15]–[18]. However, as Anth absorb radiation primarily in the green spectral range around 540–560 nm, broadband

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vegetation indices that use this spectral region will respond to their presence.

In this study, we evaluated the sensitivity of four vegetation indices that use the green spectral region in their formulation to foliar Anth content. These indices were chosen not only because they can be easily obtained from reflectance data acquired by current operational broadband satellite sensor systems (e.g., Moderate Resolution Imaging Spectroradiometer (MODIS), Landsat Thematic Mapper, and Landsat Enhanced Thematic Mapper Plus) but also because they have the potential to be used for the remote estimation of Anth content. While this study only evaluated index sensitivity to Anth content at the leaf level, this constitutes a fundamental step toward the development of procedures for the remote estimation of Anth content not only in leaves but also in entire canopies.

II. Methods

This study used data sets of five species acquired in different studies to assess foliar pigment composition through reflectance spectroscopy [12], [19]–[21]. While there is some variation among data sets because the studies were performed at different times and in different places, the techniques employed constitute standard procedures. The leaves studied include Norway maple (*Acer platanoides* L.; $n = 78$), horse chestnut (*Aesculus hippocastanum* L.; $n = 42$), beech (*Fagus sylvatica* L.; $n = 38$), Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.; $n = 74$), and dogwood (*Cornus alba* L.; $n = 23$). Information on these leaves, as well as specific details of the procedures for pigment extraction and spectral reflectance measurements, is given in [12] and [19]–[21]. To summarize, one or two disks (1.6 cm in diameter) were cut from the leaves and grounded with mortar and pestle in ca. 5–8 mL of methanol with $CaCO_3$ to prevent Chl pheophytization. Homogenates were centrifuged for 3–4 min at 3000 g. The resulting extracts were immediately assayed spectrophotometrically using specific absorption coefficients of chlorophyll-a, chlorophyll-b, total carotenoids (Car) [22], and total Anth [23]. For the determination of Anth content, the extract was acidified with concentrated HCl. Pigment content was expressed on a leaf area basis (i.e., mg/m^2).

Adaxial spectral reflectance measurements were obtained using the following: 1) a 150-20 Hitachi (Japan) spectrophotometer equipped with a 150-mm-diameter integrating sphere attachment for maple, chestnut, and creeper leaves—measurements were carried out in Russia; 2) a Shimadzu spectrophotometer for beech leaves—measurements were carried out in Germany; and 3) an Ocean Optics 2000 radiometer attached to a leaf clip for dogwood leaves—measurements were carried out in the U.S. Spectral reflectance factors (ρ) were obtained by normalizing the upwelling reflected radiation from the leaves by that from a diffuse reflectance standard (i.e., barium sulphate). The data were sampled at 2-nm intervals in the 350–800-nm range. Using ρ integrated over broad spectral bands in the blue (460–480 nm), green (540-560 nm), red (620-680 nm), and NIR (750–800 nm) spectral regions, we calculated four different indices, namely, the green normalized difference vegetation index (gNDVI), the green chlorophyll index (CI_g), $R{:}G_{\mathrm{ratio}}$, and the visible atmospherically resistant vegetation index (VARI), as

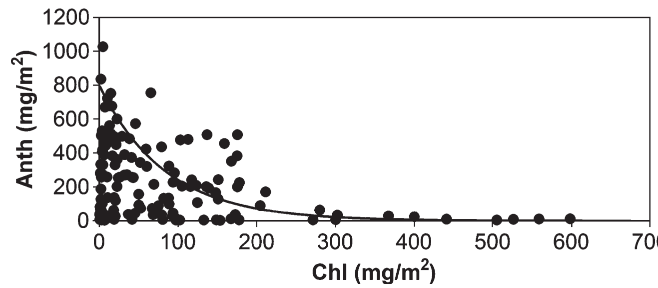

Fig. 1. Relationship between anthocyanin and total chlorophyll content (i.e., Chl-a + Chl-b) in the leaves studied.

$$gNDVI = (\rho_{\mathrm{NIR}} - \rho_{\mathrm{Green}})/(\rho_{\mathrm{NIR}} + \rho_{\mathrm{Green}}) \quad (1)$$

$$CI_g = (\rho_{\mathrm{NIR}}/\rho_{\mathrm{Green}}) - 1 \quad (2)$$

$$R{:}G_{\mathrm{ratio}} = (\rho_{\mathrm{Red}}/\rho_{\mathrm{Green}}) \quad (3)$$

$$VARI = (\rho_{\mathrm{Green}} - \rho_{\mathrm{Red}})/(\rho_{\mathrm{Green}} + \rho_{\mathrm{Red}} - \rho_{\mathrm{Blue}}). \quad (4)$$

