

This document is the Accepted Manuscript version of a Published Work that appeared in final form in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, copyright © American Chemical Society after peer review and technical editing by the publisher.

To access the final edited and published work see
<http://pubs.acs.org/doi/pdf/10.1021/jf405099n>

This document is confidential and is proprietary to the American Chemical Society and its authors. Do not copy or disclose without written permission. If you have received this item in error, notify the sender and delete all copies.

The potential of a multiparametric optical sensor for determining in situ the maturity components of red and white *Vitis vinifera* wine grapes

Journal:	<i>Journal of Agricultural and Food Chemistry</i>
Manuscript ID:	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Agati, Giovanni; CNR, IFAC D'Onofrio, Claudio; University of Pisa, Department of Agriculture, Food and Environment Ducci, Eleonora; University of Pisa, Department of Agriculture, Food and Environment Cuzzola, Angela; University of Pisa, Department of Agriculture, Food and Environment Tuccio, Lorenza; CNR, IFAC, Remorini, Damiano; University of Pisa, Department of Agriculture, Food and Environment Lazzini, Francesca; Università degli Studi di Firenze, Di.S.P.A.A. Mattii, Giovanni; Università degli Studi di Firenze, Di.S.P.A.A.

SCHOLARONE™
Manuscripts

1 **The potential of a multiparametric optical sensor for determining in situ the**
2 **maturity components of red and white *Vitis vinifera* wine grapes**

3 Giovanni Agati*[†], Claudio D'Onofrio[‡], Eleonora Ducci[‡], Angela Cuzzola[‡], Damiano Remorini[‡],

4 Lorenza Tuccio^{†‡}, Francesca Lazzini[§], Giovanni Mattii[§]

5

6 [†]Istituto di Fisica Applicata 'N. Carrara' – CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino

7 (FI), Italy

8 [‡]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80,

9 56124 Pisa, Italy

10 [§]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Viale delle

11 Idee 30, 50019 Sesto Fiorentino (FI), Italy

12

13 Corresponding Author

14 *Phone: + 39 055 5225 306. Fax: + 39 055 5225 305. E-mail: g.agati@ifac.cnr.it

15

16

17 ABSTRACT: A non-destructive fluorescence-based technique for evaluating *Vitis vinifera* L. grape
18 maturity using a portable sensor (Multiplex[®]) is presented. It provides indices of anthocyanins and
19 chlorophyll in Cabernet Sauvignon, Merlot and Sangiovese red grapes and of flavonols and
20 chlorophyll in Vermentino white grapes. The good exponential relationship between the
21 anthocyanin index and the actual anthocyanin content determined by wet chemistry was used to
22 estimate grape anthocyanins from *in field* sensor data during ripening. Marked differences were
23 found in the kinetics and the amount of anthocyanins between cultivars and between seasons. A
24 sensor-driven mapping of the anthocyanin content in the grapes, expressed as g·kg⁻¹ fresh weight,

25 was performed on a 7-ha vineyard planted with Sangiovese. In the Vermentino, the flavonol index
26 was favorably correlated to the actual content of berry skin flavonols determined by means of
27 HPLC analysis of skin extracts. It was used to make a non-destructive estimate of the evolution in
28 the flavonol concentration in grape berry samplings. The chlorophyll index was inversely correlated
29 in linear manner to the total soluble solids (°Brix): it could, therefore, be used as a new index of
30 technological maturity. The fluorescence sensor (Multiplex) possesses a high potential for
31 representing an important innovative tool for controlling grape maturity in precision viticulture.

32

33 KEYWORDS: Anthocyanins, chlorophyll fluorescence, flavonols, grape maturity, mapping, non-
34 destructive sensors, *Vitis vinifera*.

35

36 INTRODUCTION

37 In the world of increasing international competition, the challenge to produce high-quality
38 wines requires the introduction of innovative techniques during all phases of the production chain.
39 These range from the training and management of the vines to the quality control and selection of
40 grapes. Temporal and spatial heterogeneity in the characteristics of the raw material, namely grapes,
41 is a fundamental parameter to be controlled and taken into account before and at harvest time, in
42 order to better support decisions relative to the timing of the harvest¹.

43 Optical techniques can unquestionably provide useful and innovative tools for achieving this
44 task within the sphere of precision viticulture. Reflectance spectroscopy has been widely employed
45 for controlling vine vigor at different scales, using different tools ranging from satellite², airborne³
46 or Unmanned Aerial Vehicle (UAV)⁴ multispectral imaging, to active optical ground sensing devices
47 (GreenSeeker)⁵. Thermal and multispectral imagery using an UAV have made it possible to assess
48 and map the water status of vineyards⁶. Remotely-sensed multispectral imaging has been proposed
49 for predicting anthocyanins (Anth) and total phenolics in grapes by using the normalized difference
50 vegetation index (NDVI, a common indicator of plant chlorophyll content)^{3,7}. However, the Pearson

51 correlation coefficients (r) between the NDVI and the grape composition were no better than -0.66
52 at maturity, and varied depending on the date of the image acquisition and after canopy trimming.

53 Recently, new fluorescence-based techniques have been introduced in viticulture for the
54 proximal sensing of phenolic accumulation⁸, the forecasting of nitrogen content⁹, and the control of
55 disease in the plants¹⁰⁻¹². It was possible to obtain estimates of Anth and flavonols (Flav) by using
56 the non-destructive chlorophyll (Chl) fluorescence excitation screening method¹³: the larger the
57 Anth or Flav concentration in the berry skin, the lower the Chl fluorescence signal¹⁴. Following
58 these studies, portable LED-based sensors, namely the Multiplex[®] (FORCE-A, Orsay, France),
59 were developed and used directly in the field to for the manual measurement of the temporal Anth
60 accumulation of a high number of clusters per plot^{15,16}. In this way, it was possible to evaluate the
61 spatial heterogeneity of the Anth content, the latter of which is well correlated to total phenolic
62 compounds¹⁷, and to report the results on vineyard maps by using the sensor either manually^{18,19} or
63 mounted on a harvester for on-the-go sensing²⁰. This represents a particularly useful piece of
64 information in precision viticulture if we consider that phenols have greater variability than do
65 technological parameters, such as berry sugar concentration and juice pH²¹.

66 A rapid non-invasive index of phenolic maturity in the vineyard is useful for monitoring the
67 variations in Anth accumulation induced by the current severe climate changes as related to
68 irradiance^{22,23}, temperature²⁴, water availability²⁵, land characteristics (soils, topography)²⁶, and the
69 presence of viruses in the vines²⁷. The timing and severity of water deficits during maturation can
70 have positive or negative effects on the phenolic content²⁸. Common cultural practices such as
71 cluster thinning can advance fruit ripening²⁹, but their effects may depend significantly on seasonal
72 environmental conditions^{30,31}.

73 At present, the *in situ* spectroscopic method based on Chl fluorescence is calibrated for Pinot
74 Noir and Pinot Meunier from the Champagne region in France^{15,32}, the Aleatico cultivar from
75 Tuscany, Italy¹⁶, Shiraz in Australia²⁰, Tempranillo in Spain¹⁹, and the Nero d'Avola cultivar from

76 Sicily, Italy³³. Further investigations will be needed in order to validate the technique in other
77 widespread *V. vinifera* cultivars (cvs).

