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Nondestructive Estimation of Anthocyanin Content in Autumn Sugar Maple Leaves

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Abstract. **A field-portable tool for nondestructive foliar anthocyanin content estimation would be beneficial to researchers in many areas of plant science. An existing commercial chlorophyll content meter was modified to measure an index of anthocyanin content. The ability of the experimental anthocyanin meter (ACM) to estimate total extractable anthocyanin content was tested in sugar maple (*Acer saccharum* Marsh.) leaves representing several subjective color categories collected from a variety of field sites in northwestern Vermont on several dates in Autumn 2003. Overall, there was a significant linear relationship between anthocyanin content index (ACI) and total extractable anthocyanin content (r^2 = 0.872, P < 0.001). Therefore, the ACM appears to be an effective tool for estimation of relative anthocyanin content in large samples of autumn sugar maple leaves.**

Handheld chlorophyll meters have been demonstrated to be effective tools for rapid, nondestructive estimation of total chlorophyll content in a number of agricultural (Dwyer et al., 1991; Marquard and Tipton, 1987; Yamamoto et al., 2002) and forest (Cate and Perkins, 2003; Richardson et al., 2002) species. Anthocyanins are water-soluble flavonoid pigments (Strack, 1997) which often appear red in color. In addition to their transient appearance in juvenile and senescing leaves of many species, anthocyanin accumulation has been demonstrated to be influenced by environmental conditions (Sibley et al., 1999) and indicative of physiological stress (Chalker-Scott, 1999). Thus, a similar nondestructive method of anthocyanin content estimation would be extremely valuable for research in many areas of plant physiology. In addition, such an instrument would be especially useful for investigations of spring and autumn leaf phenology, for which repeated measurements of pigment changes in individual intact leaves over time have not previously been possible.

Some prior investigation into nondestructive anthocyanin estimation has been conducted. Moore (1997) was able to successfully predict anthocyanin content in red raspberry fruit using a color meter. Gitelson et al. (2001) developed an index to predict leaf anthocyanin content using reflectance values obtained with a spectroradiometer. A less expensive, compact tool for nondestructive anthocyanin estimation suitable for use in field studies is highly desirable. Thus, the objective of this study was to determine if a commercially available handheld chlorophyll meter could be modified to estimate relative anthocyanin content in autumn sugar maple (*Acer saccharum*) leaves.

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Materials and Methods

Anthocyanin meter. Previous research has demonstrated that the CCM-200 Chlorophyll Content Meter (CCM) (OptiSciences, Tyngsboro, Mass.) accurately estimates relative chlorophyll content in sugar maple leaves (Cate and Perkins, 2003; van den Berg and Perkins, 2004.). The CCM uses the ratio of optical absorbance at 655 nm to that at 940 nm to calculate an index of chlorophyll content (CCI). To produce an experimental anthocyanin meter (ACM) for this study, the 655 nm light-emitting diode (LED) of the CCM was replaced by the manufacturer with a standard 530 nm LED in order to measure absorbance near the wavelength at which the primary anthocyanin in autumn sugar maple leaves, cyanidin-3-glucoside (C3G) (Ji et al., 1992) absorbs maximally in solution (Windholz (ed.), 1983).

Plant material. Leaf collections were made on four different dates in September and October 2003 from 15 to 20 individual trees at 6 to10 sites ranging from 90 m to 400 m in elevation in the Champlain Valley and western Green Mountains in Vermont. During each collection, 10 healthy sugar maple leaves were collected from each of the following five subjective color categories: 100% green, 5% to 25% red, 26% to 50% red, 51% to 75% red, and 76% to 100% red.

For each leaf, five measurement areas, each about the same size as the measuring head of the meters, were delineated with a marking pen. Major veins and damaged areas of the leaves were avoided. Using the delineated areas as guides, five measurements per leaf were made each with the CCM and the experimental ACM.

Pigment characterization. Following CCM and ACM measurements, two 6.45-mm-diameter leaf disks were collected from each delineated leaf area for each pigment extraction, chlorophyll and anthocyanin. Disks were combined for a total of 10 disks per extracting solution. The disks were chopped into fine pieces with a razor blade before being immersed in 5 mL of the appropriate extracting solution. Chlorophyll was extracted with an 80% acetone–deionized water solution. Anthocyanins were extracted with acidified 80% methanol (Gould et al., 2000). All extractions were completed in the dark at 4 °C. Spectrophotometric analyses of the pigments were performed using a Spectronic Genesys 8 spectrophotometer (Thermo Electron Corp., Waltham, Mass.) with a 10 mm light path. Total chlorophyll (Chl) (chlorophyll $a + b$) was determined following methods and equations of Lichtenthaler and Wellburn (1983) and was expressed on a leaf area basis. Anthocyanin content (Anth) was estimated as $A_{530} - 0.24A_{653}$ following the methods of Murray and Hackett (1991) and Gould et al. (2000).

Data analysis. Data were analyzed using JMPIN software version 4.0.4 (SAS Institute, Cary, N.C.). Simple linear regression was used to test for significant correlations between anthocyanin content index (ACI) as measured with the ACM and Anth measured via extraction and spectroscopy for combined data from the overall experiment and within individual color categories.

Results and Discussion

Actual Anth and Chl content increased and decreased, respectively, in conjunction with the increasing percentage red of the subjective color categories (Table 1). Mean ACI increased with increasing Anth content, and mean CCI decreased with decreasing Chl content.

