Noninvasive Evaluation of the Degree of Ripeness in Grape Berries (*Vitis Vinifera* L. Cv. Bacchus and Silvaner) by Chlorophyll Fluorescence

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The use of chlorophyll fluorescence measurements to noninvasively evaluate degrees of ripeness was investigated in berries at various stages of ripening from two white grapevine cultivars (*Vitis vinifera* L. Cv. Bacchus and Silvaner). Berries were characterized by diameter, weight, and density and by concentrations of fructose, glucose, sucrose, and total sugars, as well as fructose/glucose ratios, and also by chlorophyll fluorescence at F_0 and F_M levels and the fluorescence ratio F_V/F_M. Pearson product moment correlation analysis on data from both cultivars revealed clear negative associations between F_0 and concentrations of fructose, glucose, and total sugars, and fructose/glucose ratios (correlation coefficient < -0.89). Curvilinear trend-lines were established for plots of F_0 versus concentrations of fructose, glucose, and total sugars, but a linear relationship between F_0 and fructose/glucose ratios was found: the corresponding coefficients of determination were always >0.82. Therefore, chlorophyll fluorescence measurements are well-suited to determine noninvasively sugar accumulation in grape berries during ripening.

KEYWORDS: Berry; chlorophyll; fluorescence; fructose; glucose; grapevine; method; sucrose; sugar; viticulture; *Vitis vinifera* L

INTRODUCTION

The development of a grape berry is divided into two growth phases that are separated by a period of little gain in size and weight: the process of ripening occurs during the second growth phase (*1*, *2*). Ripening comprises profound changes in biochemistry and physiology that bring about the typical signs of ripe berries such as enzymatic softening of the tissue (*3*, *4*), hexose accumulation (*5*−*7*), and, in red varieties, anthocyanin formation (*8*, *9*).

Information on the progress and degree of berry ripeness is of pivotal importance for grape growers to optimize farming and harvest date with the objective of producing high quality grapes. In the vineyard, the standard method to monitor ripening is refractometrical determination of sugar concentrations in juice samples from individual berries (*2*). This method, as well as other destructive analyses, does not permit continuous monitoring of the course of ripening in an individual berry by repetitive measurements; also, it is difficult to investigate the known variations of the degree of ripeness within a bunch and between bunches. Thus, measurements by destructive methods performed on many berries in both the vineyard or the winery allow only an average ripening status to be derived.

The desire for more detailed information on the grape ripening process has led to the development of nondestructive methods to measure ripening: Coombe and Bishop (*10*) used berry deformability and diameter to examine grape growth, while visual evaluation of berry color change has also been utilized to assess the onset of grape ripening (*11*). Moreover, near-infrared spectroscopy was established to accurately determine sugar contents of intact berries (*12*), and magnetic resonance imaging has been used to visualize heterogeneous sugar accumulation in berries of a cluster (*13*).

Because chlorophyll concentrations in grapes decline during ripening (*14*−*16*), chlorophyll fluorescence determinations could also be another nondestructive way to examine grape ripening because the intensity of chlorophyll fluorescence emanating from a tissue is almost certainly influenced by the chlorophyll concentration. Chlorophyll fluorescence measurements are not only noninvasive but are simple and rapidly taken, and portable chlorophyll fluorometers for field measurements are now readily available (*17*, *18*). Moreover, chlorophyll fluorescence can be measured from a distance, and in fact, camera systems to record chlorophyll fluorescence remotely have been developed to assess the quality of apples and lemons after harvest (*19*, *20*). Furthermore, based on progress in remote sensing of chlorophyll fluorescence of plant leaves (*21*, *22*), future fluorescence techniques might simultaneously analyze many fruits of a plant or of a plant group; hence, fluorescence-derived information

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on spatio-temporal variations of fruit ripening could become a powerful tool to monitor ripening and determine harvest times in precision farming.

The fluorescence intensity emanating from a fruit, however, depends not only on chlorophyll concentration but also on the quantum yield for chlorophyll fluorescence. In dark-adapted tissue, this quantum yield can assume values between 2% (F_0 fluorescence) and 10% (F_M fluorescence) depending on the functional state of PS II reaction centers that, in the open state, trap excitation energy efficiently and thereby diminish fluorescence yield but, in the closed state, enhance chlorophyll fluorescence (*23*).

