ESTIMATING LEAF NITROGEN OF EASTERN COTTONWOOD TREES WITH A CHLOROPHYLL METER

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Abstract—The utility of the SPAD-502 chlorophyll meter for nondestructive and rapid field determination of leaf nitrogen (N) has been demonstrated in agricultural crops, but this technology has not yet been extended to woody crop applications. Upper canopy leaves from a 5-year-old plantation of two eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) clones established on a former agricultural field were collected to determine the relationship between SPAD values and foliar N. The two clones, Stoneville-66 and Stoneville-75, differed in chlorophyll concentrations but had similar leaf N concentrations; relationships between N, chlorophyll, and SPAD values were not influenced by clone. Total chlorophyll was closely associated with total N concentration (mass basis) and content (area basis) (r^2 = 0.76 and 0.66, respectively), and SPAD values accurately predicted total N concentration and content (r^2 = 0.74 and 0.67, respectively). Results from this study indicate that SPAD-502 values < 32 signify a N deficiency level severe enough to limit the productivity of eastern cottonwood. The SPAD-502 chlorophyll meter shows promise as a tool for rapid field estimation of leaf N concentration or content in eastern cottonwood trees. Our findings support the development of this technology for use in eastern cottonwood or other short-rotation woody crop applications. Future investigations should be expanded to examine other eastern cottonwood clones on other site types and to standardize sampling protocols for various stages of tree phenology.

INTRODUCTION

Previous research has demonstrated the utility of the SPAD-502 chlorophyll meter (Soil-Plant Analysis Development Section, Minolta Corp., Osaka, Japan) for nondestructive and rapid estimation of leaf nitrogen (N) in agricultural crops, including corn (*Zea mays* L.), rice (*Oryza sativa* L.), and cotton (*Gossypium hirsutum* L.) (Dwyer and others 1994; Peng and others 1993; Turner and Jund 1991; Wood and others 1992a, 1992b). Using this tool to schedule fertilizer applications in a production setting allows managers to increase yields while reducing excessive fertilization that may contaminate surface and ground water (MacKown and Sutton 1998, Shapiro 1999). In spite of the development of this technology in the agricultural community, few studies have explored the value of the SPAD-502 for estimating leaf N of woody crop species. Yet, there is a need for rapid estimation of leaf N in managing short-rotation woody crops; foliar N pools are critical for maximizing tree productivity for fiber, biofuel, and carbon sequestration purposes. Previous work conducted on apple (*Malus domestica* Borkh.) and red maple (*Acer rubrum* L.) illustrates both the potential and the limitations for extending this technology to applications that involve tree species (Campbell and others 1990, Neilsen and others 1995, Sibley and others 1996).

Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) is a fast-growing, broadleaf tree species endemic to alluvial sites in the Central and Southeastern United States (Cooper 1990). Because of its rapid growth rate, eastern cottonwood is cultivated extensively in plantations for pulp fiber and timber production throughout regions of North America and Asia (Cao and Conner 1999, Krinard and Johnson 1980). Additionally, there has been a recent surge of interest in using eastern cottonwood plantations for biofuel production, carbon sequestration, soil quality improvement, and catalyzing forest ecosystem restoration on land formerly in row crop production (Stanturf and others 1998, Thornton and others 1998). Compared with other plantation tree species, eastern cottonwood makes high demands on soil nutrients, requiring high levels of available soil N to maximize growth (Blackmon and White 1972, Carter and White 1971). Eastern cottonwood requires leaf N concentrations of at least 2 percent to maximize volume production (Blackmon and White 1972).

When plantations are established on former agricultural fields where soil N has been depleted by previous row cropping, N is commonly the limiting nutrient (Blackmon 1977, Blackmon and White 1972), and fertilization is often needed to reach maximum productivity. Plantation managers needing to assess cottonwood nutrient status lack a rapid field technique for estimating leaf N levels. The objective of this study was to determine if the SPAD-502 chlorophyll meter could be used to estimate leaf N concentration and N content in eastern cottonwood trees established for fiber production on a former agricultural field in the Lower Mississippi River Alluvial Valley.

