EVALUATION OF TWO TRANSMITTANCE METERS IN ESTIMATING CHLOROPHYLL AND NITROGEN CONCENTRATIONS IN GRAPEVINE CULTIVARS

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ABSTRACT

Two transmittance-based chlorophyll meters (SPAD-502 and CCM-200) were evaluated in estimating chlorophyll (Chl) and nitrogen (N) levels in grapevine leaves. The study was conducted in a fertilization experiment [0 (N0), 60 (N1) and 120 (N2) kg N/ha] during the summer 2009, in two commercial vineyards located in Northern Greece and planted with cvs Cabernet-Sauvignon and Xinomavro (*Vitis vinifera* L.). When data were pooled over cultivars and samplings, leaves of N2 vines had the highest N and Chl content, as well as SPAD and CCM readings, followed by the respective values of N1. However, neither of the devices could detect the seasonal decline in leaf N and Chl content. Significant relationships between extracted Chl and measured leaf N were found in both cultivars. A strong linear function related SPAD and CCM readings in both cultivars. Total Chl and N were strongly correlated with SPAD and CCM readings in Cabernet Sauvignon (p<0.001) while relationships were poor for SPAD and not significant for CCM in Xinomavro. The results suggest that non-destructive chlorophyll estimations by transmittance-based meters are not applicable in all situations without specific calibrations necessary to improve their utility and accuracy over grapevine cultivars.

KEYWORD

SPAD-502 – CCM-200 – chlorophyll – nitrogen – grapevine – N fertilization

INTRODUCTION

Nitrogen (N) is the most important nutrient in grapevine, as it participates in many physiological processes and has the potential to manipulate vine growth and productivity with significant implications for grape and wine composition (Bell and Henschke, 2005). Monitoring of vine N status in the field can be important in determining N fertilizer amount and time of application. Since chlorophyll (Chl) is a nitrogenous pigment, leaf Chl content provides an indirect estimation of N plant status (Steele *et al*., 2008). However, conventional extraction of leaf Chl with various organic solvents is laborious, time consuming and destructive, thus not adapted for N fertilization scheduling. Recently, non-destructive leaf "greenness" measurements have been advocated for rapid determination of leaf Chl and N status, mainly in annual crops (Filella *et al*., 1995; Bullock and Anderson, 1998) while fewer studies have been conducted on woody species as grapevine (Fanizza *et al.*, 1991).

The aim of the present study was to evaluate the utility of two handheld transmittance-based Chl content meters in estimating Chl and N levels in intact leaves of two grapevine cultivars, in an N fertilization experiment.

MATERIALS AND METHODS

The study was conducted in two commercial vineyard blocks located in Goumenissa (Northern Greece) in the summer of 2009, planted with cvs Cabernet-Sauvignon and Xinomavro (*Vitis vinifera* L.) respectively and grafted onto 1103P. Vines were spaced 2.2 × 1.3 m and trained on a spur-pruned bilateral cordon. Nitrogen in the form of NH_4NO_3 and corresponding to three rates [0 (N0), 60 (N1) and 120 (N2) kg/ha of N] was applied at budburst. The experimental design was that of completely randomised blocks with three replications. Individual plots consisted of 6 vines distributed on two adjacent rows, and were separated by at least 6 border vines within a row.

Two handheld chlorophyll meters were evaluated: soil-plant analysis development meter (SPAD-502, Minolta Co., Osaka, Japan) and chlorophyll content meter (CCM-200, Opti-Sciences, Tyngsboro, USA). Measurements with the SPAD and CCM devices were conducted on three exterior, fully expanded leaves per plot, located on the basal shoot nodes, on four occasions during the growing season [berry set (d1), bunch closure (d2), veraison (d3) and harvest (d4)]. For each leaf, three readings on separate lobs were averaged to represent one observation. Immediately following Chl meter readings, leaves were cut, sealed in plastic bags and transported to the laboratory in a cooler for Chl and N determination. For each of the leaves sampled, three 1 cm^2 disks were cut from the same leaf areas used for Chl meter readings, weighed and extracted for 3 h in 80% ethanol solution, at 78°C in a J.P. Selecta Precisterm bath (Barcelona, Spain). Chl *a* and Chl *b* concentration of the aliquot was estimated according to the equations proposed by Arnon (1949) after measurement of the optical density at 645 and 663 nm using a 6305 UV/VIS mini spectrophotometer (Jenway Ltd, Essex, UK), and results were expressed on a fresh weight basis. The remaining leaf tissues were dried at 70°C and used for total leaf nitrogen measurement (% dry weight) by an automated combustion elemental analyzer (PDZ Europa, Cheshire, UK).

