Journal for Nature Conservation 17 (2009) 125—129

Available online at www.sciencedirect.com
ScienceDirect

Journal for Nature Conservation

www.elsevier.de/jnc

SHORT COMMUNICATION

Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research

Tracy S. Hawkins*, Emile S. Gardiner, Greg S. Comer[1]

USDA Forest Service, 432 Stoneville Road, Stoneville, MS 38776, USA

Received 27 May 2008; accepted 19 December 2008

KEYWORDS
Chlorophyll;
Federally listed;
Lauraceae;
Lindera;
Pondberry;
SPAD-502;
Spicebush

Summary

Handheld chlorophyll meters have proven to be useful tools for rapid, non-destructive assessment of chlorophyll and nutrient status in various agricultural and arborescent plant species. We proposed that a SPAD-502 chlorophyll meter would provide valuable information when monitoring life cycle changes and intraspecific variation in endangered plant populations, whereby, destruction of plants to obtain this information is impractical. Further, use of this instrument would augment leaf morphometric measurements collected during controlled studies, circumventing the need for leaf harvest. We developed a regression model relating foliar chlorophyll concentration and content to SPAD chlorophyll content indices for a genetically diverse population of the federally listed *Lindera melissifolia*. Application of the regression to four additional *L. melissifolia* populations, and to the ecologically widespread congener *L. benzoin*, proved the SPAD-502 to be an effective tool for non-destructive estimation of total foliar chlorophyll concentration ($r^2 = 0.8230$) and content ($r^2 = 0.9029$) across a range of plant ages, growing conditions, and genotypes.
Published by Elsevier GmbH.

Introduction

Methods used to monitor and assess threatened and endangered plants often are limited to non-destructive techniques in order to minimise disturbance to naturally occurring populations. In turn, emphasis is placed on morphometric measurements, such as stem height, leaf number, leaf area, and stem diameter, then supplemented by

*Corresponding author. Current address: USDA Forest Service, Center for Bottomland Hardwoods Research, Box 9681, Mississippi State, MS 39762, USA. Tel.: +1 662 347 8639; fax: +1 662 686 3195.
E-mail address: tracyhawkins@fs.fed.us (T.S. Hawkins).
[1]Current address: National Park Service, Yellowstone National Park, WY 82190, USA.

1617-1381/$ - see front matter Published by Elsevier GmbH.
doi:10.1016/j.jnc.2008.12.007

qualitative descriptions of life cycle phenology. However, when studying threatened and endangered plant species, a potentially useful and perhaps under utilised instrument, is the SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan). This instrument provides a rapid, non-destructive method for estimating foliar chlorophyll and other related variables, thus providing an avenue for immediate assessment of physiological variables, and for monitoring physiological changes over time.

Chlorophyll meters are used extensively in agriculture for estimation of foliar chlorophyll and nitrogen in numerous crop species (Marquard & Tipton 1987), as well as, arborescent species (Sibley et al. 1996). On the other hand, some researchers have presented evidence of inherent limitations in the use of chlorophyll meters, finding that mathematical relationships between SPAD-502 readings and foliar chlorophyll and/or nitrogen may vary with plant growth stage (Chapman & Barreto 1997), growing conditions (Campbell et al. 1990), growing season (Bullock & Anderson 1998) and genotype (Sibley et al. 1996). The predictive value of SPAD-502 readings has also been found to be of limited use when applied across species (Marquard & Tipton 1987).

The objective of our research was to determine the mathematical relationship between SPAD-502 readings and total foliar chlorophyll for plants of the federally endangered shrub *Lindera melissifolia* (Walt.) Blume with differing origin (naturally occurring vs. micropropagated), ontogeny, and growing conditions. Further, we investigated mathematical relationships across species with the ecologically widespread congener *L. benzoin* (L.) Blume.

The success of plant conservation efforts is often measured by establishment and protection of self-sustaining populations. We propose that if utility outweighs limitations, the SPAD-502 chlorophyll meter will provide a necessarily needed non-destructive technique for quantifying physiological variables and temporal changes in *L. melissifolia* that reflect trends in the general health of the population. This approach may then prove to be useful in plant conservation efforts for other threatened and endangered plant species where destructive research and monitoring techniques are unlawful or impractical.

