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Potential of a Multiparametric Optical Sensor for Determining in Situ the Maturity Components of Red and White *Vitis vinifera* Wine Grapes

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ABSTRACT: A nondestructive fluorescence-based technique for evaluating *Vitis vinifera* L. grape maturity using a portable sensor (Multiplex) is presented. It provides indices of anthocyanins and chlorophyll in Cabernet Sauvignon, Merlot, and Sangiovese red grapes and of flavonols and chlorophyll in Vermentino white grapes. The good exponential relationship between the anthocyanin index and the actual anthocyanin content determined by wet chemistry was used to estimate grape anthocyanins from *in field* sensor data during ripening. Marked differences were found in the kinetics and the amount of anthocyanins between cultivars and between seasons. A sensor-driven mapping of the anthocyanin content in the grapes, expressed as $g \cdot kg^{-1}$ fresh weight, was performed on a 7-ha vineyard planted with Sangiovese. In the Vermentino, the flavonol index was favorably correlated to the actual content of berry skin flavonols determined by means of HPLC analysis of skin extracts. It was used to make a nondestructive estimate of the evolution in the flavonol concentration in grape berry samplings. The chlorophyll index was inversely correlated in a linear manner to the total soluble solids (°Brix): it could, therefore, be used as a new index of technological maturity. The fluorescence sensor (Multiplex) possesses a high potential for representing an important innovative tool for controlling grape maturity in precision viticulture.

KEYWORDS: *anthocyanins, chlorophyll fluorescence, flavonols, grape maturity, mapping, nondestructive sensors, Vitis vinifera*

INTRODUCTION

In the world of increasing international competition, the challenge to produce high-quality wines requires the introduction of innovative techniques during all phases of the production chain. These range from the training and management of the vines to the quality control and selection of grapes. Temporal and spatial heterogeneity in the characteristics of the raw material, namely grapes, is a fundamental parameter to be controlled and taken into account before and at harvest time, in order to better support decisions relative to the timing of the harvest.[1]

Optical techniques can unquestionably provide useful and innovative tools for achieving this task within the sphere of precision viticulture. Reflectance spectroscopy has been widely employed for controlling vine vigor at different scales, using different tools ranging from satellite,[2] airborne,[3] or Unmanned Aerial Vehicle (UAV)[4] multispectral imaging, to active optical ground sensing devices (GreenSeeker).[5] Thermal and multispectral imagery using an UAV has made it possible to assess and map the water status of vineyards.[6] Remotely sensed multispectral imaging has been proposed for predicting anthocyanins (Anth) and total phenolics in grapes by using the normalized difference vegetation index (NDVI, a common indicator of plant chlorophyll content).[3,7] However, the Pearson correlation coefficients (r) between the NDVI and the grape composition were no better than −0.66 at maturity, and they varied depending on the date of the image acquisition and after canopy trimming.

Recently, new fluorescence-based techniques have been introduced in viticulture for the proximal sensing of phenolic accumulation,[8] the forecasting of nitrogen content,[9] and the control of disease in the plants.[10−12] It was possible to obtain estimates of Anth and flavonols (Flav) by using the nondestructive chlorophyll (Chl) fluorescence excitation screening method:[13] the larger the Anth or Flav concentration in the berry skin, the lower the Chl fluorescence signal.[14] Following these studies, portable LED-based sensors, namely the Multiplex (FORCE-A, Orsay, France), were developed and used directly in the field for the manual measurement of the temporal Anth accumulation of a high number of clusters per plot.[15,16] In this way, it was possible to evaluate the spatial heterogeneity of the Anth content, the latter of which is well correlated to total phenolic compounds,[17] and to report the results on vineyard maps by using the sensor either manually[18,19] or mounted on a harvester for on-the-go sensing.[20] This represents a particularly useful piece of information in precision viticulture if we consider that phenols have greater variability than do technological parameters, such as berry sugar concentration and juice pH.[21]

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A rapid noninvasive index of phenolic maturity in the vineyard is useful for monitoring the variations in Anth accumulation induced by the current severe climate changes as related to irradiance,[22,23] temperature,[24] water availability,[25] land characteristics (soils, topography),[26] and the presence of viruses in the vines.[27] The timing and severity of water deficits during maturation can have positive or negative effects on the phenolic content.[28] Common cultural practices such as cluster thinning can advance fruit ripening,[29] but their effects may depend significantly on seasonal environmental conditions.[30,31]

At present, the *in situ* spectroscopic method based on Chl fluorescence is calibrated for Pinot Noir and Pinot Meunier from the Champagne region in France,[15,32] the Aleatico cultivar from Tuscany, Italy,[16] Shiraz in Australia,[20] Tempranillo in Spain,[19] and the Nero d'Avola cultivar from Sicily, Italy.[33] Further investigations will be needed in order to validate the technique in other widespread *V. vinifera* cultivars (cvs).