Data were subjected to analysis of variance and correlation analysis using SPSS software (version 14.0, SPSS Inc., IL, USA). Only the mean of the three measurements per plot was used in data analysis. Comparison of means was performed using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Leaf N concentration was reduced significantly with the progress of growing season in both varieties, especially until veraison (d3). N fertilization significantly increased leaf N concentration with higher levels in N2 vines for both cultivars (Fig. 1). When data were pooled over samplings, N concentration was 1.96 % in N0, 2.09 % in N1 and 2.38 % in N2 in Cabernet Sauvignon ($p<0.001$) and 1.94 %, 2.08 % and 2.21 % respectively, in Xinomavro ($p<0.001$). ANOVA did not detect any sampling × fertilization interaction in any of the varieties studied. Direct comparisons among cultivars could not be made, as cultivars were located in separate blocs.

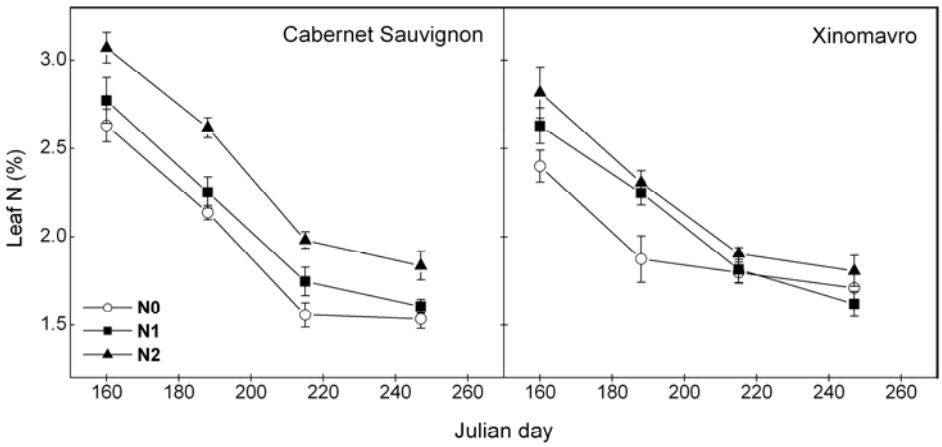

Figure 1. Seasonal variation of leaf N concentration (% dry weight) in Cabernet Sauvignon and Xinomavro; N0, N1 and N2 correspond to 0, 60 and 120 kg N/ha, respectively.

Total Chl (*a*+*b*) followed a similar seasonal pattern with leaf N, decreasing values from d1 to d4 in all N treatments (Fig. 2). Chl *a* was more intensively degraded than Chl *b* (Netto *et al*., 2005) before veraison (d3), especially in Cabernet Sauvignon, whereas Chl *a* and Chl *b* followed an opposite pattern after veraison, with increasing values for Chl *a* (Fig. 2). These results largely explain the late season increase in the Chl *a*/*b* ratio in both varieties. According to Kitajima and Hogan (2003), increase of the Chl *a*/*b* ratio is an indication of plant acclimation to N limitation, conditions that occurred during the late stages of the growing period in this study.

N application significantly increased Chl content in leaves of both varieties, with highest levels in N2 for both cultivars (4.40 and 3.83 mg/g in Cabernet Sauvignon and Xinomavro respectively, pooled data across samplings) while significant differences between N0 and N1 were only observed in Xinomavro (3.11 and 3.43 mg/g respectively). Similar results were obtained when Chl content was expressed per leaf area (data not shown).

A regression using data from the four sampling times (n=72) showed a strong positive correlation between leaf N and extracted Chl in both varieties (p<0.001; Tab. 1 and Tab. 2) suggesting a direct response of Chl synthesis to N levels in leaves (Syvertsen, 1987), with higher coefficients for Chl *a* compared to Chl *b*. All Chl traits were positively correlated with each other with few exceptions (Tab. 1 and Tab. 2). However, variation of total Chl was highly related to changes in Chl *a*, in both varieties.

Leaves of N2 vines had constantly the highest SPAD and CCM readings followed by the respective readings of N1 in both cultivars (Fig. 3). However, Chl meter readings from both devices remained relatively stable during the growth period, with no significant difference between samplings.

SPAD and CCM readings were strongly correlated with each other in both varieties (Tab. 1 and Tab. 2). Although previous studies in grapevines have reported quadratic relationships between SPAD readings and leaf traits (Steele *et al*., 2008), in our study, best-fitted curves between both Chl meter readings and leaf Chl or N were linear (Fanizza *et al*., 1991). This result is probably due to the narrow range of Chl variation in the conditions of this experiment (Jifon *et al*., 2005).