MATERIALS AND METHODS

The leaf material examined in this study was harvested from a 5-year-old eastern cottonwood plantation located on the Yazoo National Wildlife Refuge, Sharkey County, MS (32°58' N., 90°44'W.). The 24.3-ha cottonwood plantation was established in March 1995 as a component of a large-scale study on forest restoration on former agricultural land (Schweitzer and others 1997). The regeneration and cultural practices used by the Crown Vantage Paper Company

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in their fiber production operations were followed during plantation establishment; this included a deep application of soluble N prior to planting, but no subsequent fertilizer was applied [for details see Schweitzer and Stanturf (1999) and Schweitzer and others (1997)]. Sharkey clay (very fine, smectitic, thermic, Chromic Epiaquerts) was the predominant soil series on this alluvial site, and the site received an average annual precipitation of 1,318 mm (Scott and Carter 1962).

The eastern cottonwood plantation was established in three 8.1-ha stands (blocks) based on surface soil characteristics that differed according to variation in localized flooding. In early June 2000, eight sample plots were randomly delineated in each of the three stands. Four plots were installed in plantings of the commercially available clone Stoneville-66 (ST-66), while the other four were installed in plantings of the commercially available clone Stoneville-75 (ST-75). These particular clones were chosen for the current study for their widespread commercial deployment and the difference in growth they exhibit during early stand development (Schweitzer and Stanturf 1999). For example, ST-66 averaged 14.7 m tall after five growing seasons, 31 percent taller than the average height of ST-75 (11.2 m) (Fisher and others 2002).

After the sample plots were established, we collected a single leaf sample from 4 adjacent trees in each plot (96 samples: 3 blocks, 2 clones, 4 plots, 4 sample trees). We restricted sampling to mature leaves from the upper one-third of the western aspect of the crown of each sample tree. Furthermore, all leaves were sampled from the same leaf plastocron index (LPI) position (LPI 7 or 8) to ensure nutrient stability (Isebrands and Larson 1973). Sample leaves were harvested between 6 a.m. and 10 a.m. with an extension pole and clipper and were free of insect and disease damage. Dew was blotted from the leaf surface with tissue paper then three readings with a SPAD-502 chlorophyll meter were taken between the midrib and the leaf margin. After chlorophyll meter sampling, sample leaves were placed in polyethylene bags containing moist tissue paper and transported to the laboratory in a refrigerated box.

In the laboratory, the petiole and midrib were dissected from each sample leaf, creating two leaf blade halves. We measured area and green weight of each leaf blade half before randomly selecting one-half for chlorophyll analysis and the other half for N analysis. We conducted chlorophyll analysis according to the methods outlined by Hiscox and Israelstam (1979); results are presented here as total chlorophyll concentration (mass basis) (mg g_{dw}^{-1}) and total chlorophyll content (area basis) (mg dm^{-2}).

Leaf tissue used for N analysis was dried at 70 °C and ground in a ball mill to pass a 100-mesh sieve (Culmo and others 1989). Total N in sample leaf tissue and standards was assessed with a dry combustion technique using a PE 2400 Series II CHNS/O Analyzer (Perkin-Elmer Corporation, Norwalk, CT). Nitrogen analysis results are presented here as total N concentration (mass basis) (percent) and total N content (area basis) (mg dm^{-2}).

We conducted statistical analyses on leaf morphological, chlorophyll, and N variables on sample plot means (mean of four sample trees) according to a randomized block design using SAS software (SAS Institute, Inc., Cary, NC). Regression analyses were used to model relationships between leaf N and chlorophyll and between SPAD values and leaf N. N and chlorophyll values used in the modeling procedures received a natural log transformation to homogenize variance and meet normality assumptions (Myers 1990).