Up until now, less attention has been dedicated to the Flav index provided by the Multiplex sensor. In red grapes, this is because of the interference produced by the Anth content on the Flav signals.[16] However, in white grapes the Flav index could represent a new important parameter to be used to monitor the grape content of flavonoids, which are compounds that affect wine quality.[34]

In the present study, we report the results of four years of research that involved testing the potential of the Multiplex fluorescence sensor as an indicator of grape maturity on both red and white grapes. In particular, the Anth accumulation was detected in the vineyard in Cabernet Sauvignon and Merlot cvs over two consecutive seasons. The Flav index and its correlation with the actual content of flavonols in white grapes were evaluated in the Vermentino variety. An example of mapping the Anth content in a vineyard of Sangiovese is also reported. Our research was aimed at broadening our knowledge as regards the application of fluorescence-based sensors in viticulture.

MATERIALS AND METHODS

The evolution of the maturity in red wine grapes was followed in Cabernet Sauvignon (CS) and Merlot (ME) cvs during the 2008 and 2009 seasons in a commercial vineyard at the Tenuta dell'Ornellaia, Bolgheri (Livorno, Italy) (43°14′N, 10°36′E). Vines had been grafted onto 1103 P rootstock and planted, in 1999, in a clay-loam soil. Vines of both varieties had a between-row and within-row spacing of 2.00 m × 0.70 m, respectively, and the row orientation was east to west. The vines were spur-pruned (12 buds per meter of row) in a single cordon and trained in accordance with an upward vertical shoot positioning trellis system. The trellis featured a supporting wire at 0.70 m, two wires at 1.10 m aboveground for protection against wind damage, and a pair of movable shoot-positioned wires at 1.65 m. The vines were not irrigated during the growing season. The shoots were trimmed once at the end of June, after fruit set, leaving 10−12 leaves per shoot. In 2008, veraison took place around 20 July for the ME and 1 August for the CS, while in 2009, it occurred for both cvs approximately 5 days later than it did in 2008. The grapes were harvested on the dates and at the concentrations of total soluble solids (TSS), expressed as °Brix, as reported in Table 1.

Nondestructive optical measurements were performed in the vineyard once a week from the end of July (day of the year (DOY) 206−209) to mid-September (DOY 257−262). In 2008, two adjacent rows per cultivar were chosen and scanned using the Mx fluorimeter sensor, which operated in proximal sensing at a distance of 10 cm from the grape bunches on both sides of the rows. A total number of 160 and 100 bunches for the CS and the ME, respectively, which were distributed equally on the two sides of each row, were sampled once a week. In 2009, 100 bunches for both cvs from both sides of a single row were measured for each measuring date. All measurements were performed in the morning between 9:00 a.m. and 12:00 noon. In order to calibrate the fluorescence sensor for the Anth berry content during the 2008 season, samplings of CS and ME, 3 bunches per cultivar (cv), were randomly collected once a week from veraison until harvest. The grape bunches were measured by the fluorescence sensor before harvest. From each bunch, 19 berries, that is, those filling the 5×10^3 mm^2 circular area of the sensor window, were collected and processed in the laboratory for the extraction and analysis of Anth in accordance with the Glories method.[35] At harvest, the Anth grape concentration of both cvs was determined in 5 samples, each one made up of 150 berries randomly collected all over the vineyard by using the same wet chemistry procedure. Total Anth was expressed as mg per kg of berry fresh weight (FW).

Table 1. Cabernet Sauvignon and Merlot Grape Parameters at Harvest

cultivar, season	harvest time (DOY)	TSS[a] (°Brix)	Anth[a] (mg/kg)	yield[b] (kg/vine)
CS 2008	274	26.8 ± 0.3	2334 ± 309	0.951 ± 0.405 b
CS 2009	272	23.2 ± 0.8	1206 ± 299	1.386 ± 0.316 a
ME 2008	249	26.6 ± 0.7	2471 ± 70	0.736 ± 0.097 c
ME 2009	239	26.1 ± 1.0	1380 ± 204	0.840 ± 0.082 b

[a]Each value is the average (±SD) of $n = 5$ samples. [b]Each value is the average (±SD) of $n = 10$ samples. Means with different letters are significantly different at $P \leq 0.05$.

The concentration of TSS (°Brix) was measured using a PCE-Oe hand refractometer (PCE Italia, Lucca, Italy).