The broad spectral bands used in this study are intended to be "generic" thus, they do not specifically match any particular broad spectral band on current operational satellite sensor systems (particularly the NIR). The gNDVI was found to be sensitive to changes in Chl content and was proposed as an alternative index to be used in MODIS onboard the National Aeronautics and Space Administration's Terra and Aqua satellites [24]. CI_g is based on a conceptual model developed at the leaf level [25] and has been shown to linearly respond to the amount of Chl not only in leaves [26], [27] but also in crop canopies [17]. $R{:}G_{\mathrm{ratio}}$, which compares the reflectance in the red region (where Chl absorption occurs) to the reflectance in the green region (where absorption of both Chl and Anth occurs), was proposed as a surrogate of foliar anthocyanin content [28]. The VARI constitutes a proxy of green canopy cover [16] and has been shown to be useful for detecting changes associated with crop phenology [5], relative greenness [29], live fuel moisture [30], and water stress [31]. These indices were related with absolute and relative foliar Anth contents, expressed as the ratio of Anth content to the total pigment content (i.e., the sum of the contents of chlorophyll-a, chlorophyll-b, carotenoids, and anthocyanins).

III. Results and Discussion

Anth content tends to exhibit an exponential decay with increases in Chl content (Fig. 1). At Chl content above 300 mg/m^2, foliar Anth drops below 20% of the total pigment content, and the leaves are Chl dominated (Fig. 1). Importantly, however, there is a substantial variability in foliar Anth content that is not directly related with the amount of foliar Chl content. Therefore, the contents of both pigments seem to be partially independent, particularly for Chl below 300 mg/m^2. This has an important negative effect on the nondestructive estimation of Chl content by means of vegetation indices, such as the gNDVI and CI_g, which use the green region of the electromagnetic spectrum. In Anth-free leaves, both indices showed

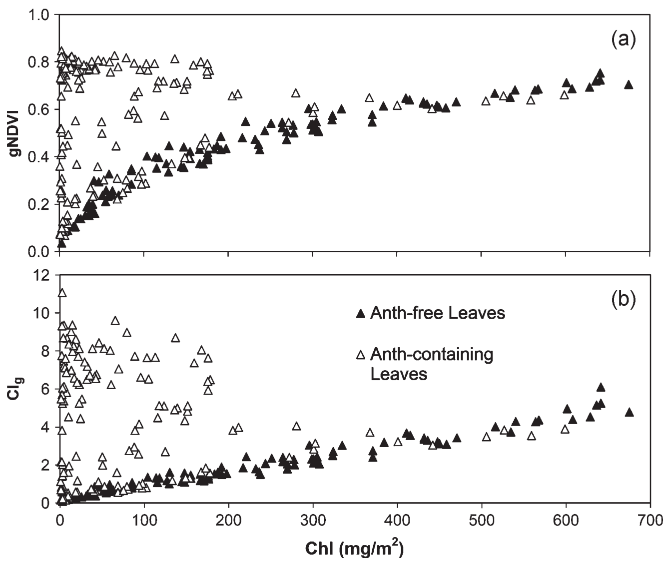

Fig. 2. Relationships of (a) gNDVI and (b) CI_g versus total chlorophyll content (i.e., Chl-a + Chl-b) for anthocyanin-free (i.e., Anth content below 3.0 mg/m^2) and anthocyanin-containing leaves.

a high sensitivity to changes in Chl content along the entire range of Chl measured, with gNDVI exhibiting a nonlinear relationship, while CI_g exhibited a linear relationship (Fig. 2). However, both gNDVI and CI_g also showed sensitivity to foliar Anth, as Anth-containing leaves exhibited higher gNDVI and CI_g values than Anth-free leaves with the same Chl content (Fig. 2). This sensitivity is due to Anth absorption in the green range of the electromagnetic spectrum, which decreases the green reflectance. The sensitivity of these indices to Anth is particularly pronounced at low-to-moderate Chl contents (< 300 mg/m^2; Fig. 2), when absorption in the green range tends to be dominated by Anth. In leaves with Chl $>$ 300 mg/m^2, Chl absorption is the main factor governing green reflectance, and the indices increase as Chl increases in both Anth-containing and Anth-free leaves (Fig. 2). The sensitivity of gNDVI and CI_g to Anth content reduces their usefulness as proxies of Chl content in Anth-containing vegetation.

While sensitive to the presence of Anth and exhibiting significant relationships with absolute Anth content [Fig. 3(a) and (b)], gNDVI and CI_g do not constitute suitable surrogates of absolute foliar Anth content. This is because both of these indices varied widely for the same Anth content at values below 200 mg/m^2 and became virtually insensitive to changes in Anth content at moderate-to-high Anth contents [i.e., exceeding 300 mg/m^2; Fig. 3(a) and (b)]. The other two broadband vegetation indices evaluated, $R{:}G_{\mathrm{ratio}}$ and the VARI, also have limitations for the nondestructive estimation of Anth content. While $R{:}G_{\mathrm{ratio}}$ exhibited a linear relationship and the VARI exhibited an exponential decay, both showed a drastic scattering of the points from the regression line [Fig. 3(c) and (d)]. Therefore, for the same Anth content, the index values might change two-fold or three-fold.