RESULTS AND DISCUSSION

The two eastern cottonwood clones studied in this experiment, ST-66 and ST-75, exhibited differences in leaf morphology and chemistry. ST-75 developed leaf blades with a 25-percent larger area and a 23-percent greater mass than those of ST-66, but leaf mass per area remained similar between the two clones (table 1). N concentrations in leaf tissues were similar for each clone and averaged 1.6 percent across the study site (table 1). Foliar N levels in this study were equivalent to those reported for other eastern cottonwood plantations established on former agricultural fields (Blackmon and White 1972), but were below the optimal 2 percent defined by Blackmon and White (1972) for maximizing productivity. Similar N concentrations and leaf mass per area led to comparable levels of N content per leaf among clones. However, chlorophyll concentration and chlorophyll content of ST-75 leaves were 14 percent and 16 percent greater, respectively, than those of ST-66 (table 1). These findings indicate that ST-75 has a greater proportion of leaf N associated with the chlorophyll fraction than does ST-66. In

Table 1—Leaf morphology, nitrogen and chlorophyll characteristics of two eastern cottonwood clones growing in a 5-year-old plantation, Sharkey County, MS

Variable	Clone ST-66	Clone ST-75	*P*-value
	- - - - - - - *mean ± standard error*[a] - - - - - - -		
Blade area (dm^2)	0.66 ± 0.03 b	0.83 ± 0.05 a	0.0027
Blade mass (g_{dw})	0.64 ± 0.03 b	0.79 ± 0.06 a	0.0022
Blade mass per area (g_{dw} dm^{-2})	0.97 ± 0.02 a	0.96 ± 0.01 a	0.4734
Total nitrogen concentration (percent)	1.55 ± 0.10 a	1.67 ± 0.05 a	0.0845
Total nitrogen content (mg dm^{-2})	15.0 ± 0.71 a	15.9 ± 0.58 a	0.1209
Total chlorophyll concentration (mg g_{dw}^{-1})	2.69 ± 0.16 b	3.08 ± 0.15 a	0.0125
Total chlorophyll content (mg dm^{-2})	3.65 ± 0.19 b	4.22 ± 0.19 a	0.0025

[a] The sample size for each mean is 12 (3 blocks x 4 plots), and means in a row followed by the same letter do not differ at the 0.05 probability level.

support of the this finding, calculation of N:chlorophyll ratios revealed that ST-66 had 1 mg of chlorophyll for every 4.1 mg of leaf N, while leaves of ST-75 had 1 mg of chlorophyll for every 3.7 mg of leaf N.

Several different models were fit to leaf data to describe relationships between total N (percent and mg dm^{-2}) and total chlorophyll (mg g_{dw}^{-1} and mg dm^{-2}), and between SPAD values and total N (percent and mg dm^{-2}). A curvilinear relationship represented by equation 1 consistently provided the highest coefficient of determination (r^2) values for all relationships.

$$y = \exp\left[a + bx + cx^2\right] \quad (1)$$

Simple linear and curvilinear functions have been used to model these relationships in agronomic crops, as several factors including edaphic conditions, plant phenology, and temporal variation can influence response (Westcott and Wraith 1995; Wood and others 1992a, 1992b). Though the two cottonwood clones exhibited different chlorophyll levels and apparently partitioned differing proportions of N in their chlorophyll fractions, intercept and slope coefficients from models of individual clones did not differ from coefficients of models in which clones were pooled. Thus, results presented here are from the pooled datasets that were used to fit the single curves presented in figures 1A, 1B, 2A, and 2B. Model coefficients for the line in figure 1A are a = -0.399398, b = 1.161110, and c = -0.160611. Model coefficients for the line in figure 1B are a = -0.999735, b = 0.241596, and c = -0.005651. Model coefficients for the line in figure 2A are a = -2.682481, b = 0.196621, and c = -0.002897. Model coefficients for the line in figure 2B are a = 0.596826, b = 0.125604, and c = -0.001680.