A second experiment was conducted during the 2010 and 2011 seasons at the Bulichella farm, Suvereto (Livorno), (43°04′N, 10°41′E) on the white-grape Vermentino (VE) cultivar. Vines were grafted onto 110R rootstock, planted in a clay-loam soil, and trained as an upward single spur cordon, with approximately 12 buds per meter of row. The vines had a between-row and within-row spacing of 2.5 and 1.0 m, respectively, and were north−south oriented. No water was supplied to the vines during the growing season. The shoots were trimmed once at the end of June, after fruit set, leaving around 12 leaves per shoot. Grapes were harvested at 24°Brix. In order to calibrate the Mx sensor for the Flav content, samples of berries from bunches with differing sun exposure (from 100% sun-exposed to almost complete shade) were collected on different days in 2010 and 2011, so as to cover the widest possible Flav concentration range. Samplings made of 19 grape berries, that is, those filling the 5×10^3 mm^2 circular area of the sensor window, were first measured by the fluorescence sensor and were then extracted and analyzed for their phenolic content as described below. The seasonal time evolution of Flav was followed by means of nondestructive optical measurements on samplings of 150 berries collected at intervals of 10−11 days, from 2 July (DOY 183) to 14 September (DOY 257) 2010. Four replicates per date were used. The sensor data were converted into Flav berry skin concentrations by using the calibration curve derived previously.

The concentration of TSS (°Brix) was measured by means of an OPTECH K71319 hand refractometer (Optical Technology, Munich, Germany).

Fluorescence-Based Sensor. The Multiplex fluorimetric sensor has previously been described in detail.[15] It is based on the detection of fluorescence emitted by Chl in the red (RF) and far-red (FRF) spectral regions, under excitation with different LED sources in the UV (375 nm) and visible (blue at 450 nm, green at 515 nm, and red at 630 nm). The basis of the fluorescence method applied by the Multiplex sensor is schematized in Figure 1. The intensity of the chlorophyll fluorescence (ChlF) emitted by a grape berry depends on the amount of excitation light able to reach the Chl pigment present inside the chloroplasts of the berry cells.[36] By considering a cross section of a red berry skin, we can model the Anth-containing cell layers localized above the Chl-containing cell layers. Anth can then attenuate part of the incident light before this can reach the Chl molecules.

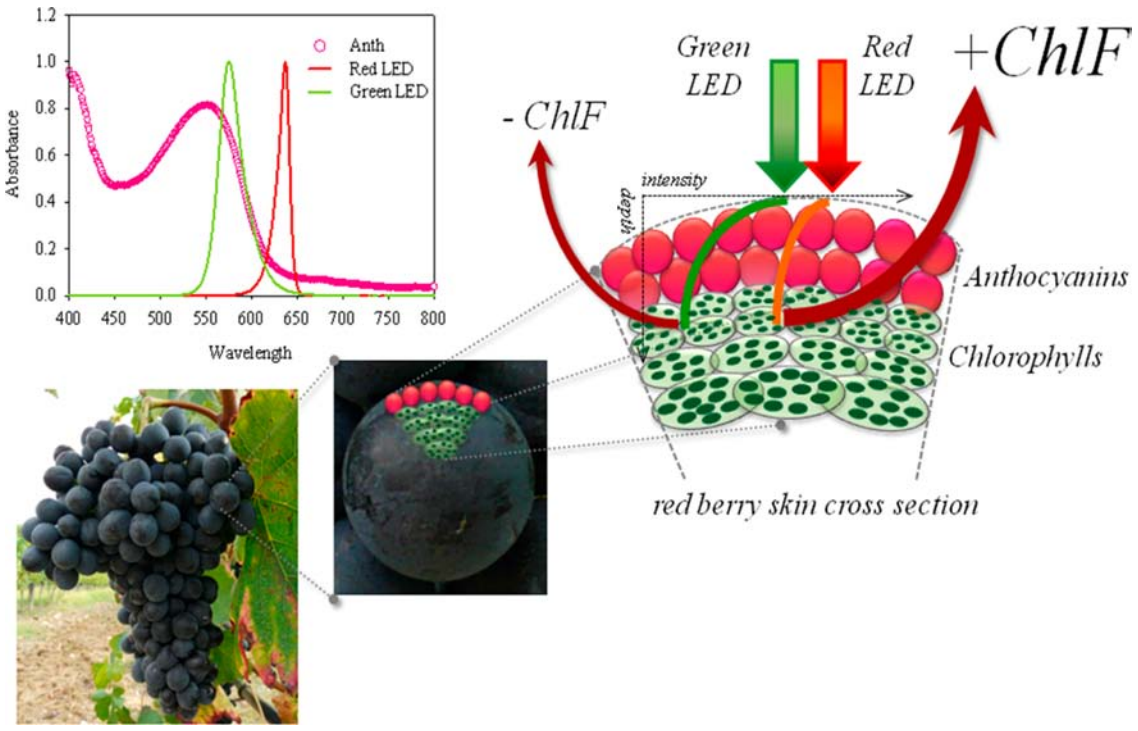

Figure 1. Schematization of the Chl fluorescence screening method based on the filtering effect of excitation light by compounds in the grape berry exocarp located above the Chl layer. Anthocyanins are located in the vacuoles of the cells, whereas chlorophylls are located in the internal membranes of the chloroplasts. The intensity of the excitation lights is exponentially attenuated as a function of the depth inside the berry. The extent of attenuation depends on the concentration of anthocyanins and the waveband of irradiation. Absorption spectrum of anthocyanins and emission bands of green and red LEDs (left-top). Higher (+ChlF) and lower (-ChlF) Chl fluorescence correspond to the lesser and greater attenuation of red and green lights, respectively.