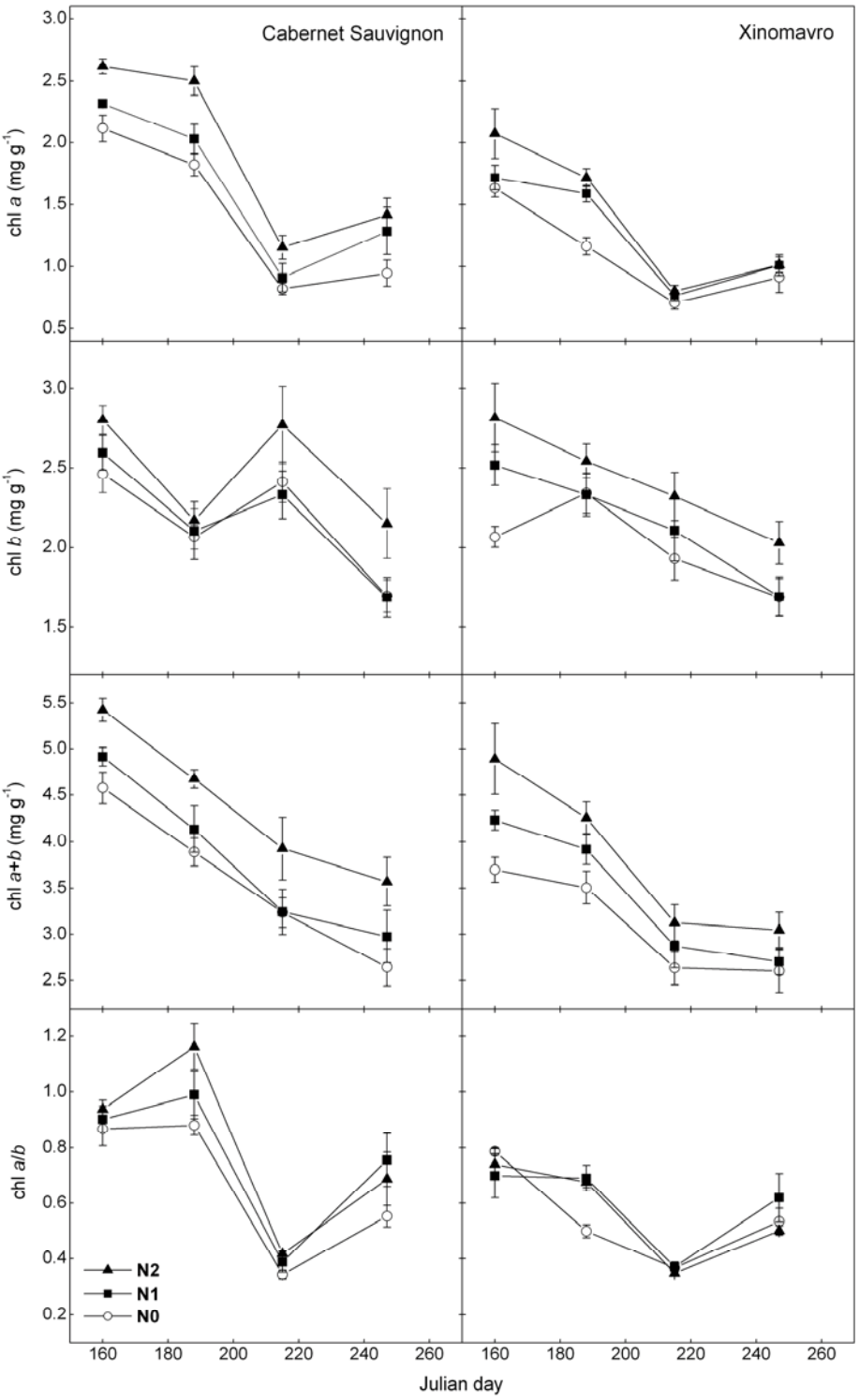

Figure 2. Seasonal variation of leaf chlorophyll [Chl *a*, Chl *b*, Chl (*a*+*b*)] concentration and Chl *a*/*b* ratio in Cabernet Sauvignon and Xinomavro; N0, N1 and N2 correspond to 0, 60 and 120 kg N/ha, respectively.