Total chlorophyll concentration of eastern cottonwood leaves was strongly associated with leaf N concentration

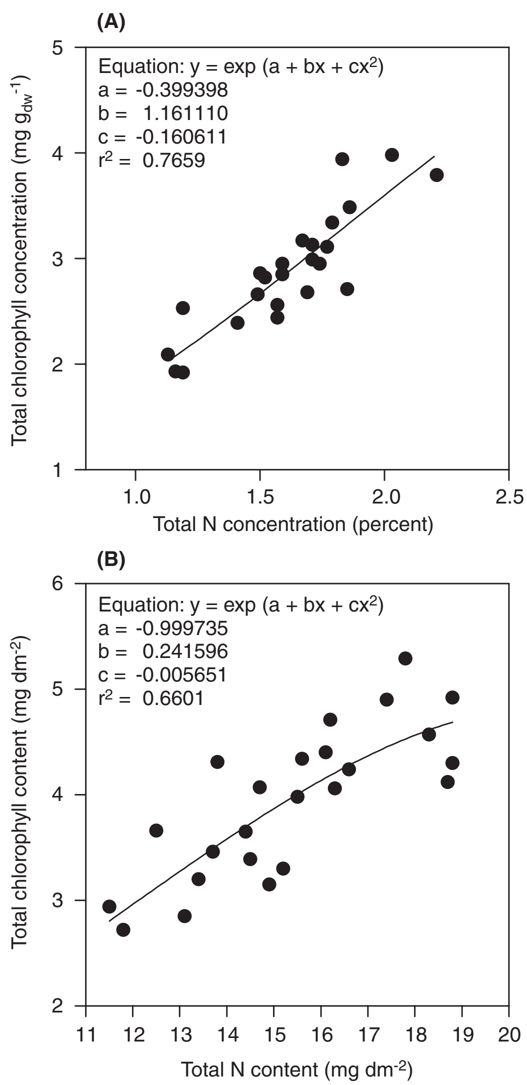

Figure 1—Relationships between total chlorophyll concentration and total nitrogen concentration (A) and between total chlorophyll content and total nitrogen content (B) of eastern cottonwood leaves, Sharkey County, MS.

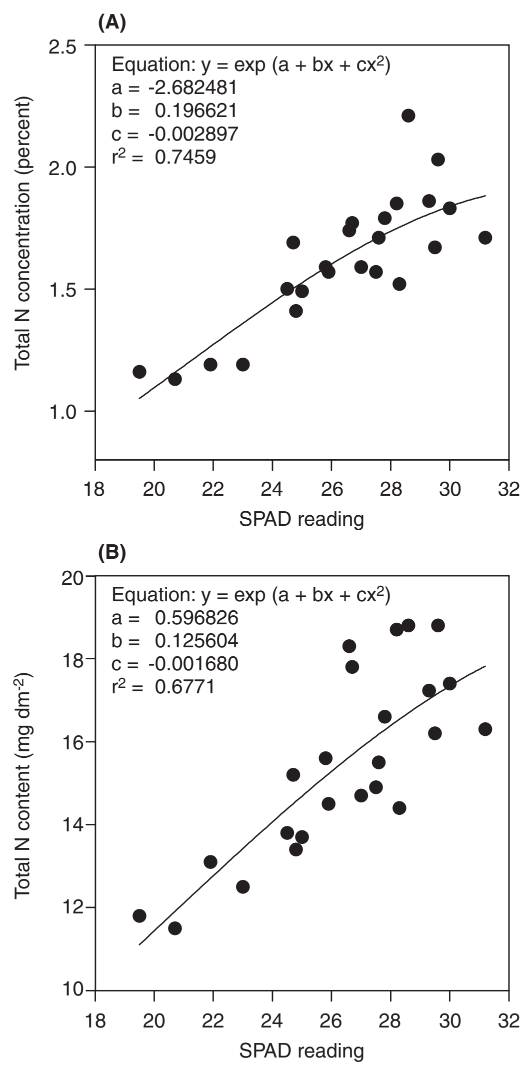

Figure 2—Relationships between total nitrogen concentration and SPAD readings (A) and between total nitrogen content and SPAD readings (B) for eastern cottonwood leaves, Sharkey County, MS.

(P = 0.0001, r^2 = 0.76) (fig. 1A). Within the range of our data, a 0.5-percent increase in N concentration yielded a 0.93-mg g_{dw}^{-1} rise in total chlorophyll concentration. As with N concentration, a significant amount of the variation in leaf chlorophyll content could be attributed to N content (P = 0.0001, r^2 = 0.66) (fig. 1B). However, a greater degree of unexplained variation was associated with this area-based relationship than with the previously described mass-based relationship (figs. 1A and 1B).

