Assessing Petiole Iron Content in *Vitis vinifera* ‘Chardonnay’ Using Reflectance Based Hyperspectral Indices

C. Gonzalez-Flor, G. Goreks and L. Serrano
Departament d’Enginyeria Agroalimentària i Biotecnologia
Universitat Politècnica de Catalunya
Barcelona
Spain

Keywords: iron chlorosis, chlorophyll indices, petiole analysis, precision viticulture, canopy, vines

Abstract

Iron chlorosis is a relevant nutritional disorder in vineyards grown in the Mediterranean area, which affects both berry yield and quality. Reflectance based remote sensing techniques might provide a valuable tool to characterize iron chlorosis incidence through the assessment of chlorophyll content. The study was conducted in ten vineyards (*Vitis vinifera* ‘Chardonnay’) to test the capability of reflectance based chlorophyll indices at assessing petiole iron content. Significant relationships emerged between petiole Fe concentration and reflectance based chlorophyll indices. Among the chlorophyll indices tested, the modified Normalized Difference 705 (mND705) and the modified Simple Ratio 705 (mSR705) were found to be significantly related (p < 0.01) to petiole iron concentration with $r^2 = 0.82$ and $r^2 = 0.72$, respectively. In addition, the Chlorophyll Normalized Difference Index was also significantly related to petiole iron concentration although in a lesser extent ($r^2 = 0.53$, p<0.05). The results obtained suggest that narrow-band chlorophyll indices derived from canopy reflectance might provide a useful tool to assess petiole iron concentration in ‘Chardonnay’ vineyards.

INTRODUCTION

Iron chlorosis is a nutritional disorder that affects a wide range of crops in semiarid environments like the Mediterranean region. Since iron is a constituent of chlorophyll thylacoid membranes, its deficiency affects photosynthesis processes (Terry and Abadia, 1986). Particularly, in vineyards, Fe deficiency has been reported to decrease plant vigour while affecting yield and berry composition (Diaz et al., 2010; Martin et al., 2007). In general, iron chlorosis leads to a decrease of must sugar content while total acidity increases or does not vary (Martin et al., 2007).

Traditional methods for assessing iron chlorosis, as blades or petiole analysis, are destructive, time consuming and often inaccurate (i.e. limited number of samples). Alternatively, and because Fe deficiency finally results in an inhibition of chlorophyll synthesis, optical methods might be a valuable tool for early detection of iron chlorosis occurrence while being a rapid and non destructive technique. Attempts to estimate petiole Fe concentration using optical methods such as the SPAD chlorophyll meter (i.e. absorbance in the red and infrared regions) have reported poor correlation between chlorophyll meter readings and petiole Fe concentration (Diaz et al. 2009, 2010). In contrast, recent studies conducted in vineyards have shown the feasibility of reflectance based chlorophyll indices at different scales (from leaf to landscape) to assess iron-induced chlorosis (Zarco-Tejada et al., 2005; Martin et al., 2007; Gil-Pérez et al., 2010). However, to our knowledge, reflectance based chlorophyll indices have not been tested against measurements of petiole iron concentration.

The objective of this study was to test the suitability of reflectance based chlorophyll indices at assessing petiole iron concentration in *Vitis vinifera* ‘Chardonnay’ at the canopy level.
MATERIAL AND METHODS

The study took place in ten commercial vineyards cultivated with Vitis vinifera L. 'Chardonnay'. Plots were located in Alt Penedès region (Barcelona, Spain). Average annual temperatures (1998-2007) range from 13.7 to 15.8°C while cumulative annual precipitation ranges from 266.2 to 612.6 mm.

Vineyard planting characteristics are summarized in Serrano et al. (2012). Spectral data were collected at veraison (16-22 July 2007) on three vines per vineyard using a narrow-bandwidth spectroradiometer (UNISPEC, PP Systems Ltd., Havervill MA, USA) using a 12° field of view forcups (UNI-710, PP Systems Ltd., Havervill MA, USA) positioned 0.75 m above the canopy and oriented to the vine. Irradiance was measured using a cosine corrected lens (UNI-685, PP Systems Ltd., Havervill MA, USA) oriented to the sky. Several reflectance chlorophyll indices were derived from apparent reflectance as reported in Table 1.

Petiole iron concentration (Fe_p) was determined in July 2007. A hundred petiodes were randomly selected within each vineyard. The Fe_p was determined by spectrophotometry (PerkinElmer Model 4300 DV ICP/OES, PerkinElmer, Massachusets, USA), after petiode wet digestion with HNO₃ and H₂O₂.

Analysis of variance and correlation were executed using the statistical packages SPSS 17.0 and SPSS 19.0 (SPSS Inc., Chicago ILL, USA). Vineyard was considered as a source of variation in ANOVA analyses. Means were compared using the Tukey's test and correlation analyses were used to study the relationships between spectral indices and petiole iron concentration.

RESULTS AND DISCUSSION

Petiole iron concentration (Fe_p) ranged between 31 and 66 mg/kg which is considered as medium and above medium content (Calhoun, 1985). Nonetheless, in our study, Fe_p was slightly lower than that reported in previous studies conducted on vineyards grown on calcareous soils without fertilizer application (Díaz et al., 2010).