78 Up until now, less attention has been dedicated to the Flav index provided by the Multiplex
79 sensor. In red grapes, this is because of the interference produced by the Anth content on the Flav
80 signals¹⁶. However, in white grapes the Flav index could represent a new important parameter to be
81 used to monitor the grape content of flavonoids, which are compounds that affect wine quality³⁴.

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

89

90 MATERIALS AND METHODS

91 The evolution of the maturity in red wine grapes was followed in Cabernet Sauvignon (CS) and
92 Merlot (ME) cvs during the 2008 and 2009 seasons in a commercial vineyard at the Tenuta
93 dell'Ornellaia, Bolgheri (Livorno, Italy), (43°14'N, 10°36'E). Vines had been grafted onto 1103 P
94 rootstock and planted, in 1999, in a clay-loam soil. Vines of both varieties had a between-row and
95 within-row spacing of 2.00 m x 0.70 m, respectively, and the row orientation was east to west. The
96 vines were spur-pruned (12 buds per meter of row) in a single cordon and trained in accordance
97 with an upward vertical shoot positioning trellis system. The trellis featured a supporting wire at
98 0.70 m, two wires at 1.10 m aboveground for protection against wind damage, and a pair of
99 movable shoot-positioned wires at 1.65 m. The vines were not irrigated during the growing season.
100 The shoots were trimmed once at the end of June, after fruit set, leaving 10-12 leaves per shoot. In
101 2008, veraison took place around 20 July for the ME and 1 August for the CS, while in 2009, it

102 occurred for both cvs approximately 5 days later than it did in 2008. The grapes were harvested on
103 the dates and at the concentrations of total soluble solids (TSS), expressed as °Brix, as reported in
104 Table 1.

105 Non-destructive optical measurements were performed in the vineyard once a week from the
106 end of July (day of the year (DOY) 206-209) to mid-September (DOY 257-262). In 2008, two
107 adjacent rows per cultivar were chosen and scanned using the Mx fluorimeter sensor, which
108 operated in proximal sensing at a distance of 10 cm from the grape bunches on both sides of the
109 rows. A total number of 160 and 100 bunches for the CS and the ME, respectively, which were
110 distributed equally on the two sides of each row, were sampled once a week. In 2009, 100 bunches
111 for both cvs from both sides of a single row were measured for each measuring date. All
112 measurements were performed in the morning between 9:00 a.m. and 12:00 noon. In order to
113 calibrate the fluorescence sensor for the Anth berry content during the 2008 season, samplings of
114 CS and ME, 3 bunches per cultivar (cv), were randomly collected once a week from veraison until
115 harvest. The grape bunches were measured by the fluorescence sensor before harvest. From each
116 bunch, 19 berries, that is, those filling the $5 \times 10^3 \text{ mm}^2$ circular area of the sensor window, were
117 collected and processed in the laboratory for the extraction and analysis of Anth in accordance with
118 the Glories method³⁵. At harvest, the Anth grape concentration of both cvs was determined in 5
119 samples, each one made up of 150 berries randomly collected all over the vineyard by using the
120 same wet chemistry procedure. Total Anth were expressed as mg per kg of berry fresh weight (FW).
121 The concentration of TSS (°Brix) was measured using a PCE-Oe hand refractometer (PCE Italia,
122 Lucca, Italy).

123 A second experiment was conducted during the 2010 and 2011 seasons at the Bulichella
124 farm, Suvereto (Livorno), (43°04'N, 10°41'E) on the white-grape Vermentino (VE) cultivar. Vines
125 were grafted onto 110R rootstock, planted in a clay-loam soil, and were trained as an upward single
126 spur cordon, with approximately 12 buds per meter of row. The vines had a between-row and
127 within-row spacing of 2.5 m and 1.0 m, respectively, and were north-south oriented. No water was

128 supplied to the vines during the growing season. The shoots were trimmed once at the end of June,
129 after fruit set, leaving around 12 leaves per shoot. Grapes were harvested at 24 °Brix. In order to
130 calibrate the Mx sensor for the Flav content, samples of berries from bunches with differing sun
131 exposure (from 100% sun-exposed to almost complete shade) were collected on different days in
132 2010 and 2011, so as to cover the widest possible Flav concentration range. Samplings made of 19
133 grape berries, that is, those filling the $5 \cdot 10^3 \text{ mm}^2$ circular area of the sensor window, were first
134 measured by the fluorescence sensor and were then extracted and analyzed for their phenolic
135 content as described below. The seasonal time evolution of Flav was followed by means of non-
136 destructive optical measurements on samplings of 150 berries collected at intervals of 10-11 days,
137 from 2 July (DOY 183) to 14 September (DOY 257) 2010. Four replicates per date were used. The
138 sensor data were converted into Flav berry skin concentrations by using the calibration curve
139 derived previously.

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

142

143 **Fluorescence-based sensor.** The Multiplex fluorimetric sensor has previously been described in
144 detail¹⁵. It is based on the detection of fluorescence emitted by Chl in the red (RF) and far-red
145 (FRF) spectral regions, under excitation with different LED sources in the UV (375 nm) and visible
146 (blue at 450 nm, green at 515 nm, and red at 630 nm). The basis of the fluorescence method applied
147 by the Multiplex sensor is schematized in **Figure 1**. The intensity of the chlorophyll fluorescence
148 (ChlF) emitted by a grape berry depends on the amount of excitation light able to reach the Chl
149 pigment present inside the chloroplasts of the berry cells³⁶. By considering a cross section of a red
150 berry skin, we can model the Anth-containing cell layers localized above the Chl-containing cell
151 layers. Anth can then attenuate part of the incident light before this can reach the Chl molecules.
152 Consequently, the higher the Anth concentration, the lower the ChlF intensity. The extent of the
153 Anth attenuation also depends on the spectral band of the excitation light. Since Anth absorbs

154 mainly in the green around 520 nm (see the absorption spectrum in the left-top corner of **Figure 1**),
155 green excitation light will be attenuated more than, e.g., red light, which is in a spectral zone
156 characterized by a weak Anth absorption. The ChlF detected will be significantly lower under green
157 excitation (-ChlF) than under red light excitation (+ChlF). By comparing the two fluorescence
158 signals, it is possible to obtain an index that is proportional to the berry skin Anth content.

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

$$160 \quad ANTH_{GR} = -ANTH_{RG} = \log (FRF_G/FRF_R) \quad (1)$$

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

$$167 \quad FLAV = \log (FRF_R/FRF_{UV}) \quad (2)$$

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

$$169 \quad CHL = FRF_R/RF_R \quad (3)$$

170 which was previously denoted as the simple fluorescence ratio (SFR_R), can be used as a Chl
171 index, due to the partial reabsorption of RF by the Chl itself³⁷.