Minolta SPAD-502 values showed a strong positive association to total N concentration in eastern cottonwood leaves (P = 0.0001, r^2 = 0.74) (fig. 2A). Fit of prediction curves for eastern cottonwood leaves were in the range reported for several apple cultivars (Neilsen and others 1995). Furthermore, our findings are in contrast to those of Sibley and others (1996), who were unable to demonstrate a significant relationship between SPAD-502 values and foliar N in several red maple cultivars, though others have reported r^2 values as ≥ 0.90 for corn and tall fescue (*Festuca arundinacea* Schreb.) (Kantety and others 1996, Wood and others 1992a). Peng and others (1995), who studied rice, suggested that foliar N predictions could be improved if SPAD values were used to estimate N content (area basis) rather than concentration. Findings from this study do not support those of Peng and others (1995); predicting total N content of eastern cottonwood leaves with the SPAD-502 was not as precise as predicting total N concentration (figs. 2A and 2B).

Research conducted by Blackmon and White (1972) and Blackmon (1977) suggested that volume production in eastern cottonwood is greatest when foliar N levels exceed 2 percent. Our observations of leaf N concentrations from the plantation were generally well below this critical amount. The lack of observations > 2 percent prevented us from identifying a SPAD-502 value that would define sufficient foliar N for maximizing eastern cottonwood productivity. However, based on our observations, SPAD-502 values < 32, when measured in the upper canopy, are probably indicative of a N deficiency severe enough to limit eastern cottonwood volume production.

CONCLUSIONS

We used SPAD-502 chlorophyll meter values to accurately determine foliar N concentrations and contents in two clones of 5-year-old eastern cottonwood trees. Though leaves from ST-66 and ST-75 exhibited different amounts of chlorophyll, the coefficients of the regression equations used to predict leaf N were not influenced by clonal variety. Results from this study indicate that SPAD-502 values < 32 are indicative of foliar N concentrations under 2 percent, the threshold N concentration below which volume production of eastern cottonwood is N limited. This research demonstrates the potential utility of the SPAD-502 chlorophyll meter for rapid field determination of foliar N concentration and content in eastern cottonwood trees. This technology could provide short-rotation woody crop managers a quick screening method to identify and quantify N deficiencies in eastern cottonwood stands. Future investigations should be expanded to examine other eastern cottonwood clones on other site types and to standardize sampling protocols for various stages of tree phenology.

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LITERATURE CITED

Blackmon, B.G. 1977. Effects of fertilizer nitrogen on tree growth, foliar nitrogen, and herbage in eastern cottonwood plantations. Soil Science Society of America Journal. 41: 992-995.

Blackmon, B.G.; White, E.H. 1972. Nitrogen fertilization increases cottonwood growth on old-field soil. Res. Note SO-143. New Orleans: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 5 p.

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.

Cao, F.L.; Conner, W.H. 1999. Selection of flood-tolerant *Populus deltoides* clones for reforestation projects in China. Forest Ecology and Management. 117: 211-220.

Carter, M.C.; White, E.H. 1971. Dry weight and nutrient accumulation in young stands of cottonwood (*Populus deltoides* Bartr.). Circ. 190. Auburn, AL: Agricultural Experiment Station, Auburn University, 14 p.

Cooper, D.T. 1990. *Populus deltoides* Bartr. ex Marsh. eastern cottonwood. In: Burns, R.M.; Honkala, B.H., tech. coords. Silvics of North America: hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service. 877 p. Vol. 2.

Culmo, R.F.; Swanson, K.J.; Brennan, W.P. 1989. For organic element analysis: it's the number of particles not the sample size that counts. Elemental Analysis Newsletter. [Number of pages unknown].

Dwyer, L.M.; Anderson, A.M.; Ma, B.L. [and others]. 1994. Quantifying the nonlinearity in chlorophyll meter response to corn leaf nitrogen concentration. Canadian Journal of Plant Science. 75: 179-182.

Fisher, R.K.; Gardiner, E.S.; Stanturf, J.S.; Portwood, C.J. 2002. Disking effects on fifth-year volume production of four eastern cottonwood clones established on an afforestation site, Sharkey County, Mississippi. In: Outcalt, K.W., ed. Proceedings of the eleventh biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-48. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 515-519.