With respect to the relative foliar Anth content (i.e., Anth/total pigments), gNDVI and CI_g exhibited poor relationships [Fig. 4(a) and (b)]. $R{:}G_{\mathrm{ratio}}$ showed a nonlinear relationship with low sensitivity at relative Anth contents below

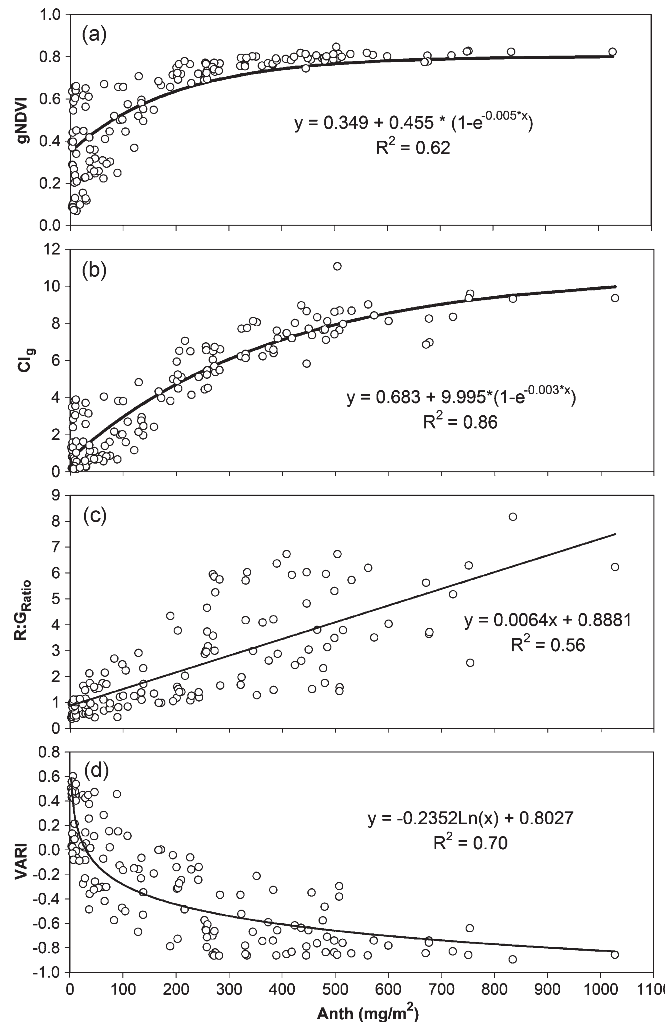

Fig. 3. Relationships of (a) gNDVI, (b) CI_g, (c) $R{:}G_{\mathrm{ratio}}$, and (d) VARI versus absolute foliar anthocyanin content. Regression lines represent best-fit functions.

0.4 and with a wide scattering of the points from the regression line at relative Anth contents above 0.5 [Fig. 4(c)]. In contrast, the relationship between VARI and the relative Anth content was linear, with a high coefficient of determination [Fig. 4(d)]. This significant linear relationship makes VARI a suitable surrogate of the relative foliar Anth content. Using species as a dummy variable, the relationship between VARI and Anth/total pigments was found to be not species specific (at least for the species evaluated) since the dummy coefficient was not significant ($p > 0.05$).

The relationship VARI versus the relative foliar Anth content was inverted in order to generate an empirical predictive model. This predictive model was validated by splitting the data set into two parts, one to be used for calibration (ca. two-thirds of the leaves) and the remainder (ca. one-third of the leaves) for validation. In order to reduce the dependence on a single random partition into calibration and validation data sets, 10 000 different random partitions were performed. Estimates (i.e., mean ±95% confidence intervals) of model coefficients (i.e., slope and intercept), the coefficient of determination, and the root-mean-square error (RMSE) were obtained from these 10 000 random partitions. The average RMSE of the relative