172 Further details on the origin of the above equations can be found in the literature^{14,15,38}.

173

174 **Mapping of Anth grape content.** In 2012, a 7-ha vineyard at the 'Cantina Vignaioli del Morellino
175 di Scansano Soc. Coop Agricola' in Valle Maggiore (Grosseto, Italy), planted with Sangiovese
176 vines, with vine and row spacing of 0.6 and 3 m, respectively, was manually scanned by the Mx on
177 a 15m x 15m sampling grid. For each spatial point, the first bunch close to the cordon on three
178 adjacent vines was measured and the average of the three measurements was then computed. The
179 plot was measured within 8 h on 19 September, 2012, just prior to harvest. The $ANTH_{GR}$ values

180 were converted into Anth concentration (g kg^{-1} FW) by using a calibration curve acquired
181 previously that is specifically for the Sangiovese cultivar. A specific software (Surface Mapping
182 System Surfer 11.0.642, Golden Software, Inc.) was used for the geostatistical analysis, by
183 computing the variogram that represented the best model fit of data and mapping the Anth values by
184 means of kriging analysis. Thanks to the knowledge of the Global Position System (GPS)
185 coordinates of each point measured by the Mx sensor, it was also possible to export data to a virtual
186 globe information software (Google Earth, Mountain View, California, USA), in order to
187 superimpose maps on satellite or aerial photography images.

188

189 **Analysis of berry Flav content.** The concentrations of Flav, expressed as $\mu\text{g/g}$ of skins, equivalents
190 of quercetin-3-O-glucoside, were obtained by means of HPLC analysis and following a slightly
191 modified Downey procedure^{39,40} by using a LC1260 system with DAD detection and a Poroshell
192 120 EC-C18 column (4.6x150 mm, 2.7 μm) (both from Agilent Technologies, Palo Alto, CA, USA).
193 In brief, about 0.4 g of frozen ground grape skin powder were extracted with 2 ml of 30 % methanol
194 in water. The supernatant (50 μl) was injected in the HLPC system. Chromatographic separation
195 was achieved by using a linear gradient from 10% formic acid in water to 10% formic acid in
196 methanol, at a flow rate of 0.8 ml/min. Flavonols were monitored by DAD detection at 354 nm.
197 Identification of the individual components peaks was performed by making a comparison of
198 retention times and UV-Vis absorption data with those found in the literature⁴⁰. Quantification was
199 performed by using quercetin-3-O-glucoside as an external standard for building the calibration
200 curve.

201

202 **Statistical analysis.** Statistical analysis and curve fitting were carried out with SigmaPlot for
203 Windows Version 11.0 (Systat Software, Inc.). The results are given as mean \pm standard deviations
204 (SD).

205

206

207 **RESULTS AND DISCUSSION**

208

209 **Calibration curve for the Anth index.** The *in-field* non-destructive evaluation of the Anth grape
210 concentration required a calibration of the Multiplex indices by means of wet chemistry. In **Figure**
211 **2**, the relationship between the *in situ* ANTH_{GR} index and the relative Anth content determined by
212 the destructive analysis of the same grape bunches is reported for the CS and ME cvs. Data were
213 satisfactorily fitted (coefficient of determination (R^2) of 0.83 and 0.75 for ME and CS, respectively)
214 by means of a rising exponential curve. The increase in ANTH_{GR} with an increase in the Anth
215 concentration was in accordance with all other studies on different cvs in which the opposite Anth
216 index, ANTH_{RG}, was calibrated against wet chemistry^{16,19,20}. The curvilinear relationship between
217 ANTH_{GR} (or ANTH_{RG}) and the Anth berry content was predicted by the theory based on the
218 absorbance spectral properties of Anth¹⁵. Our data represent the first report of Anth index
219 calibration curves for the CS and ME cvs.

220

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

232

233 **Anth spatial distribution.** The Multiplex sensor could also be employed for mapping grape
234 quality, more precisely Anth content, over large areas of the vineyard. In **Figure 4**, an example of
235 the spatial distribution of the Anth bunch concentration obtained using the hand-held Multiplex
236 detector for the Sangiovese cv is presented. One thousand and sixty-three clusters over a 7-ha plot
237 were measured. **Figure 4A** shows, using a colorimetric scale, the spatial heterogeneity of the Anth
238 bunch content within the vineyard. It was possible to identify two areas, in red, in which the
239 phenolic maturity was higher as compared to the rest of the vineyard, with an Anth concentration of
240 around 1.8 g kg^{-1} FW. Four localised areas, in blue, indicate low phenolic maturity, with an Anth
241 content of $1\text{-}1.2 \text{ g kg}^{-1}$ FW. The producer will be able to use this information in order to examine the
242 origin of the Anth spatial differences in terms of soil nutrition and drainage, stress factors, vigour
243 management, diseases, and to operate in such a way as to make the vineyard more homogeneous.
244 By means of segmentation, which signifies dividing all the Anth values into two parts based on the
245 median or a value considered significant by the producer, it was possible to represent the Anth
246 content map in two coloured zones (**Figure 4C**), with higher and lower phenolic content. This
247 makes it possible to proceed with a selective harvest in order to produce two wines differing in
248 quality and, consequently, in price. **Figures 4B** and **4D** show the two colorimetric maps directly on
249 Google Earth images. By downloading them into a smartphone, the producer would be able to go
250 directly to his vineyard and identify, with great precision, the areas in which to operate.

251

252 **Calibration curve for the Flav index.** The main flavonol compounds found in white-grape berry
253 skins by means of the HPLC analysis were quercetin-3-O-glucuronide and quercetin-3-O-glucoside,
254 which accounted on average for 67% and 23%, respectively, of the total. Kaempferol-3-O-
255 galactoside, kaempferol-3-O-glucuronide, and kaempferol-3-O-glucoside were present as minor
256 compounds, namely as 6%, 2% and 2%, respectively, of the total. The large variability found in the

257 Flav content of berry samples, i.e. from 200 to 2700, was mainly due to the well-known response of
258 flavonol accumulation to exposure to sunlight in grapevine leaves^{10,42} and berries⁴³⁻⁴⁶.
259 The non-destructive FLAV index was satisfactorily correlated directly to the Flav berry skin content
260 determined by the destructive analysis and computed as the sum of the quercetin and kaempferol
261 glycoside compounds (Figure 5). The best fitting curve was exponential (R^2 of 0.766), even if the
262 linear regression $FLAV = 0.674 + 1.717 \cdot 10^{-4} \cdot Flav$ was similarly valid ($R^2 = 0.764$). This result
263 supported the effectiveness of the fluorescence sensor in determining the flavonol content in the
264 berry skin of white grapes.

265

266 **Evolution of flavonols in white grapes.** Inversion of the above calibration curve made it possible
267 to estimate the Flav content from the FLAV non-destructive index detected in the berry samplings.
268 The time course of the Flav concentration estimated by the fluorescence sensor measurements for
269 VE during the 2010 season is reported in **Figure 6**. Flav remained almost constant at around 550
270 $\mu\text{g/g}$ FW until the beginning of veraison (around DOY 215), a sharp increase to around 800 $\mu\text{g/g}$
271 FW, was then observed within 10 days. A plateau was maintained, within experimental errors, until
272 DOY 246, when the Flav concentration dropped rapidly to less than 20% of the maximum.