Methods

Chlorophyll measurement and extraction

Source plants used to develop the regression originated from micropropagation of *Lindera melissifolia* stockplants (20 genotypes) collected from selected bottomland forests in the Lower Mississippi Alluvial Valley (Hawkins et al. 2007). Micropropagules were grown for approximately six months in a climate controlled greenhouse with a 14 h light ($\geqslant 300\,\mu mol\,m^{-2}\,s^{-1}$):10 h dark photoperiod and mean temperature of $78 \pm 6\,^{\circ}C$. On 13 December 2005, one fully developed leaf was harvested from each of 75 randomly chosen *L. melissifolia* plants. Three readings were taken with a SPAD-502 chlorophyll meter between the base and apex of the left half of each leaf blade, and the mean chlorophyll content index (CCI) reading was recorded.

A 0.08 cm^2 disk was cut from each leaf blade half and weighed to the nearest 0.001 g. Leaf disks were immediately placed in vials containing DMSO, and chlorophyll extraction followed the methods outlined by Hiscox and Israelstam (1979). Total chlorophyll concentration and content were determined with a Spectronic 21D spectrophotometer using equations derived by Barnes et al. (1992) for DMSO extraction of chlorophylls *a* and *b*.

Leaves were harvested from four additional *L. melissifolia* populations and one population of *L. benzoin* to test the applicability of the regression developed from the initial *L. melissifolia* leaf harvest. Table 1 summarises harvest abbreviations, collection dates, ages, locations, and growing conditions for plants from which these leaves were harvested. Leaf harvesting, measurements, and chlorophyll extraction followed the same protocol as that outlined for leaves collected on 13 December 2005.

Statistical analysis

Regression analysis was used to evaluate the relationship between extracted chlorophyll values (dependent variable) and SPAD CCI (independent variable) using only LMC (*L. melissifolia*, climate controlled) samples. We evaluated actual values and estimated values (fitted from the LMC based equations) separately for LM1 (*L. melissifolia*, bottomland forest), LM2 (*L. melissifolia*, 95% shade), LM3 (*L. melissifolia*, 63% shade), LM4 (*L. melissifolia*, 30% shade), and LB (*L. benzoin*, bottomland forest). Mean chlorophyll content (mg/cm^2) and chlorophyll concentration (mg/g) among harvests were compared by a one-way analysis of variance (ANOVA; factor = plant population, variable = chlorophyll). Protected least significant difference test (PLSD, $p = 0.05$) was used as the multiple comparison procedure. The SAS

Table 1. Plant age, leaf sample size, leaf collection date, leaf collection location, and plant growing conditions for *Lindera melissifolia* and *L. benzoin* from which leaves were harvested for SPAD-502 measurements and chlorophyll extraction.

Population	Age	*N*	Collection date	Location[a]	Growing conditions
L. melissifolia (LMC)[b]	6 months	75	2005-December	Washington	Greenhouse
L. melissifolia (LM1)	>1 yr	20	2006-June	Bolivar	Bottomland forest
L. melissifolia (LM2)	1 yr	10	2006-June	Sharkey	Ambient, 95% shade
L. melissifolia (LM3)	1 yr	10	2006-June	Sharkey	Ambient, 63% shade
L. melissifolia (LM4)	1 yr	10	2006-June	Sharkey	Ambient, 30% shade
L. benzoin (LB)	>1 yr	20	2006-June	Bolivar	Bottomland forest

[a]Mississippi counties.
[b]Micropropagated plants.

Table 2. Range of SPAD-502 readings, mean (±SE) chlorophyll concentration (mg/g) and chlorophyll content (mg/cm^2) for leaves of *Lindera melissifolia* (LMC, LM1, LM2, LM3, LM4) and *L. benzoin* (LB).

Population	SPAD range	Chl_{a+b} (mg/g)	Chl_{a+b} (mg/cm^2)
LMC	3.8–47.3	2.50 ± 0.11^a	0.03 ± 0.00^a
LM1	13.4–37.5	3.57 ± 0.23^b	0.02 ± 0.00^a
LM2	33.6–44.6	7.68 ± 0.36^c	0.04 ± 0.00^b
LM3	25.7–36.1	2.59 ± 0.08^a	0.03 ± 0.00^a
LM4	22.8–35.9	2.61 ± 0.13^a	0.03 ± 0.00^a
LB	22.0–45.4	4.48 ± 0.31^d	0.03 ± 0.01^a

Within a column, values with different lowercase letters are significantly different ($p < 0.05$, LSD).

procedures GLM and REG were used to perform statistical analyses (SAS Institute 2001).