As iron is a constituent of the chloroplasts thylakoid membrane, iron chlorosis might be assessed through changes in chlorophyll content (Martín et al., 2007). Previous studies have tested the capability of spectral indices at estimating variation in chlorophyll content and concentration in several species (Sims and Gamon, 2002; LéMarie et al., 2004; Serrano, 2008), including studies conducted in vineyards (Steele et al., 2008; Martin et al., 2007; Gil-Pérez et al., 2010). However, in these studies, chlorophyll spectral indices were found to be poorly related to tissue Fe concentration. In contrast, in our study, chlorophyll spectral indices provided confident estimates of Fe_p concentration. Indeed, among the indices tested, Fe_p was found to be closely related to mSR₇⁰₅ and mND₇⁰₅ (R² = 0.72, R² = 0.82; P<0.01; respectively) and in a lesser extend to Chl NDI (R²=0.53; P<0.05) (Fig. 1).

The lack of correlation between tissue Fe concentration and chlorophyll concentration is attributed to a phenomenon known as 'iron chlorosis paradox' (Bavaresco et al., 1999), which describes plants that present chlorotic leaves with high Fe concentration as a result of severe growth inhibition. Thus, the close correlation between chlorophyll spectral indices and Fe_p found in our study might be attributed to the low incidence of iron deficiency so that, reduction in vine growth was probably minor.

Previous studies have documented a close relationship between chlorophyll and leaf blade Fe concentration (Terry and Abadia, 1987) as well as between mineral composition in both blade and petiole tissues (Bavaresco et al., 1997). Therefore, the capability of chlorophyll spectral indices to track Fe_p suggests that, under the conditions of our study, variation in leaf chlorophyll content was largely associated to changes in petiole iron concentration.

In conclusion, in our study, chlorophyll indices derived from spectral reflectance successfully estimated petiole iron concentration. However, due to the low incidence of iron chlorosis found in our study, characterization of the relationships between reflectance based chlorophyll indices with respect to variations in Fe_p over a wide range of chlorosis
occurrence is needed. More studies, particularly at larger scales, are necessary in order to confirm the capability of the reflectance based chlorophyll indices at assessing Fe₆ concentration.

CONCLUSIONS
Our study shows the reliability of three chlorophyll indices, mSR, mND, and Chl NDI, derived from reflectance measurements at the canopy level to assess petiole iron concentration in field grown vineyards. More studies are needed to confirm the results obtained by considering a wider range of iron-induced chlorosis occurrence and cultivars.

ACKNOWLEDGEMENTS
We thank Dr. Ramon Josa for his advice in the field sampling and lab analysis assistance. We also thank to Cidorniu S.L. and, particularly Mr. Antoni Abad, for providing us petiole analysis data.

Literature Cited
Table 1. Summary of the hyperspectral chlorophyll indices tested in this study and their formulation. References are reported in LeMaric et al. (2004) and Serrano et al. (2008).

<table>
<thead>
<tr>
<th>Red-NIR (Red Edge) indices</th>
<th>[ \frac{(R_{750} - R_{705})}{(R_{750} - R_{705})} ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl NDI</td>
<td>[ \frac{R_{750}}{R_{750}} ]</td>
</tr>
<tr>
<td>Chl Index</td>
<td>[ \frac{R_{680}}{R_{680}} ]</td>
</tr>
<tr>
<td>PSSRa</td>
<td>[ \frac{R_{660}}{R_{660}} ]</td>
</tr>
<tr>
<td>PSSRb</td>
<td>[ \frac{R_{900}}{R_{900}} ]</td>
</tr>
<tr>
<td></td>
<td>[ \frac{R_{750}}{R_{750}} ]</td>
</tr>
<tr>
<td>Visible indices</td>
<td>[ \frac{R_{550}}{R_{660}} ]</td>
</tr>
<tr>
<td>G</td>
<td>[ \frac{R_{440}}{R_{680}} ]</td>
</tr>
<tr>
<td>G (Lich)</td>
<td></td>
</tr>
<tr>
<td>Scatter-adjusted indices</td>
<td>[ \frac{(R_{750} - R_{705})}{(R_{750} - R_{705} - 2R_{440})} ]</td>
</tr>
<tr>
<td></td>
<td>[ \frac{(R_{750} - R_{705})}{(R_{750} - R_{440})} ]</td>
</tr>
<tr>
<td></td>
<td>[ \frac{R_{680}}{R_{350} \times R_{750}} ]</td>
</tr>
<tr>
<td></td>
<td>[ \frac{R_{675}}{R_{750}} ]</td>
</tr>
<tr>
<td>First derivative indices</td>
<td>[ \text{mean } [R_{715}; R_{720} / \text{mean } [R_{700}; R_{715}]] ]</td>
</tr>
<tr>
<td>D715/D705</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Relationships between petiole Fe content (Feₚ) and the Chlorophyll Normalized Difference Index (Chl NDI), Modified Simple Ratio 705 (mSR₇₀₅) and Modified Normalized Difference 705 (mNDI₇₀₅). Data are the mean ± SEM of 3 measurements (n=10).