273 A similar time course for the Flav had previously been observed in the Erbaluce white grape cv by
274 using wet chemistry⁴⁷. Explaining the change in the grape Flav concentration during the entire
275 season is difficult, since single compounds may follow different kinetics. Gregan et al.³⁴ observed
276 that, in the Sauvignon Blanc cv, the quercetin-3-O-glucuronide decreased from veraison to harvest,
277 while the quercetin-3-O-glucoside and kaempferol-3-O-glucoside increased through development
278 and reached a maximum at harvest time. Furthermore, Flav accumulation and degradation⁴⁸ can
279 clearly be affected by climatic conditions and sun exposure. The increase in Flav observed during
280 veraison is consistent with the evolution in the expression of flavonol synthase genes observed in
281 the Chardonnay and Shiraz cvs⁴⁹. On the other hand, we are at present unable to offer any
282 explanation for the drop in Flav that occurred at harvest.

283 Since the level of Flav in the clusters reached at harvest was maintained in the derived
284 wines⁴³, the possibility of controlling *in vivo* these compounds, with their very healthy properties⁵⁰,
285 appears to be of considerable importance.

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

290

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

301 The time course of CHL for the VE cv in 2010 is shown in **Figure 7B**. Here, we have a
302 complete representation of the Chl change in berries from pre-veraison to harvest time. It is
303 worthwhile drawing attention to the prominent change, during veraison, in the CHL curve slope
304 between DOY 215 and 225. CHL can then be used as an alternative and more precise indicator of
305 the veraison setting. The time evolution of the Chl content predicted by the Multiplex sensor was
306 similar to that obtained by means of destructive analyses on the Erbaluce, Barbera, and Nebbiolo
307 Italian varieties⁴⁷.

308

309 **Technological maturity parameters.** Basing ourselves on previous observations of the presence of
310 an inverse relationship between Chl fluorescence and sugar contents in the Bacchus and Silvaner
311 cvs⁵³, and by considering the time evolution of CHL, we checked to see whether this index could
312 provide information on the technological maturity of grapes. Indeed, we found a good inverse
313 correlation between the CHL index and the TSS measured as °Brix on the same berry samples
314 (**Figure 8**). This had previously been observed for Pinot cvs¹⁵. Here, we have presented evidence
315 that the CHL index can be a good proxy of TSS (°Brix) for both red (CS and ME) and white (VE)
316 grape cultivars, as reported in **Figure 8**. The advantage of this technique in evaluating the berry
317 sugar content with respect to the destructive refractometric method lies in the possibility that it
318 offers of increasing the size of the sampling evaluation in the same time interval. In fact, there is no
319 need for berry collection and sample squeezing, and several berries (19 berries, that is, those filling
320 the $5 \cdot 10^3 \text{ mm}^2$ circular area of the sensor window) can be measured within 1 s.

321

322 **Conclusion.** The good correlation found between wet chemistry and Anth Multiplex data during the
323 berry ripening of the CS and ME cvs and those previously reported in the literature suggests this
324 new fluorimetric technique as a green analytical tool in alternative to time-consuming, costly and
325 environmental-unfriendly standard laboratory phenolic analyses. Portable optical sensors not only
326 solve the problem of analyzing a large number of samples, or even the whole crop, directly in the
327 vineyard, but also offer the possibility of following the same bunches during the whole season and
328 of repeating the optical measurements with greater frequency. Combining the assessment of the
329 Anth and Chl grape constituents under temporal and spatial monitoring provides a complete set of
330 decision supports in viticulture for defining the best harvest time and the best place for harvesting.
331 Mounting the fluorescence sensor on a tractor makes it possible to obtain information on the Anth
332 bunch content on large areas of a vineyard, in order to control viticultural practices or to have a
333 general characterization of the vineyard in question.

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

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

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

344

345

346 **ABBREVIATIONS USED**

347 Anth, anthocyanin(s); ANTH_{GR}, anthocyanin non-destructive index; Chl, chlorophyll; CHL,
348 chlorophyll non-destructive index; ChlF, chlorophyll fluorescence; CS, Cabernet Sauvignon; cv(s),
349 cultivar(s); DOY, day of the year; Flav, flavonol(s); FLAV, flavonol non-destructive index; FW,
350 fresh weight; ME, Merlot; Mx, Multiplex; R², coefficient of determination; TSS, total soluble
351 solids; VE, Vermentino.

352

353 **ACKNOWLEDGMENT**

354 The authors are grateful to the Bulichella, Tenuta dell'Ornellaia and to the Cantina Vignaioli del
355 Morellino di Scansano Soc. Coop Agricola wineries for the utilization of their vineyards. We also
356 wish to thank the Fondazione Bertarelli (Grosseto), and the Provincia di Grosseto POR FSE 2007-
357 2013 for their financial support. This work was supported in part by the Italian Consiglio Nazionale
358 delle Ricerche through the 'Ricerca Spontanea a Tema Libero' grant.

359

360

361 **REFERENCES**

- 362 (1) Trought, M. C. T.; Bramley, R. G. V., Vineyard variability in Marlborough, New Zealand:
363 characterising spatial and temporal changes in fruit composition and juice quality in the vineyard. *Australian*
364 *Journal of Grape and Wine Research* **2011**, 17, (1), 72-82.
- 365 (2) Johnson, L. F.; Roczen, D. E.; Youkhana, S. K.; Nemani, R. R.; Bosch, D. F., Mapping vineyard leaf
366 area with multispectral satellite imagery. *Comput. Electron. Agric.* **2003**, 38, (1), 33-44.
- 367 (3) Hall, A.; Lamb, D. W.; Holzappel, B. P.; Louis, J. P., Within-season temporal variation in correlations
368 between vineyard canopy and winegrape composition and yield. *Precis. Agric.* **2011**, 12, (1), 103-117.
- 369 (4) Johnson, L. F.; Herwitz, S.; Dunagan, S.; Lobitz, B.; Sullivan, D.; Slyea, R., Collection of ultra high
370 spatial and spectral resolution image data over California vineyards with a small UAV. In *Proceedings Int'l*
371 *Symposium on Remote Sensing of Environment*, Honolulu, HI, 2003; pp 10-14.
- 372 (5) Mazzetto, F.; Calcante, A.; Mena, A.; Vercesi, A., Integration of optical and analogue sensors for
373 monitoring canopy health and vigour in precision viticulture. *Precis. Agric.* **2010**, 11, (6), 636-649.
- 374 (6) Baluja, J.; Diago, M. P.; Balda, P.; Zorer, R.; Meggio, F.; Morales, F.; Tardaguila, J., Assessment of
375 vineyard water status variability by thermal and multispectral imagery using an unmanned aerial vehicle
376 (UAV). *Irrigation Science* **2012**, 30, (6), 511-522.
- 377 (7) Lamb, D. W.; Weedon, M. M.; Bramley, R. G. V., Using remote sensing to predict grape phenolics
378 and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and
379 optimising image resolution. *Australian Journal of Grape and Wine Research* **2004**, 10, (1), 46-54.
- 380 (8) Cerovic, Z. G.; Moise, N.; Agati, G.; Latouche, G.; Ben Ghazlen, N.; Meyer, S., New portable
381 optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. *J. Food*
382 *Comp. Anal.* **2008**, **21**, 650-654.
- 383 (9) Serrano, E.; Dias, F.; Biais, T.; Dufourcq, T., Les nouvelles technologies pour renseigner du statut
384 azote des raisins, Recherche de modèles de prédiction à l'aide du capteur multiplex. In *Actes du Colloque*
385 *Mondiaviti, Vinitech 2010*, Bordeaux, 2010; pp 103-111.