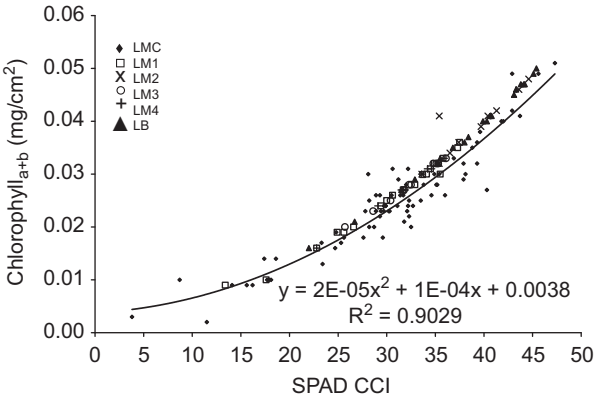

Figure 1. Relationship of extractable foliar chlorophyll content (LMC) determined by spectrophotometry and fitted values (LM1, LM2, LM3, LM4, LB) with SPAD-502 readings (CCI).

Results

Actual mean foliar chlorophyll concentration ranged from 2.5±0.11 mg/g to 7.68±0.36 mg/g, and mean foliar chlorophyll content ranged from 0.02±0.00 mg/cm^2 to 0.04+0.00 mg/cm^2 among all harvested leaves (Table 2). Mean chlorophyll concentration for LM1, LM2, and LB was significantly greater than that of LMC, LM3, and LM4. Mean foliar chlorophyll content for LM2 was significantly greater than that of all other harvested leaves (Table 2).

Using the LMC leaves to develop a regression, several models were tested to describe the relationship between chlorophyll variables and SPAD CCI. Best fit was a second order polynomial relating SPAD CCI to chlorophyll content (Figure 1) or chlorophyll concentration (Figure 2). There were no significant differences in slope or intercept among regression estimates and actual chlorophyll concentration for LM1, LM2, LM3, and LM4; however, the slopes for actual and estimated chlorophyll concentration for LB were statistically significant (Table 3). Slopes for actual and

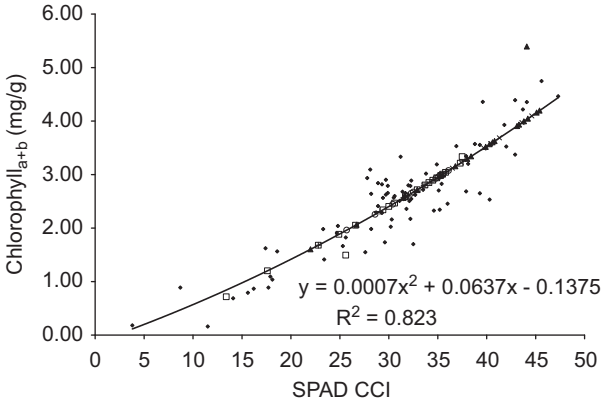

Figure 2. Relationship of extractable foliar chlorophyll concentration (LMC) determined by spectrophotometry and fitted values (LM1, LM2, LM3, LM4, LB) with SPAD-502 readings (CCI).

Table 3. Regression parameters for the relationship between estimated and actual foliar chlorophyll content (mg/cm^2) and actual foliar chlorophyll concentration (mg/g) for leaves of *Lindera melissifolia* (LM1, LM2, LM3, LM4) and *L. benzoin* (LB).

Population	*N*	Chlorophyll content (mg/cm^2) *p*-value			Chlorophyll concentration (mg/g) *p*-value		
		Slope	Intercept	*F*	Slope	Intercept	*F*
LM1	20	0.0303	0.9394	238.63	0.8842	0.6633	740.76
LM2	10	0.2298	0.8556	18.75	0.4469	0.7431	116.67
LM3	10	0.3487	0.3241	52.62	0.5708	0.6609	73.73
LM4	10	0.0922	0.0918	13.72	0.7705	0.8833	44.16
LB	20	0.1947	0.5937	69.17	0.0068	0.2134	498.03

estimated chlorophyll content for LM1 were significant (Table 3).

Discussion

Among harvests, foliar chlorophyll concentration (mg/g) for *Lindera melissifolia* generally increased with decreased light availability. With the exception of LM2, the range of values for chlorophyll concentration was comparable to that reported for 12 *Acer rubrum* L. cultivars (Sibley et al. 1996). Further, chlorophyll concentration for LMC, LM1, LM3, and LM4, and chlorophyll content for leaves of all *L. melissifolia* and *L. benzoin* harvests were similar to that reported for two clones of *Populus deltoides* Bartram ex. Marsh (Moreau et al. 2004).