Consequently, the higher the Anth concentration, the lower the ChlF intensity. The extent of the Anth attenuation also depends on the spectral band of the excitation light. Since Anth absorbs mainly in the green around 520 nm (see the absorption spectrum in the left-top corner of Figure 1), green excitation light will be attenuated more than, e.g., red light, which is in a spectral zone characterized by a weak Anth absorption. The ChlF detected will be significantly lower under green excitation (-ChlF) than under red light excitation (+ChlF). By comparing the two fluorescence signals, it is possible to obtain an index that is proportional to the berry skin Anth content.

Here, instead of the former definition of the Anth index as $ANTH_{RG}$, we used the opposite formula:

$$ANTH_{GR} = -ANTH_{RG} = \log(FRF_G/FRF_R) \quad (1)$$

where FRF_G and FRF_R are the far-red ChlF excited in the green and red, respectively, in order to obtain an index that increases monotonically with the Anth concentration from complete veraison to harvest. The definition of a fluorescence index for flavonols in the berry skin follows the same principle as above. In this case, since Flav attenuate ultraviolet radiation (they attain maximum absorption at 350 nm), the Flav index is obtained by comparing the ChlF signals excited by UV and by red light.

$$FLAV = \log(FRF_R/FRF_{UV}) \quad (2)$$

The ratio between FRF and RF under red excitation, namely,

$$CHL = FRF_R/RF_R \quad (3)$$

which was previously denoted as the simple fluorescence ratio (SFR_R), can be used as a Chl index, due to the partial reabsorption of RF by the Chl itself.[37]

Further details on the origin of the above equations can be found in the literature.[14,15,38]

Mapping of Anth Grape Content. In 2012, a 7-ha vineyard at the 'Cantina Vignaioli del Morellino di Scansano Soc. Coop Agricola' in Valle Maggiore (Grosseto, Italy), planted with Sangiovese vines, with vine and row spacing of 0.6 and 3 m, respectively, was manually scanned by the Mx sensor on a 15 m × 15 m sampling grid. For each spatial point, the first bunch close to the cordon on three adjacent vines was measured and the average of the three measurements was then computed. The plot was measured within 8 h on 19 September, 2012, just prior to harvest. The $ANTH_{GR}$ values were converted into Anth concentration (g kg^{-1} FW) by using a calibration curve acquired previously that is specifically for the Sangiovese cultivar. A specific software (Surface Mapping System Surfer 11.0.642, Golden Software, Inc.) was used for the geostatistical analysis, by computing the variogram that represented the best model fit of data and mapping the Anth values by means of kriging analysis. Thanks to the knowledge of the Global Position System (GPS) coordinates of each point measured by the Mx sensor, it was also possible to export data to a virtual globe information software (Google Earth, Mountain View, CA, USA), in order to superimpose maps on satellite or aerial photography images.

Analysis of Berry Flav Content. The concentrations of Flav, expressed as μg/g of skins, equivalents of quercetin-3-*O*-glucoside, were obtained by means of HPLC analysis and following a slightly modified Downey procedure[39,40] by using a LC1260 system with DAD detection and a Poroshell 120 EC-C18 column (4.6 mm × 150 mm, 2.7 μm) (both from Agilent Technologies, Palo Alto, CA, USA). In brief, about 0.4 g of frozen ground grape skin powder were extracted with 2 mL of 30% methanol in water. The supernatant (50 μL) was injected in the HLPC system. Chromatographic separation was achieved by using a linear gradient from 10% formic acid in water to 10% formic acid in methanol, at a flow rate of 0.8 mL/min. Flavonols were monitored by DAD detection at 354 nm. Identification of the individual component peaks was performed by making a comparison of retention times and UV−vis absorption data with those found in the literature.[40] Quantification was performed by using quercetin-3-*O*-glucoside as an external standard for building the calibration curve.

Statistical Analysis. Statistical analysis and curve fitting were carried out with SigmaPlot for Windows Version 11.0 (Systat Software, Inc.). The results are given as mean ± standard deviations (SD).

RESULTS AND DISCUSSION

Calibration Curve for the Anth Index. The *in-field* nondestructive evaluation of the Anth grape concentration required a calibration of the Multiplex indices by means of wet chemistry. In Figure 2, the relationship between the *in situ*

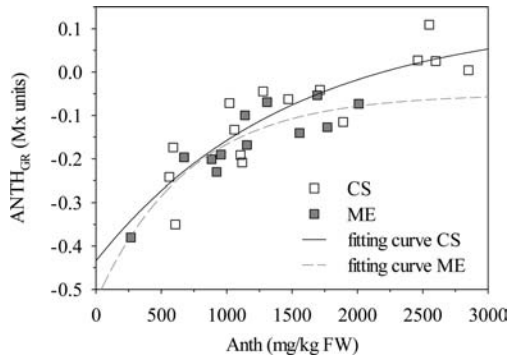

Figure 2. Calibration curves for the Mx ANTH$_{GR}$ index computed by the Anth content (mg/kg FW) results of the destructive analysis. Fitting curve equations were ANTH$_{GR}$ (ME) = −0.527 + 0.476[1 − exp (−0.001Anth)] and ANTH$_{GR}$ (CS) = −0.434 + 0.56[1 − exp(−6.8 × 10^{-4})Anth], with R^2 of 0.83 and 0.75, respectively.