- 386 (10) Agati, G.; Cerovic, Z. G.; Dalla Marta, A.; Di Stefano, V.; Pinelli, P.; Traversi, M. L.; Orlandini, S.,
387 Optically-assessed preformed flavonoids and susceptibility of grapevine to *Plasmopara viticola* under
388 different light regimes. *Functional Plant Biology* **2008**, 35, (1), 77-84.
- 389 (11) Bellow, S.; Latouche, G.; Brown, S. C.; Poutaraud, A.; Cerovic, Z. G., In vivo localization at the
390 cellular level of stilbene fluorescence induced by *Plasmopara viticola* in grapevine leaves. *Journal of*
391 *Experimental Botany* **2012**, 63, (10), 3697-3707.
- 392 (12) Latouche, G.; Bellow, S.; Poutaraud, A.; Meyer, S.; Cerovic, Z. G., Influence of constitutive phenolic
393 compounds on the response of grapevine (*Vitis vinifera* L.) leaves to infection by *Plasmopara viticola*. *Planta*
394 **2013**, 237, (1), 351-361.
- 395 (13) Bilger, W.; Rolland, M.; Nybakken, L., UV screening in higher plants induced by low temperature in
396 the absence of UV-B radiation. *Photochem Photobiol Sci* **2007**, 6, (2), 190-195.
- 397 (14) Agati, G.; Meyer, S.; Matteini, P.; Cerovic, Z. G., Assessment of anthocyanins in grape (*Vitis vinifera*
398 L.) berries using a non-invasive chlorophyll fluorescence method. *J. Agric. Food Chem.* **2007**, 55, 1053-
399 1061.
- 400 (15) Ben Ghazlen, N.; Cerovic, Z. G.; Germain, C.; Toutain, S.; Latouche, G., Non-destructive optical
401 monitoring of grape maturation by proximal sensing. *Sensors* **2010**, 10, (11), 10040-10068.
- 402 (16) Tuccio, L.; Remorini, D.; Pinelli, P.; Fierini, E.; Tonutti, P.; Scalabrelli, G.; Agati, G., Rapid and non-
403 destructive method to assess in the vineyard grape berry anthocyanins under different seasonal and water
404 conditions. *Australian Journal of Grape and Wine Research* **2011**, 17, (2), 181-189.
- 405 (17) Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L., Effect of maturity and vine water status on
406 grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002**, 53, 268-274.
- 407 (18) Cerovic, Z. G.; Goutouly, J. P.; Hilbert, G.; Destrac-Irvine, A.; Martinon, V.; Moise, N., Mapping
408 winegrape quality attributes using portable fluorescence-based sensors In *Frutic 09*, Best, S., Ed. Progap
409 INIA: Conception, Chile, 2009; pp 301-310.
- 410 (19) Baluja, J.; Diago, M. P.; Goovaerts, P.; Tardaguila, J., Assessment of the spatial variability of
411 anthocyanins in grapes using a fluorescence sensor: relationships with vine vigour and yield. *Precis. Agric.*
412 **2012**, 13, (4), 457-472.

- 413 (20) Bramley, R. G. V.; Le Moigne, M.; Evain, S.; Ouzman, J.; Florin, L.; Fadaili, E. M.; Hinze, C. J.;
414 Cerovic, Z. G., On-the-go sensing of grape berry anthocyanins during commercial harvest: development and
415 prospects. *Australian Journal of Grape and Wine Research* **2011**, 17, (3), 316-326.
- 416 (21) Bramley, R., Understanding variability in winegrape production systems. 2. Within vineyard
417 variation in quality over several vintages. *Australian Journal of Grape and Wine Research* **2005**, 11, 33-42.
- 418 (22) Bergqvist, J.; Dokoozlian, N.; Ebisuda, N., Sunlight exposure and temperature effects on berry
419 growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of
420 California. *Am. J. Enol. Vitic.* **2001**, 52, (1), 1-7.
- 421 (23) Downey, M. O.; Dokoozlian, N. K.; Krstic, M. P., Cultural practice and environmental impacts on
422 the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.* **2006**, 57, 257-
423 268.
- 424 (24) Yamane, T.; Jeong, S. T.; Goto-Yamamoto, N.; Koshita, Y.; Kobayashi, S., Effects of temperature on
425 anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Vitic.* **2006**, 57, (1), 54-59.
- 426 (25) Deluc, L. G.; Quilici, D. R.; Decendit, A.; Grimplet, J.; Wheatley, M. D.; Schlauch, K. A.; Merillon,
427 J. M.; Cushman, J. C.; Cramer, G. R., Water deficit alters differentially metabolic pathways affecting
428 important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*
429 **2009**, 10, 212-245.
- 430 (26) Yokotsuka, K.; Nagao, A.; Nakazawa, K.; Sato, M., Changes in anthocyanins in berry skins of
431 Merlot and Cabernet Sauvignon grapes grown in two soils modified with limestone or oyster shell versus a
432 native soil over two years. *Am. J. Enol. Vitic.* **1999**, 50, (1), 1-12.
- 433 (27) Guidoni, S.; Mannini, F.; Ferrandino, A.; Argamante, N.; Di Stefano, R., The effect of grapevine
434 leafroll and rugose wood sanitation on agronomic performance and berry and leaf phenolic content of a
435 Nebbiolo clone (*Vitis vinifera* L.). *Am. J. Enol. Vitic.* **1997**, 48, (4), 438-442.
- 436 (28) Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A., Influence of pre- and postvéraison
437 water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis*
438 *vinifera* L., cv Shiraz. *Am. J. Enol. Vitic.* **2002**, 53, 261-267.
- 439 (29) Guidoni, S.; Allara, P.; Schubert, A., Effect of cluster thinning on berry skin anthocyanin
440 composition of *Vitis vinifera* cv. Nebbiolo. *Am. J. Enol. Vitic.* **2002**, 53, (3), 224-226.