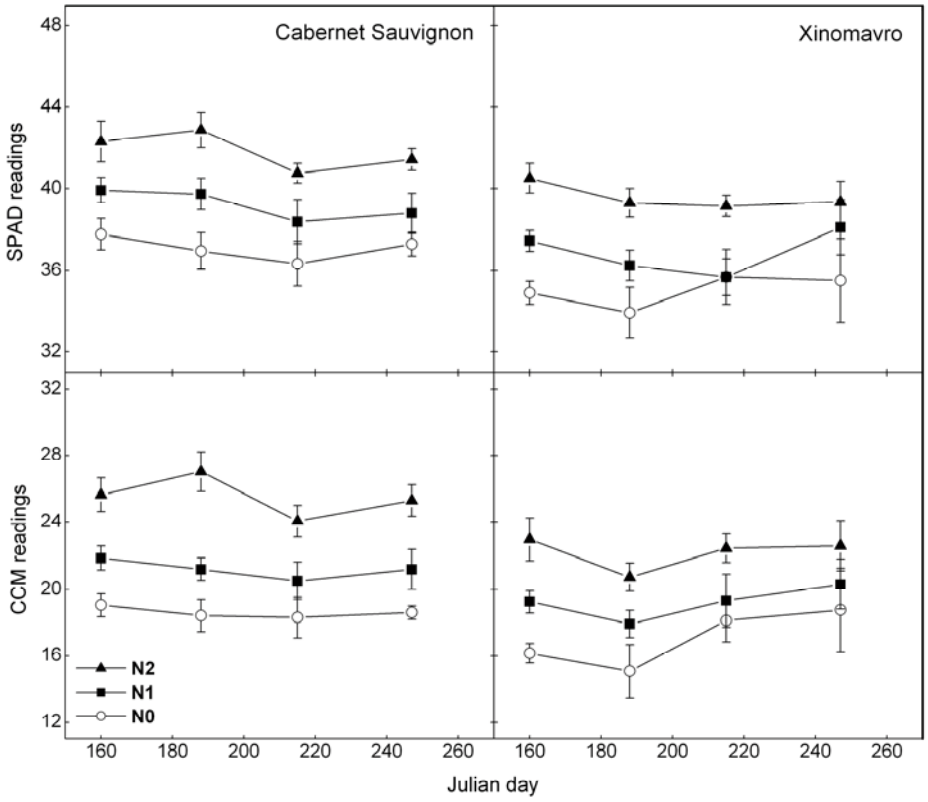

Figure 3. Seasonal variation of SPAD and CCM readings in Cabernet Sauvignon and Xinomavro; N0, N1 and N2 correspond to 0, 60 and 120 kg N/ha, respectively.

In Cabernet Sauvignon, SPAD and CCM readings were significantly correlated with leaf Chl traits and N concentration, although correlation coefficients were low (Tab. 1). Moreover, Chl meter readings were better correlated with Chl *a* than Chl *b*, as previously reported in other plants (Madeira *et al*., 2003). In Xinomavro, relationships were generally poor for SPAD and not significant for CCM (Tab. 2), possibly due to the thicker leaves of this variety (Jiffon *et al*., 2005).

Table 1. Correlation coefficients and significance level for the leaf traits determined in Cabernet Sauvignon. *, **, ***: significant coefficients at $p<0.05$, $p<0.01$, or $p<0.001$, respectively, ns: not significant ($n=72$); Chl *a*, Chl *b*, Chl (*a*+*b*): (mg g^{-1} fresh weight); N (% dry weight).

	Chl *a*	Chl *b*	Chl *a*/*b*	N	SPAD	CCM
Chl (*a*+*b*)	0.878***	0.740***	0.543***	0.878***	0.490***	0.461***
Chl *a*		0.329**	0.868***	0.886***	0.484***	0.437***
Chl *b*			ns	0.488***	0.286*	0.295*
Chl *a*/*b*				0.644***	0.371***	0.329**
N					0.464***	0.419***
SPAD						0.952***

Table 2. Correlation coefficients and significance level for the leaf traits determined in Xinomavro. *, **, ***: significant coefficients at $p<0.05$, $p<0.01$, or $p<0.001$, respectively, ns: not significant ($n=72$); Chl *a*, Chl *b*, Chl (*a*+*b*): (mg g^{-1} fresh weight); N (% dry weight).

	Chl *a*	Chl *b*	Chl *a*/*b*	N	SPAD	CCM
Chl (*a*+*b*)	0.907***	0.893***	0.507***	0.824***	0.348**	ns
Chl *a*		0.620***	0.811***	0.825***	0.294*	ns
Chl *b*			ns	0.659***	0.305**	ns
Chl *a*/*b*				0.545***	ns	ns
N					0.345**	0.246*
SPAD						0.903***

CONCLUSIONS

Leaf Chl and N concentration were linearly related to SPAD and CCM readings in grapevine. However, correlation coefficients were stronger in Cabernet Sauvignon than Xinomavro, especially for the CCM device. Moreover, none of the devices was able to detect the seasonal N and Chl pattern. The results suggest that non-destructive chlorophyll estimations by transmittance-based meters are not applicable in all situations for accurate N status monitoring during vine growth cycle, and that specific calibrations are recommendable to improve their utility and accuracy across grapevine cultivars. However, both devices accurately distinguished N application levels and thus can serve as an indicator of seasonal N nutritional status.

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