ANTH$_{GR}$ index and the relative Anth content determined by the destructive analysis of the same grape bunches is reported for the CS and ME cvs. Data were satisfactorily fitted (coefficient of determination (R^2) of 0.83 and 0.75 for ME and CS, respectively) by means of a rising exponential curve. The increase in ANTH$_{GR}$ with an increase in the Anth concentration was in accordance with all other studies on different cvs in which the opposite Anth index, ANTH$_{RG}$, was calibrated against wet chemistry.[16,19,20] The curvilinear relationship between ANTH$_{GR}$ (or ANTH$_{RG}$) and the Anth berry content was predicted by the theory based on the absorbance spectral properties of Anth.[15] Our data represent the first report of Anth index calibration curves for the CS and ME cvs.

***In-Field* Estimate of Anth Bunch Content.** Inversion of the calibration curves made it possible to estimate the content of Anth from the ANTH$_{GR}$ index detected in the vineyards. The time course of the Anth estimated from the fluorescence sensor measurements for CS and ME during two consecutive seasons is reported in Figure 3. For both cvs, the biosynthesis of Anth in 2009 was begun earlier as compared to 2008. While for the ME similar mean values of total Anth were found during both seasons, the CS Anth accumulation in 2009 was markedly lower than that in 2008. Large seasonal variability in the CS Anth had been reported previously.[21,41] It is likely that the lesser accumulation of Anth in 2009 with respect to 2008 was due to a faster vine growth determined by more favorable environmental conditions. In fact, 2009 was characterized by higher rainfalls and milder summer temperatures as compared with 2008, thus leading to both greater vegetative growth and yield, and, consequently, lower berry concentrations, as reported in Table 1.

Anth Spatial Distribution. The Multiplex sensor could also be employed for mapping grape quality, more precisely Anth content, over large areas of the vineyard. In Figure 4, an

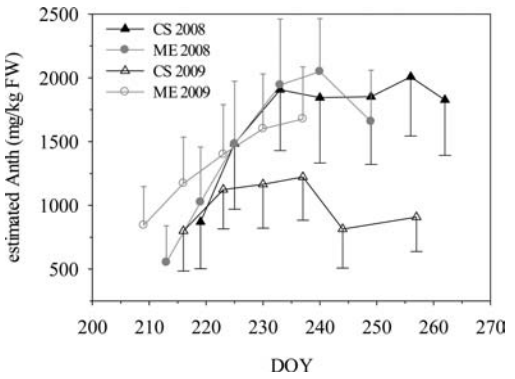

Figure 3. Time evolution of the Anth berry concentration estimates determined by the *in-field* Mx measurements and the inverted calibration curves for both the ME and the CS during the 2008 and 2009 seasons. Each point of CS 2008 is the mean of 160 bunches equally distributed over two adjacent rows, both sides per row. For CS 2009, ME 2008, and ME 2009, each point is the mean of 100 bunches. The last points of ME were measured at harvest, and the last points of CS were measured 2-weeks before harvest. The bars represent SDs.

example of the spatial distribution of the Anth bunch concentration obtained using the hand-held Multiplex detector for the Sangiovese cv is presented. One thousand and sixty-three clusters over a 7-ha plot were measured. Figure 4A shows, using a colorimetric scale, the spatial heterogeneity of the Anth bunch content within the vineyard. It was possible to identify two areas, in red, in which the phenolic maturity was higher as compared to the rest of the vineyard, with an Anth concentration of around 1.8 g kg^{-1} FW. Four localized areas, in blue, indicate low phenolic maturity, with an Anth content of 1−1.2 g kg^{-1} FW. The producer will be able to use this information in order to examine the origin of the Anth spatial differences in terms of soil nutrition and drainage, stress factors, vigor management, and diseases and to operate in such a way as to make the vineyard more homogeneous. By means of segmentation, which signifies dividing all the Anth values into two parts based on the median or a value considered significant by the producer, it was possible to represent the Anth content map in two colored zones (Figure 4C), with higher and lower phenolic content. This makes it possible to proceed with a selective harvest in order to produce two wines differing in quality and, consequently, in price. Figure 4B and D shows the two colorimetric maps directly on Google Earth images. By downloading them into a smartphone, the producer would be able to go directly to his vineyard and identify, with great precision, the areas in which to operate.