- 441 (30) Keller, M.; Mills, L. J.; Wample, R. L.; Spayd, S. E., Cluster thinning effects on three deficit-
442 irrigated *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.* **2005**, 56, 91-103.
- 443 (31) Guidoni, S.; Ferrandino, A.; Novello, V., Effects of seasonal and agronomical practices on skin
444 anthocyanin profile of Nebbiolo grapes. *Am. J. Enol. Vitic.* **2008**, 59, (1), 22-29.
- 445 (32) Ben Ghazlen, N.; Moise, N.; Latouche, G.; Martinon, V.; Mercier, L.; Besançon, E.; Cerovic, Z. G.,
446 Assessment of grapevine maturity using new portable sensor: Non-destructive quantification of
447 anthocyanins. *Journal International Des Sciences De La Vigne Et Du Vin* **2010**, 44, 1-8.
- 448 (33) Fierini, E.; Varner, M.; Pangrazzi, P.; Agati, G., Valutazione *in situ* mediante un sensore di
449 fluorescenza del contenuto di antociani nelle varietà Nero d'Avola, Shiraz e Teroldego presso il Gruppo
450 Mezzacorona. *Acta Italus Hortus* **2012**, 3, 469-474.
- 451 (34) Gregan, S. M.; Wargent, J. J.; Liu, L.; Shinkle, J.; Hofmann, R.; Winefield, C.; Trought, M.; Jordan,
452 B., Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of
453 Sauvignon Blanc grapes. *Australian Journal of Grape and Wine Research* **2012**, 18, (2), 227-238.
- 454 (35) Saint-Cricq de Gaulejac, N.; Vivas, N.; Glories, Y., Maturité phénolique: définition et contrôle.
455 *Revue française d'oenologie* **1998**, 173, 22-25.
- 456 (36) Palliotti, A.; Silvestroni, O.; Petoumenou, D., Seasonal patterns of growth rate and
457 morphophysiological features in green organs of Cabernet Sauvignon grapevines. *Am. J. Enol. Vitic.* **2010**,
458 61, (1), 74-82.
- 459 (37) Buschmann, C., Variability and application of the chlorophyll fluorescence emission ratio red/far-red
460 of leaves. *Photosynthesis Research* **2007**, 92, 261-271.
- 461 (38) Cerovic, Z. G.; Ounis, A.; Cartelat, A.; Latouche, G.; Goulas, Y.; Meyer, S.; Moya, I., The use of
462 chlorophyll fluorescence excitation spectra for the non-destructive *in situ* assessment of UV-absorbing
463 compounds in leaves. *Plant, Cell and Environment* **2002**, 25, 1663-1676.
- 464 (39) Downey, M. O.; Mazza, M.; Krstic, M. P., Development of a stable extract for anthocyanins and
465 flavonols from grape skin. *Am. J. Enol. Vitic.* **2007**, 58, (3), 358-364.
- 466 (40) Downey, M.; Rochfort, S., Simultaneous separation by reversed-phase high-performance liquid
467 chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin. *Journal*
468 *of Chromatography A* **2008**, 1201, (1), 43-47.

- 469 (41) Tesic, D.; Woolley, D. J.; Hewett, E. W.; Martin, D. J., Environmental effects on cv Cabernet
470 Sauvignon (*Vitis vinifera* L.) grown in Hawke's Bay, New Zealand.: 1. Phenology and characterisation of
471 viticultural environments. *Australian Journal of Grape and Wine Research* **2002**, 8, (1), 15-26.
- 472 (42) Kolb, C.; Käser, M. A.; Kopecky, J.; Zotz, G.; Reiderer, M.; Pfündel, E. E., Effects of natural
473 intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape
474 leaves. *Plant Physiol* **2001**, 127, (3), 863-875.
- 475 (43) Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T., Cluster sun exposure and quercetin in pinot-
476 noir grapes and wine. *Am. J. Enol. Vitic.* **1995**, 46, (2), 187-194.
- 477 (44) Haselgrove, L.; Botting, D.; van Heeswijck, R.; HØJ, P. B.; Dry, P. R.; Ford, C.; Land, P. G. I.,
478 Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of
479 *Vitis vinifera* L cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research* **2000**, 6, (2), 141-
480 149.
- 481 (45) Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J. C., Separation of sunlight and temperature
482 effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002**, 53, (3), 171-182.
- 483 (46) Kolb, C. A.; Kopecky, J.; Riederer, M.; Pfündel, E. E., UV screening by phenolics in berries of
484 grapevine (*Vitis vinifera*). *Functional Plant Biology* **2003**, 30, (12), 1177-1186.
- 485 (47) Giovanelli, G.; Brenna, O. V., Evolution of some phenolic components, carotenoids and chlorophylls
486 during ripening of three Italian grape varieties. *European Food Research and Technology* **2007**, 225, (1),
487 145-150.
- 488 (48) Liang, N. N.; He, F.; Bi, H. Q.; Duan, C. Q.; Reeves, M. J.; Wang, J., Evolution of flavonols in berry
489 skins of different grape cultivars during ripening and a comparison of two vintages. *European Food*
490 *Research and Technology* **2012**, 235, (6), 1187-1197.
- 491 (49) Downey, M. O.; Harvey, J. S.; Robinson, S. P., Synthesis of flavonols and expression of flavonol
492 synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Australian*
493 *Journal of Grape and Wine Research* **2003**, 9, 110-121.
- 494 (50) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M., Wine as a biological fluid: History, production, and
495 role in disease prevention. *Journal of Clinical Laboratory Analysis* **1997**, 11, (5), 287-313.

- 496 (51) Bureau, S. M.; Baumes, R. L.; Razungles, A. J., Effects of vine or bunch shading on the glycosylated
497 flavor precursors in grapes of *Vitis vinifera* L. Cv. Syrah. *J. Agric. Food Chem.* **2000**, 48, (4), 1290-1297.
- 498 (52) Kwasniewski, M. T.; Vanden Heuvel, J. E.; Pan, B. S.; Sacks, G. L., Timing of cluster light
499 environment manipulation during grape development affects C-13 norisoprenoid and carotenoid
500 concentrations in Riesling. *J. Agric. Food Chem.* **2010**, 58, (11), 6841-6849.
- 501 (53) Kolb, C. A.; Wirth, E.; Kaiser, W. M.; Meister, A.; Riederer, M.; Pfündel, E. E., Noninvasive
502 evaluation of the degree of ripeness in grape berries (*Vitis vinifera* L. cv. Bacchus and Silvaner) by
503 chlorophyll fluorescence. *J. Agric. Food Chem.* **2006**, 54, 299-305.
- 504

505 **FIGURE CAPTIONS**

506

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

515

516 Figure 2. Calibration curves for the M_x ANTH_{GR} index computed by the Anth content (mg/kg FW)
517 results of the destructive analysis. Fitting curve equations were ANTH_{GR} (ME) = $-0.527 + 0.476 \cdot (1 -$
518 $\exp(-0.001 \cdot \text{Anth}))$ and ANTH_{GR} (CS) = $-0.434 + 0.56 \cdot (1 - \exp(-6.8 \cdot 10^{-4} \cdot \text{Anth}))$, with R^2 of 0.83 and
519 0.75, respectively.

520

521 Figure 3. Time evolution of the Anth berry concentration estimates determined by the *in-field* M_x
522 measurements and the inverted calibration curves for both the ME and the CS during the 2008 and
523 2009 seasons. Each point of CS 2008 is the mean of 160 bunches equally distributed over two
524 adjacent rows, both sides per row. For CS 2009, ME 2008 and ME 2009, each point is the mean of
525 100 bunches. Last points of ME were measured at harvest, last points of CS were measured 2-weeks
526 before harvest. The bars represent SDs.

527

528 Figure 4. Spatial distribution of Anth bunch concentration (g kg^{-1} FW) estimated by the ANTH_{GR}
529 Multiplex index for the Sangiovese cv on a 7-ha plot in Tuscany. One thousand and sixty-three
530 clusters were measured manually with the M_x in September 2012, just before harvest time. (A)

531 Rainbow map of Anth distribution and (B) the same map exported to the corresponding Google
532 Earth image; (C) Segmented map of Anth distribution based on data median and (D) the same map
533 exported to the corresponding Google Earth image.

534

535 Figure 5. Calibration curve for the Mx FLAV index computed by using the berry skin flavonol
536 content derived from the destructive HPLC analysis of Vermentino samplings during the 2010 and
537 2011 seasons. The fitting curve equation was $FLAV = 0.659 + 1.445 \cdot (1 - \exp(-1.42 \cdot 10^{-4} \cdot Flav))$, with
538 R^2 of 0.766.