The non-linear relationship between total extractable chlorophyll and SPAD CCI is consistent with that reported by other researchers (Abdelhamid et al. 2003). Further, Marquard and Tipton (1987) reported a better correlation between SPAD CCI and total extractable chlorophyll per unit area, rather than per unit of weight, citing decreased accuracy of chlorophyll estimation at high SPAD CCI. This has been attributed to the sieve effect which results from chlorophyll being less uniformly distributed in high chlorophyll leaves due to increased chlorophyll density in chloroplasts, rather than an increase in the number of chloroplasts (Terashima & Saeki 1983). Although the relationship between SPAD CCI and foliar chlorophyll per unit area was slightly stronger than that on a per weight basis in our study, sieve effect did not appear to have a detrimental impact on the accuracy of estimated values. Our regression explained 90% of variation in the chlorophyll content-SPAD CCI relationship, and 82% of variation in the chlorophyll concentration-SPAD CCI relationship. On the other hand, the significant difference in slopes for estimated and actual chlorophyll concentration for LB suggests some decreased accuracy on the upper end of SPAD CCI values. Similarly, slopes for estimated and actual chlorophyll content in LM1 suggest some decreased accuracy on the lower end of SPAD CCI values.

Sibley et al. (1996) described the SPAD-502 as a reliable tool for estimation of chlorophyll content in *A. rubrum* cultivars grown under similar environmental conditions, but cautioned against use of the equation for cultivars grown in a different environment. In our study, the SPAD-502 proved to be an effective tool for estimating total foliar concentration and content in plants of *L. melissifolia* across a range of plant ages, growing conditions, and genotypes, as well as, in the congener *L. benzoin*. However, different SPAD-502 meters may give different meter values (Huang & Peng 2004) and we suggest standardisation of a calibration curve prior to use on a plant population. Once this is determined, the SPAD-502 chlorophyll meter may be used to gauge the progress of conservation practices by assessing and monitoring physiological variables that reflect the relative health of threatened and endangered plant species.

Acknowledgements

The authors thank Tom Dell for help with statistical analysis, US Fish and Wildlife Service for permitting, US Army Corps of Engineers for underwriting the cost of this research, and Drs. Chris Adams and Mary Anne Sword Sayer for review of an earlier draft of this manuscript.

References

Abdelhamid, M., Horiuchi, T., & Oba, S. (2003). Evaluation of the SPAD value in faba bean (*Vicia faba* L.) leaves in relation to different fertilizer applications. *Plant Production Science*, *6*, 185–189.

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environmental and Experimental Botany*, *32*, 85–100.

Bullock, D. G., & Anderson, D. S. (1998). Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. *Journal of Plant Nutrition*, *21*, 741–755.

Campbell, R. J., Mobley, K. N., Marini, R. P., & Pfeiffer, D. G. (1990). Growing conditions alter the relationship between SPAD-501 values and apple leaf chlorophyll. *HortScience*, *25*, 330–331.

Chapman, S. C., & Barreto, H. J. (1997). Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agronomy Journal*, *89*, 557–562.

Hawkins, T. S., Schiff, N. M., Gardiner, E. S., Leininger, T., Devall, M. S., Wilson, D., et al. (2007). Micropropagation of the endangered shrub pondberry (*Lindera melissifolia* [Walt.] Blume). *HortScience*, *42*, 1–3.

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, *57*, 1332–1334.

Huang, J., & Peng, S. (2004). Comparison and standardization among chlorophyll meters in their readings on rice leaves. *Plant Production Science*, *7*, 97–100.

Marquard, R. D., & Tipton, J. L. (1987). Relationship between extractable chlorophyll and an *in situ* method to estimate leaf greenness. *HortScience*, *22*, 1327.

Moreau, B., Gardiner, E.S., Stanturf, J.A., & Fisher, R.K., (2004). Estimating leaf nitrogen of eastern cottonwood trees with a chlorophyll meter. In: *Proceeding of the 12th biennial southern silvicultural research conference*. General technical report SRS-71. US Department of Agriculture, Forest Service, Asheville, North Carolina. pp. 487–491.

SAS Institute. (2001). The SAS system for Windows, release V8. SAS Institute Inc., Cary, NC.

Sibley, J. L., Eakes, D. J., Gilliam, C. H., Keever, G. J., Dozier, W. A., Jr., & Himelrick, D. G. (1996). Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. *HortScience*, *31*, 468–470.

Terashima, I., & Saeki, T. (1983). Light environment within a leaf. *Plant & Cell Physiology*, *24*, 1493–1501.