Calibration Curve for the Flav Index. The main flavonol compounds found in white-grape berry skins by means of the HPLC analysis were quercetin-3-*O*-glucuronide and quercetin-3-*O*-glucoside, which accounted on average for 67% and 23%, respectively, of the total. Kaempferol-3-*O*-galactoside, kaempferol-3-*O*-glucuronide, and kaempferol-3-*O*-glucoside were present as minor compounds, namely as 6%, 2%, and 2%, respectively, of the total. The large variability found in the Flav content of berry samples, i.e. from 200 to 2700, was mainly due to the well-known response of flavonol accumulation to exposure to sunlight in grapevine leaves[10,42] and berries.[43−46]

The nondestructive FLAV index was satisfactorily correlated directly to the Flav berry skin content determined by the destructive analysis and computed as the sum of the quercetin and kaempferol glycoside compounds (Figure 5). The best

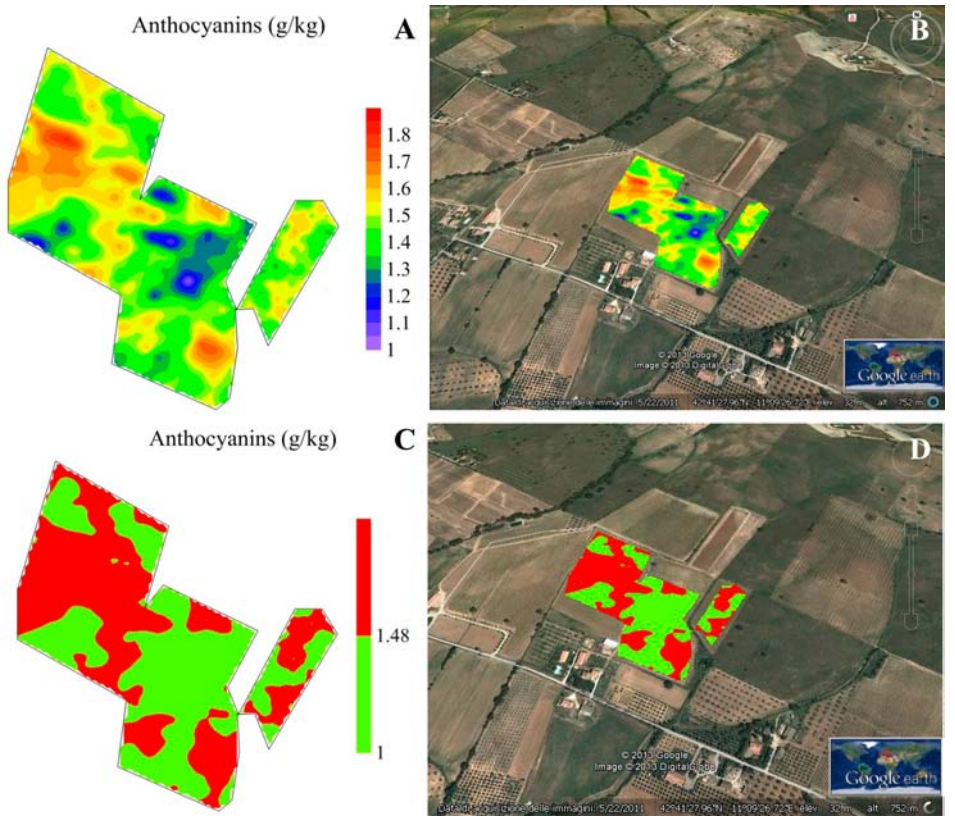

Figure 4. Spatial distribution of Anth bunch concentration (g kg^{-1} FW) estimated by the $ANTH_{GR}$ Multiplex index for the Sangiovese cv on a 7-ha plot in Tuscany. One thousand and sixthy-three clusters were measured manually with the Mx in September 2012, just before harvest time. (A) Rainbow map of Anth distribution and (B) the same map exported to the corresponding Google Earth image. (C) Segmented map of Anth distribution based on data median and (D) the same map exported to the corresponding Google Earth image.

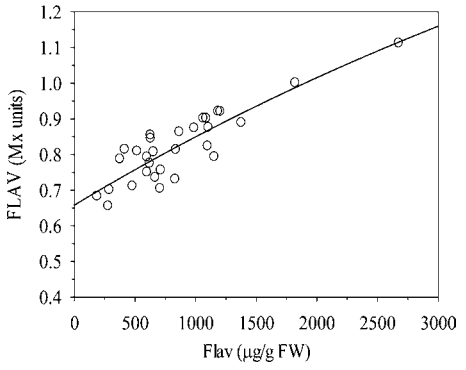

Figure 5. Calibration curve for the Mx FLAV index computed by using the berry skin flavonol content derived from the destructive HPLC analysis of Vermentino samplings during the 2010 and 2011 seasons. The fitting curve equation was FLAV = 0.659 + 1.445{1 − exp[(−1.42 × 10^{-4})Flav]}, with R^2 of 0.766.