539

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

543

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

548

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

552

553

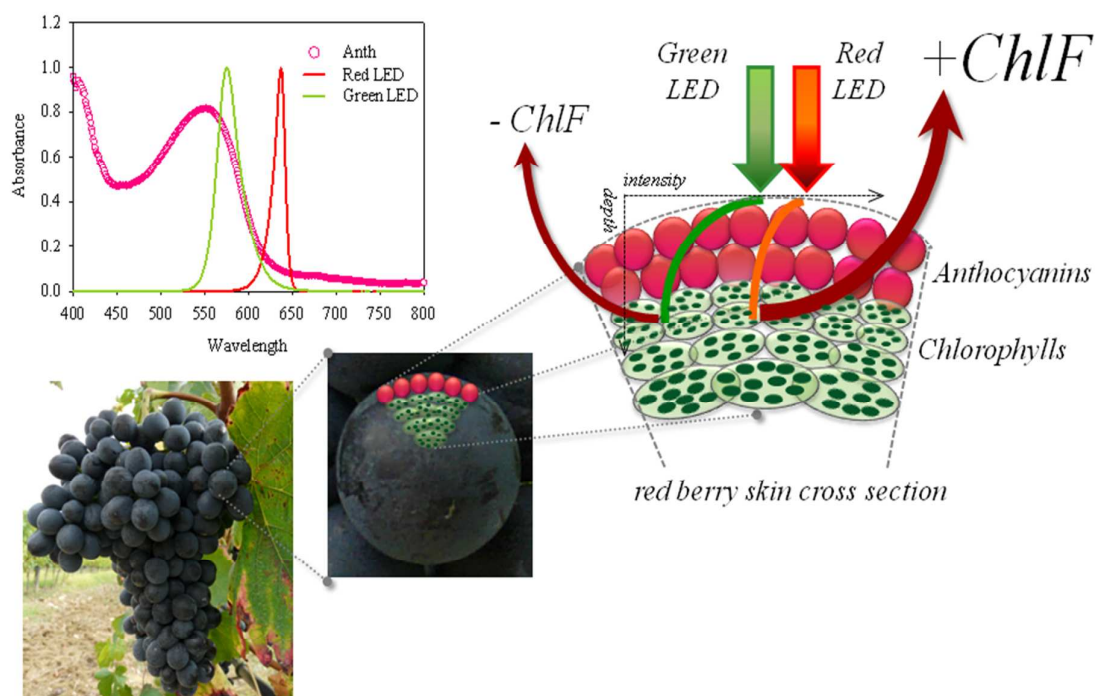
Table 1. Cabernet Sauvignon and Merlot grape parameters at harvest

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

^aEach value is the average (±SD) of n = 5 samples. ^bEach value is the average (±SD) of n = 10 samples. Means with different letters are significantly different at P ≤ 0.05

554

555



556

557

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

566

567

568

569

570

571

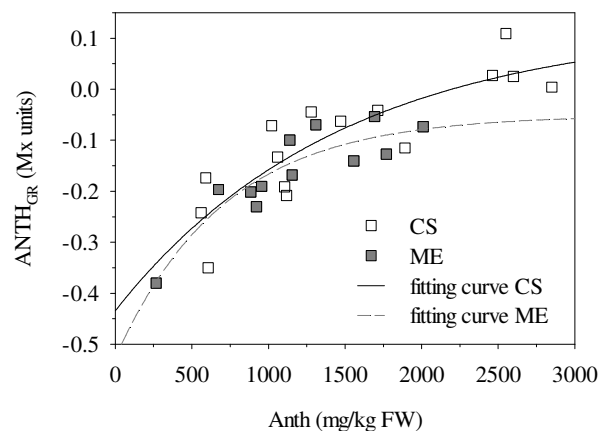
572

573

574

575

576



577 Figure 2. Calibration curves for the Mx ANTH_{GR} index computed by the Anth content (mg/kg FW)
578 results of the destructive analysis. Fitting curve equations were ANTH_{GR} (ME) = -0.527 + 0.476·(1-
579 exp (-0.001·Anth) and ANTH_{GR} (CS) = -0.434 + 0.56·(1-exp (-6.8·10⁻⁴·Anth), with R² of 0.83 and
580 0.75, respectively.