The relationship between mean ACI and extracted anthocyanin content was significantly linear when all samples collected in the experiment were analyzed together (Fig. 1). The strength of the linear relationship was marginally improved when green leaves were excluded from the analysis (Table 1). These relationships are similar to that observed between CCI and total chlorophyll in this experiment (r^2 = 0.876, P < 0.001) as well as those reported for CCI and total chlorophyll in large samples of sugar maple leaves (Cate and Perkins, 2003; van den Berg and Perkins, 2004).

Some inconsistency was observed in the strength of linear relationships when ACI versus Anth was analyzed within individual color categories. There was no significant correlation between ACI and Anth in green leaves (Table 1). However, highly significant linear relationships were detected between ACI and Anth in each of the red color categories. Though mean ACI and Anth increased in conjunction with the increasing percentage red of the color categories, the strength of the linear relationships did not increase with increasing anthocyanin content as might be expected.

The ACM was designed simply and did not subtract the interfering absorbance of

Table 1. Mean (standard error) anthocyanin content index (ACI), anthocyanin content (Anth) ($A_{530} - 0.24A_{653}$), chlorophyll content index (CCI) and total chlorophyll (Chl) ($\mu g \cdot mm^{-2}$) for sugar maple leaves from five color categories collected on four dates during Autumn 2003 in the Champlain Valley and Green Mountains of Vermont.

Category	r^2[z]	n	ACI	Anth	CCI	Chl
Combined data						
Overall experiment		200	7.79(0.29)	0.37(0.01)	4.85(0.44)	0.07(0.01)
Overall experiment—red leaves only	0.876^{***}	160	8.73(0.32)	0.44(0.02)	3.78(0.13)	0.05(0.002)
By color category						
Green	0.052^{NS}	40	4.00(0.45)	0.07(0.01)	9.12(1.44)	0.14(0.03)
5% to 25% Red	0.481^{***}	40	4.96(0.17)	0.22(0.01)	5.12(0.39)	0.08(0.01)
26% to 50% Red	0.820^{***}	40	7.05(0.53)	0.34(0.02)	4.11(0.13)	0.06(0.005)
51% to 75% Red	0.696^{***}	40	9.02(0.66)	0.48(0.01)	3.49(0.40)	0.05(0.01)
76% to 100% Red	0.571^{***}	40	13.89(0.83)	0.73(0.04)	2.38(0.28)	0.03(0.01)

[z]Correlation coefficients for regression analyses of ACI versus Anth for the complete data set with green leaves excluded and subdivided into color categories.

^{NS,***}Nonsignificant or significant at $P \leq 0.001$, respectively.

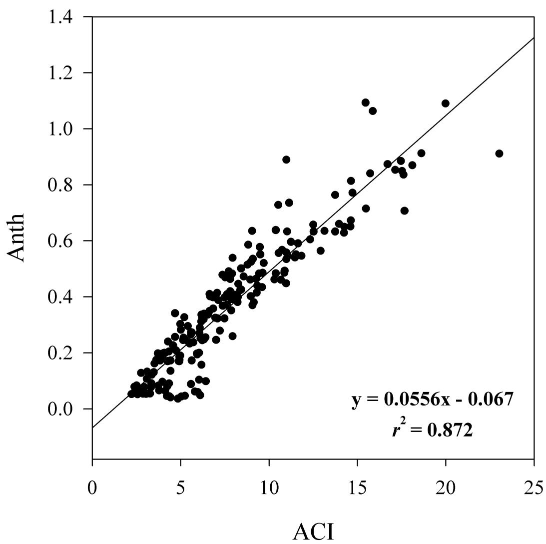

Fig. 1. Linear regression of mean anthocyanin content index (ACI) versus extracted anthocyanin content (Anth) ($A_{530} - 0.24A_{653}$) in 200 sugar maple leaves from five color categories collected on four dates during Autumn 2003 in the Champlain Valley and Green Mountains of Vermont.

chlorophyll at 530 nm (Murray and Hackett, 1991) or compensate for differences between the maximum absorbance of C3G in solution versus that in intact leaves. Gitelson et al. (2001) developed a broadly applicable anthocyanin index that compensated for both of these factors by using the inverse spectral reflectance at 550 and 700 nm. Using a similar algorithm, it is possible that the ability of the ACM to estimate anthocyanin content could be improved, particularly within leaves which are mostly green. It should also be noted that the sample population used in this study consisted of leaves from many individuals of the same species collected at different times and from different locations. Anthocyanin estimation would likely be more consistent when applied to less variable populations, at a single point in time, or to clonal stock.

In conclusion, although some inconsistency was observed in the strength of linear relationships between ACI and Anth within individual color categories, the overall relationship observed between ACI and Anth in this experiment was significantly linear and similar in strength to relationships reported between CCI and Chl in large samples of sugar maple leaves. Thus, the ACM appears to be an effective, field-portable tool for accurate estimation of relative anthocyanin content in large samples of autumn sugar maple leaves. With the general correlative relationship established, the ACM could be used in a number of management or research applications for which precise values of anthocyanin content are not necessary, such as monitoring changes in pigment content over time, assessing the effects of experimental treatments, or as a tool for assessing color in horticultural applications.

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