For mango and banana fruits, F_0 fluorescence has been suggested as an estimate for chlorophyll concentration because the quantum yield for F_0 fluorescence is relatively unaffected by various stress conditions (*18*). In fact, the F_0 decline during ripening of plantains was assumed to result mostly from decreases in chlorophyll concentration (*24*). In papaya fruits, however, variations in both F_0 and F_M fluorescence correlated with variations in ripening-associated data like skin color and fruit firmness, and it has been supposed that a decline in both fluorescence parameters is due to chlorophyll degradation (*25*). Experiments with apple fruits suggested that F_M rather than F_0 fluorescence is related to chlorophyll loss during ripening (*26*) but both, F_0 and F_M fluorescence, correlated with mostly chlorophyll-dependent color changes, measured as hue angles, during ripening of fruits from another apple cultivar (*27*).

Despite the potential of chlorophyll fluorescence measurements to nondestructively analyze the ripening of fruits, we are unaware of studies relating F_0 or F_M with the ripening of grape berries. Therefore, we examined the levels of chlorophyll fluorescence in white grapes at various degrees of ripeness and compared these data not only with sugar content, which is the standard parameter for evaluation of ripening in the field, but also with simple growth parameters including berry diameter, weight, and density.

MATERIAL AND METHODS

Plant Material. Both grapevine cultivars investigated (*Vitis vinifera* L. Cv. Bacchus and Cv. Silvaner) produce white berries and are extensively used for white wine production in Franconia (northwestern Bavaria, Germany). Berries at different ripening stages were collected from Bacchus and Silvaner vines planted either in 1980 and 1978, respectively, in north–south-directed rows in sloped vineyards close to Würzburg. In the vineyard, berries were collected at three sampling periods denoted T2, T3, and T4: Bacchus and Silvaner were evaluated on the August 7 and 8 (T2), on August 16 and 17 (T3), and on August 29 and 31, 2000 (T4), respectively. For both cultivars in the vineyard, anthesis was observed between June 1 and June 7.

To increase in our analyses the fraction of unripe berries, we also considered available data from an originally separate experiment with 2-year old potted plants of both cultivars grown in a greenhouse and then acclimated to outdoor conditions for 5 days. From these plants, berries were measured at time point T1, which corresponds to June 21, 2000. The greenhouse vines were cultivated in commercially available soil (Einheitserde Typ T, Einheitserde Werkverband, Sinntal-Jossa, Germany) to which fertilizer (Hakaphos blau, Compo, Münster, Germany) was applied once a month according to the manufacturer's instructions. In the greenhouse, night- and daytime temperatures ranged from 5 to 10 and 15–19 °C, respectively, which were adjusted by heating, shading, and ventilation. Anthesis onset in the greenhouse was not recorded, but we observed that potted vines developed about 2 weeks earlier than outdoor plants because greenhouse temperatures were warmer than field temperatures. Therefore, data from greenhouse and field-grown vines are represented as a function of our four harvest times, T1−T4, rather than as a function of a linear time scale.

Measurements started during evening hours: one bunch per plant was randomly chosen, and one healthy berry of the upper third of the exposed side of selected bunches were cut off at the peduncle. In the vineyard, only easterly sides of plants were considered. Principally, each individual berry was subjected to all measurements reported next and, in **Figures 2** and **5**, each data point corresponds to data of the same berry. The numbers (*n*) of berries analyzed for Bacchus and Silvaner, respectively, were 16 and 13 at T1 and 28 and 26 at T3. At T2 and T4, respectively, six berries per cultivar were evaluated. We used the density, derived from weight and diameter, of the individual berry to calculate absolute sugar concentrations in g L^{-1}. Because weight and diameter measurements were not available for some berries, the *n* for sugar concentrations at T1 is reduced to nine and 10 for Bacchus and Silvaner, respectively.

Measurement of Fluorescence and Growth. Prior to determining chlorophyll fluorescence, berries were stored for at least 1 h at room temperature in a dark, moist container. Chlorophyll fluorescence was recorded using a PAM-2000 fluorometer (Walz, Effeltrich, Germany) that includes fiber optics to guide excitation light to the sample and emitted fluorescence back to the instrument. During measurements, the fiber optics (active diameter at measuring end: 6 mm) was gently positioned on the berry surface opposite to the peduncle. F_0 and F_M fluorescence was probed with a measuring light generated by a 655 nm light emitting diode (LED) in the form of 3 μs pulses at frequencies of 600 Hz or 20 kHz, respectively. The LED light passes a short-pass filter (λ < 670 nm) before reaching the sample. F_M fluorescence was elicited by a strong white light pulse of 0.8 s duration and 15 000 μmol m^{-2} s^{-1} intensity. Fluorescence was detected by a PIN diode equipped with a long pass filter (λ > 700 nm) and a heat absorbing filter (see http://www.walz.com/for a detailed description of the instrument). From F_0 and F_M fluorescence values, F_V/F_M ratios were calculated ($F_V = F_{M-F_0}$), which represent an indicator for the maximum quantum yield of PS II (*28*). Identical fluorometer settings were always used, and fiber optic cleanliness was strictly maintained.