581

582

583

584

585

586

587

588

589

590

591

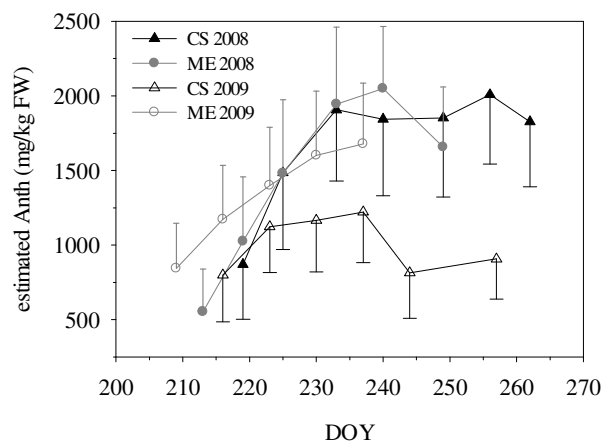
592

593

594

595

596



597

598

599

600

601

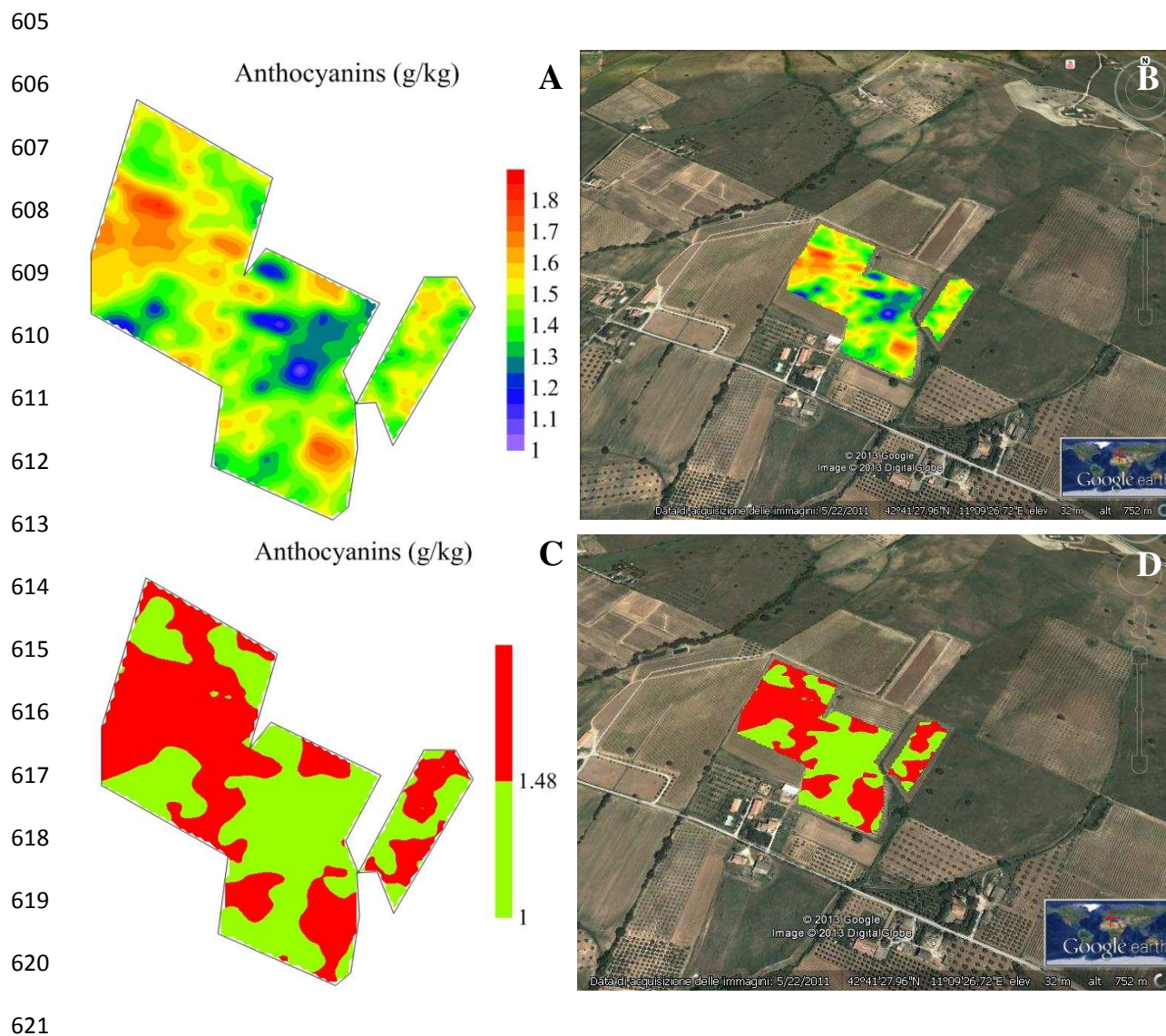
602

603

604

605

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. Last points of ME were measured at harvest, last points of CS were measured 2-weeks before harvest. The bars represent SDs.



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

630

631

632

633

634

635

636

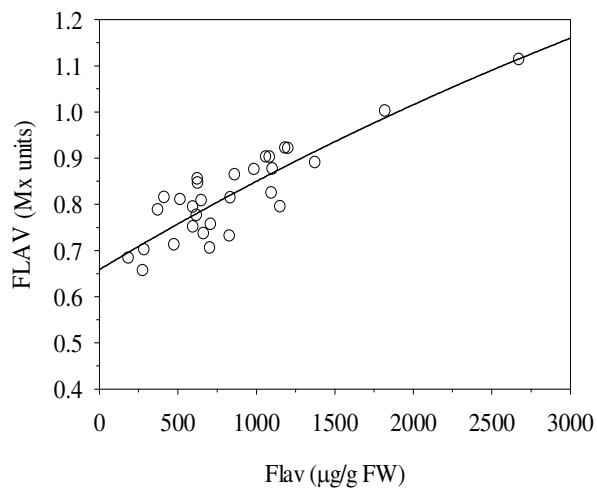
637

638

639

640

641



642 Figure 5. Calibration curve for the Mx FLAV index computed by using the berry skin flavonol
643 content derived from the destructive HPLC analysis of Vermentino samplings during the 2010 and
644 2011 seasons. The fitting curve equation was $FLAV = 0.659 + 1.445 \cdot (1 - \exp(-1.42 \cdot 10^{-4} \cdot Flav))$, with
645 R^2 of 0.766.

646

647

648

649

650

651

652

653

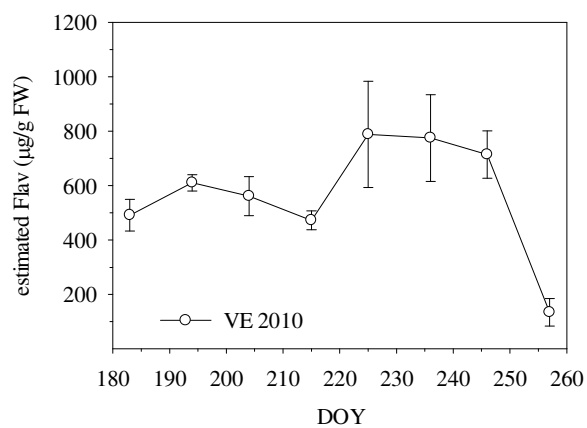
654

655

656

657

658



657

658

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

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

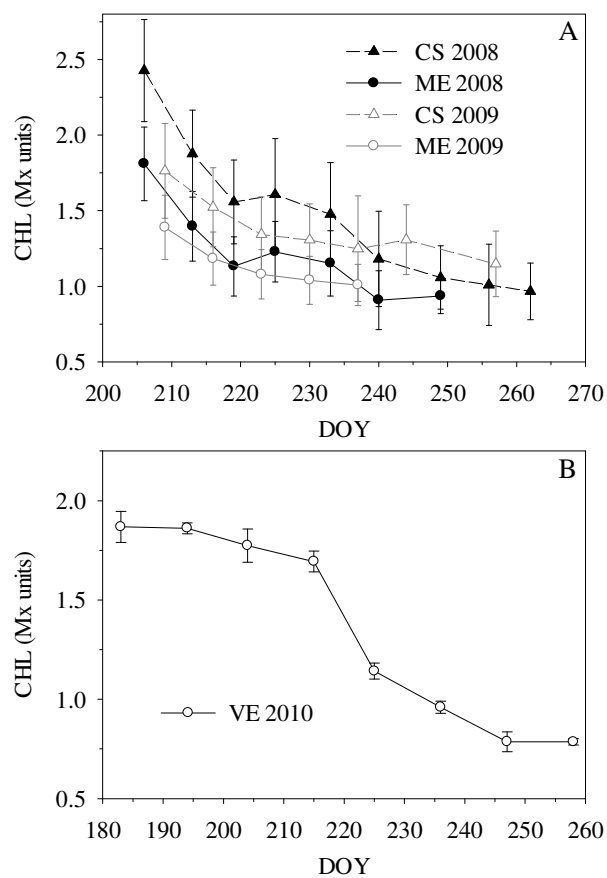
681

682

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

687

688



689

690

691

692

693

694

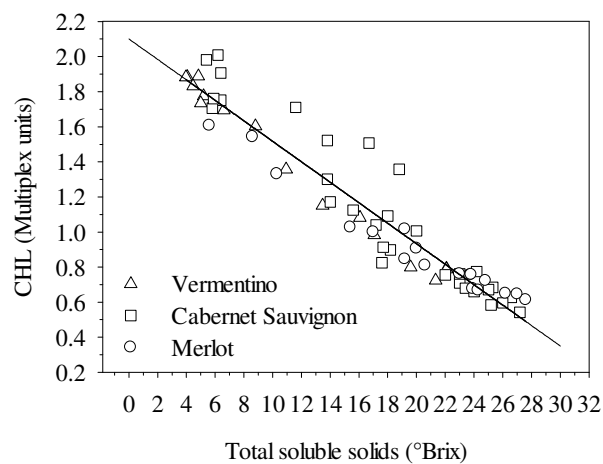
695

696

697

698

699



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

703

704

705 TOC Graphic



706

707