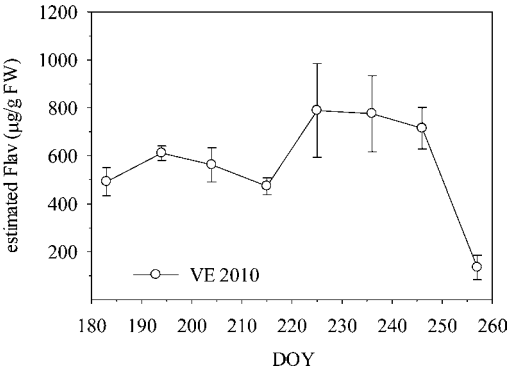

Figure 6. Time course of the Flav berry skin concentration estimates determined by the Mx measurements of samplings in 2010 and the inverted calibration curve for the VE cv. Single points are the mean (±SD) of 4 samples, each one made up of 20 berries.

fitting curve was exponential (R^2 of 0.766), even if the linear regression FLAV = 0.674 + 1.717 × 10^{-4}·Flav was similarly valid (R^2 = 0.764). This result supported the effectiveness of the fluorescence sensor in determining the flavonol content in the berry skin of white grapes.

Evolution of Flavonols in White Grapes. Inversion of the above calibration curve made it possible to estimate the Flav content from the FLAV nondestructive index detected in the berry samplings. The time course of the Flav concentration estimated by the fluorescence sensor measurements for VE during the 2010 season is reported in Figure 6. Flav remained almost constant at around 550 μg/g FW until the beginning of veraison (around DOY 215); a sharp increase to around 800 μg/g FW was then observed within 10 days. A plateau was maintained, within experimental errors, until DOY 246, when the Flav concentration dropped rapidly to less than 20% of the maximum.

A similar time course for the Flav had previously been observed in the Erbaluce white grape cv by using wet chemistry.[47] Explaining the change in the grape Flav concentration during the entire season is difficult, since single compounds may follow different kinetics. Gregan et al.[34] observed that, in the Sauvignon Blanc cv, the quercetin-3-*O*-

glucuronide decreased from veraison to harvest, while the quercetin-3-O-glucoside and kaempferol-3-*O*-glucoside increased through development and reached a maximum at harvest time. Furthermore, Flav accumulation and degradation[48] can clearly be affected by climatic conditions and sun exposure. The increase in Flav observed during veraison is consistent with the evolution in the expression of flavonol synthase genes observed in the Chardonnay and Shiraz cvs.[49] On the other hand, we are at present unable to offer any explanation for the drop in Flav that occurred at harvest.

Since the level of Flav in the clusters reached at harvest was maintained in the derived wines,[43] the possibility of controlling *in vivo* these compounds, with their very healthy properties,[50] appears to be of considerable importance.

The aroma potential of grape berries was found to be increased by exposure to sunlight.[51,52] Since Flav are also positively correlated to sun exposure, the FLAV index, as a proxy of white-grape bunch irradiation, could indirectly provide information on the level of aroma-related compounds.

Chlorophyll Index. In addition to information on the polyphenol content, the Multiplex sensor can also provide an index of the Chl concentration of grape berries (CHL). The time course of CHL for the CS and ME cvs in 2008 and 2009 is shown in Figure 7A. At the starting time of monitoring, the higher values of CHL in 2008 as compared to 2009 indicated early grape maturity in the latter season, in accordance with the decrease with ripening in the Chl berry content. We could also observe that the CHL evolution curves showed a delayed maturity in the CS as compared to the ME. This result fitted with the evidence obtained by means of the time evolution of the *in-field* estimated Anth contents reported in Figure 3. The CHL index can therefore provide complementary indications for evaluating the ripening stage of the grapes. This parameter could be particularly useful for the nondestructive optical monitoring of maturity in Anth-free white-grape cvs.

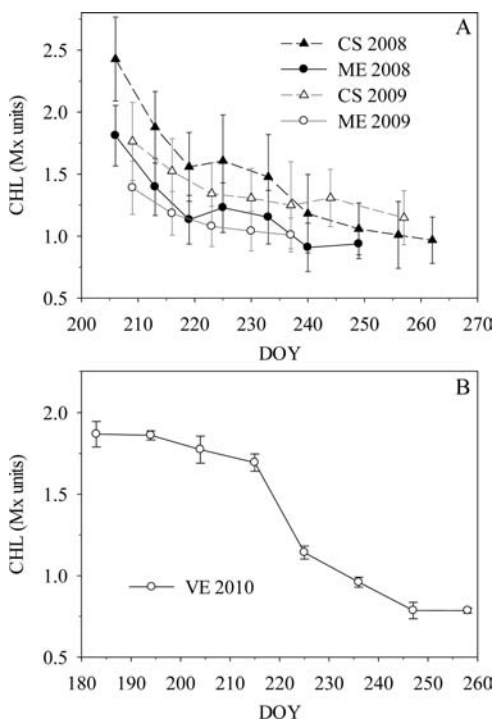

Figure 7. Time course of the Multiplex chlorophyll index (CHL) for the Cabernet Sauvignon (CS) and Merlot (ME) during the 2008 and 2009 seasons (A) and for the Vermentino (VE) in 2010 (B). Error bars are standard deviations. Measuring conditions were as in Figure 3 for the CS and ME and in Figure 6 for the VE.