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.

Isebrands, J.G.; Larson, P.R. 1973. Anatomical changes during leaf ontogeny in *Populus deltoides*. American Journal of Botany. 60: 199-208.

Kantety, R.V.; van Santen, E.; Woods, F.M.; Wood, C.W. 1996. Chlorophyll meter predicts nitrogen status of tall fescue. Journal of Plant Nutrition. 19: 881-899.

Krinard, R.M.; Johnson, R.L. 1980. Fifteen years of cottonwood plantation growth and yield. Southern Journal of Applied Forestry. 4: 180-185.

MacKown, C.T.; Sutton, T.G. 1998. Using early-season leaf traits to predict nitrogen sufficiency of burley tobacco. Agronomy Journal. 90: 21-27.

Myers, R.H. 1990. Classical and modern regression with applications. 2d ed. Boston: PWS-KENT Publishing Co. 488 p.

Neilsen, D.; Hogue, E.J.; Neilsen, G.H.; Parchomchuk, P. 1995. Using SPAD-502 values to assess the nitrogen status of apple trees. HortScience. 30: 508-512.

Peng, S.; Garcia, F.V.; Laza, R.C.; Cassman, K.G. 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agronomy Journal. 85: 987-990.

Peng, S.; Laza, R.C.; Garcia, F.V.; Cassman, K.G. 1995. Chlorophyll meter estimates leaf area-based nitrogen concentration of rice. Communications in Soil Science and Plant Analysis. 26: 927-935.

Schweitzer, C.J.; Stanturf, J.A. 1999. A comparison of large-scale reforestation techniques commonly used on abandoned fields in the Lower Mississippi Alluvial Valley. In: Haywood, J.D., ed. Proceedings of the tenth biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-30. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 136-141.

Schweitzer, C.J.; Stanturf, J.A.; Shepard, J.P. [and others]. 1997. Large-scale comparison of reforestation techniques commonly used in the Lower Mississippi Alluvial Valley: first-year results. In: Pallardy, S.G.; Cecich, R.A.; Garrett, H.G.; Johnson, P.S., eds. Proceedings, 11th central hardwood forest conference. Gen. Tech. Rep. NC-188. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 313-320.

Scott, F.T.; Carter, R.C. 1962. Soil survey of Sharkey County, Mississippi. [Location of publisher unknown]: U.S. Department of Agriculture, Soil Conservation Service. 36 p.

Shapiro, C.A. 1999. Using a chlorophyll meter to manage nitrogen applications to corn with high nitrate irrigation water. Communications in Soil Science and Plant Analysis. 30: 1037-1049.

Sibley, J.L.; Eakes, D.J.; Gilliam, C.H. [and others]. 1996. Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. HortScience. 31: 468-470.

Stanturf, J.A.; Schweitzer, C.J.; Gardiner, E.S. 1998. Afforestation of marginal agricultural land in the Lower Mississippi River Alluvial Valley, U.S.A. Silva Fennica. 32: 281-297.

Thornton, F.C.; Joslin, J.D.; Bock, B.R. [and others]. 1998. Environmental effects of growing woody crops on agricultural land: first year effects on erosion, and water quality. Biomass and Bioenergy. 15: 57-69.

Turner, F.T.; Jund, M.F. 1991. Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. Agronomy Journal. 83: 926-928.

Westcott, M.P.; Wraith, J.M. 1995. Correlation of leaf chlorophyll readings and stem nitrate concentrations in peppermint. Communications in Soil Science and Plant Analysis. 26: 1481-1490.

Wood, C.W.; Reeves, D.W.; Duffield, R.R.; Edmisten, K.L. 1992a. Field chlorophyll measurements for evaluation of corn nitrogen status. Journal of Plant Nutrition. 15: 487-500.

Wood, C.W.; Tracy, P.W.; Reeves, D.W.; Edmisten, K.L. 1992b. Determination of cotton nitrogen status with a hand-held chlorophyll meter. Journal of Plant Nutrition. 15: 1435-1448.