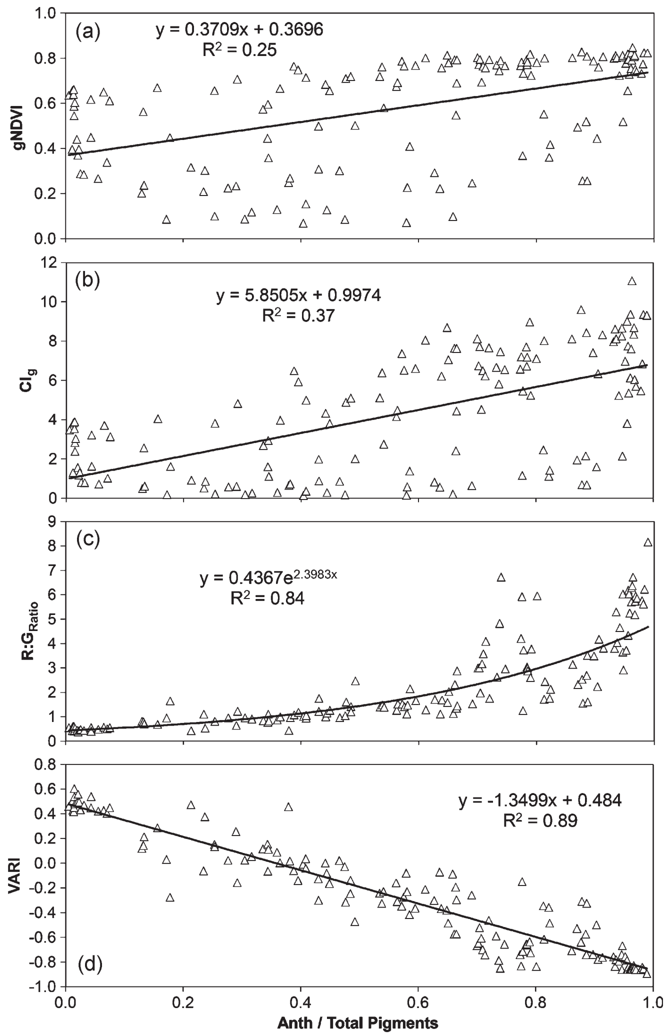

Fig. 4. Relationships of (a) gNDVI, (b) CI_g, (c) $R{:}G_{\mathrm{ratio}}$, and (d) VARI versus relative content of foliar anthocyanin (i.e., the ratio of Anth content to the total pigment content; total pigment content constitutes the sum of the contents of chlorophyll-a, chlorophyll-b, carotenoids, and anthocyanins). Regression lines represent best-fit functions.

TABLE I

ESTIMATES OF MODEL PARAMETERS OF THE LINEAR RELATIONSHIP BETWEEN THE RELATIVE CONTENT OF FOLIAR ANTHOCYANIN (I.E., ANTH/TOTAL PIGMENTS) AND VARI. MODEL PARAMETERS, AS WELL AS THE RMSE AND THE ±95% CONFIDENCE INTERVALS, WERE OBTAINED USING 10 000 RANDOM PARTITIONS OF THE DATA SET INTO CALIBRATION (2/3 OF THE LEAVES SAMPLED) AND VALIDATION (1/3 OF THE LEAVES SAMPLED)

Parameter	Value	±95% Confidence Intervals
Slope	-0.658	0.022
Intercept	0.710	0.013
R^2	0.888	0.029
RMSE	0.105	0.024

Anth content prediction was found to be ca. 0.105 (Table I), and the relative error (RMSE/mean relative Anth content) was below 20%.

IV. CONCLUSIONS

This letter has evaluated the sensitivity to foliar Anth content of some broadband vegetation indices that use spectral bands in the green region of the electromagnetic spectrum. Although gNDVI and CI_g have been suggested as proxies of foliar and canopy Chl content [17], [25], [26], they are significantly affected by the presence of foliar Anth, which reduces their usefulness as proxies of Chl content. This is important since both Anth and Chl can be present simultaneously, as Anth accumulation usually precedes Chl breakdown in senescing leaves [32]. In addition, due to its photoprotective role, Anth are produced not only during senescence but also during periods of environmental stress and in young emerging leaves [33]. However, while sensitive to the presence of Anth, these indices, as well as $R{:}G_{\mathrm{ratio}}$ (specifically suggested as a proxy of Anth content [28]) and the VARI, cannot be used for estimating absolute Anth content either. Both gNDVI and CI_g did not show significant relationships with the relative Anth content. While $R{:}G_{\mathrm{ratio}}$ showed a significant relationship with relative Anth content, it exhibited little or no sensitivity at low relative Anth content values. In contrast, the VARI exhibited a strong linear relationship with relative Anth content and was able to predict it (after model inversion) with an RMSE of *ca.* 0.1. Therefore, the VARI has potential to be used as a surrogate of the relative content of Anth.

While these results are promising, further studies are required in order to evaluate VARI as a proxy of the relative Anth content among other species, with different pigment compositions and leaf structures. In addition, the applicability of the index at canopy and regional scales, its sensitivity to background effects, and its responses through time (e.g., in different phenological stages) also need to be evaluated. With further validation, this index may well become a simple yet effective tool for the remote estimation of relative Anth content using currently operational broadband satellite sensor systems. There is still a long way to go, but the results obtained in this study are encouraging, and the potentially practical implications are worth examining.

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