The time course of CHL for the VE cv in 2010 is shown in Figure 7B. Here, we have a complete representation of the Chl change in berries from preveraison to harvest time. It is worthwhile drawing attention to the prominent change, during veraison, in the CHL curve slope between DOY 215 and 225. CHL can then be used as an alternative and more precise indicator of the veraison setting. The time evolution of the Chl content predicted by the Multiplex sensor was similar to that obtained by means of destructive analyses on the Erbaluce, Barbera, and Nebbiolo Italian varieties.[47]

Technological Maturity Parameters. Based on previous observations of the presence of an inverse relationship between Chl fluorescence and sugar contents in the Bacchus and Silvaner cvs,[53] and by considering the time evolution of CHL, we checked to see whether this index could provide information on the technological maturity of grapes. Indeed, we found a good inverse correlation between the CHL index and the TSS measured as °Brix on the same berry samples (Figure 8). This had previously been observed for Pinot cvs.[15]

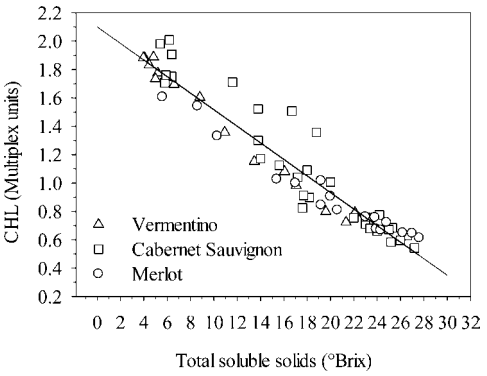

Figure 8. Relationship between the Multiplex index for chlorophyll (CHL) and berry TSS (°Brix) for the Cabernet Sauvignon (squares), Merlot (circles), and Vermentino (triangles) cvs. The solid line indicates the linear curve fitting ($y = 2.10 - 0.058x$).

Here, we have presented evidence that the CHL index can be a good proxy of TSS (°Brix) for both red (CS and ME) and white (VE) grape cultivars, as reported in Figure 8. The advantage of this technique in evaluating the berry sugar content with respect to the destructive refractometric method lies in the possibility that it offers increased size of the sampling evaluation in the same time interval. In fact, there is no need for berry collection and sample squeezing, and several berries (19 berries, that is, those filling the 5×10^3 mm^2 circular area of the sensor window) can be measured within 1 s.

Conclusion. The good correlation found between wet chemistry and Anth Multiplex data during the berry ripening of the CS and ME cvs and those previously reported in the literature suggests this new fluorimetric technique as a green analytical tool in alternative to time-consuming, costly, and environmentally-unfriendly standard laboratory phenolic analyses. Portable optical sensors not only solve the problem of analyzing a large number of samples, or even the whole crop, directly in the vineyard, but also offer the possibility of following the same bunches during the whole season and of repeating the optical measurements with greater frequency.

Combining the assessment of the Anth and Chl grape constituents under temporal and spatial monitoring provides a complete set of decision supports in viticulture for defining the best harvest time and the best place for harvesting.

Mounting the fluorescence sensor on a tractor makes it possible to obtain information on the Anth bunch content on large areas of a vineyard, in order to control viticultural practices or to have a general characterization of the vineyard in question.

Although there is no direct link between sugar content and Chl fluorescence, the Multiplex sensor can provide information on the degree of technological maturity for both red and white grapes.

The possibility of evaluating nondestructively the accumulation of flavonol compounds in white-grape berries represents quite an innovative aspect in viticulture and opens up new research lines for a better understanding of the seasonal evolution of flavonols. However, that the wet chemistry must still be used when it is desired to monitor single flavonol compounds represents a limitation of the method.

Further experimental studies in order to understand whether the Flav index in Anth-free varieties can be used to predict wine-quality parameters related to sun exposure, such as aromatic compounds, will be necessary.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

Anth, anthocyanin(s); $ANTH_{GR}$, anthocyanin nondestructive index; Chl, chlorophyll; CHL, chlorophyll nondestructive index; ChlF, chlorophyll fluorescence; CS, Cabernet Sauvignon; cv(s), cultivar(s); DOY, day of the year; Flav, flavonol(s); FLAV, flavonol nondestructive index; FW, fresh weight; ME, Merlot; Mx, Multiplex; R^2, coefficient of determination; TSS, total soluble solids; VE, Vermentino

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on December 5, 2013, with an error to Figure 8. The corrected version was published with the issue on December 18, 2013.