After chlorophyll fluorescence measurements, berries were weighed, and the maximum and minimum diameters of each berry were determined with calipers and then averaged. Because berries of both cultivars were approximately spherical, we used the textbook equation for a sphere to derive berry volumes from the mean diameters. The berries were then frozen in liquid nitrogen and stored at −80 °C for later sugar determinations. Prior to sugar extraction, seeds were removed, and the fresh weight was again established.

Sugar Determination. Sugar determinations were carried out as described earlier (*29*) with modifications: the still-frozen material was ground in a pre-cooled mortar with pestle together with a small amount of washed sea sand (Applichem, Darmstadt, Germany). Then, for each milligram of fresh weight, 4 μL of distilled water was added, and the suspension was thoroughly mixed. Solid material was removed by centrifugation at 10 000*g* for 10 min. The supernatant was boiled for 3 min to denature proteins, which were removed by centrifugation (as stated previously). Subsequently, soluble amino acids were bound to Serdolit (Serva, Boehringer Bioproducts Partnership, Ingelheim, Germany) using 10 mg for each 100 μL of supernatant. Finally, Serdolit was removed by centrifugation (as stated previously), and the supernatant was used for chromatography.

Major sugars (glucose, fructose, and sucrose) were separated by anion chromatography (100 μL injection volume, eluent 0.08 M NaOH, flow rate 1.0 mL min^{-1}, column temperature 40 °C) on a Carbopac column in combination with an Amino-trap precolumn (both Dionex, Idstein, Germany) and quantified by pulsed amperometry (Dionex 4500i, Dionex). Prior to sample analyses, one point-calibration was achieved by injecting mixtures of the previous sugars (Merck, Darmstadt, Germany) at a concentration of 50 μM each. Calibration was repeated every five sample analyses, and the same sample was measured repeatedly at different dilutions to give a detector signal comparable to that observed with the respective standard substance. Data were processed using WINPEAK-software (Biotronik, Maintal, Germany). For each berry, the sugar concentration in grams per unit fresh weight was converted to grams per liter using the density value calculated for the respective berry.
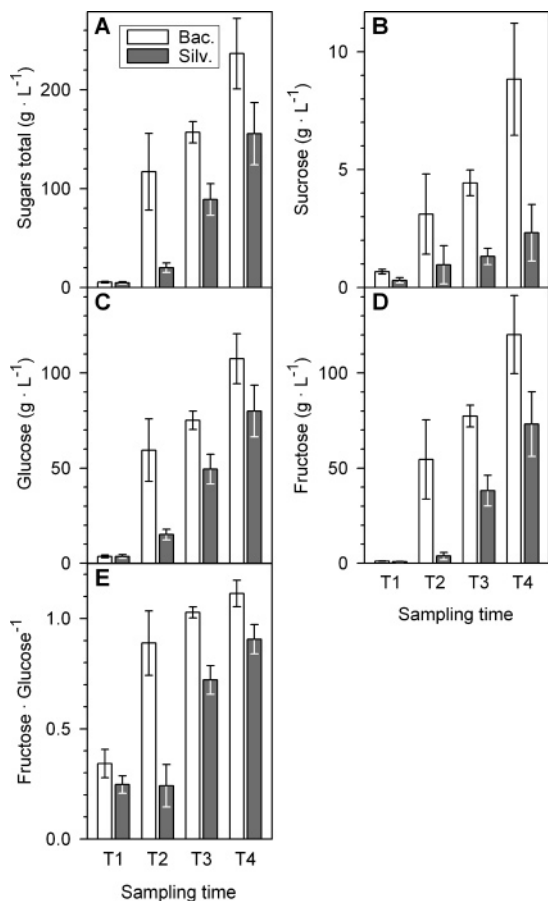
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Figure 1. Sugar concentrations of Bacchus and Silvaner berries. Both cultivars were probed at four sampling times denoted T1–T4 (abscissa) representing different ripening stages: interval periods between sampling times vary (see Materials and Methods). Ordinate data are mean values of n measurements: the *n* per cultivar was 6 at T2 and T4, >12 at T1, and >25 at T3. Open and filled bars represent data from Bacchus and Silvaner berries, respectively. Error bars indicate 95% confidence intervals. Panels A–D depict concentrations of all sugars, sucrose, glucose, and fructose, respectively. In panel E, the means of fructose/glucose ratios derived from the corresponding ratios of individual berries are shown.

Statistics. Statistical analyses were carried out using SigmaStat for Windows version 2.03 (SPSS, München, Germany): in **Figures 1**, **3**, and **4**, 95% confidence intervals are drawn to illustrate statistically significant differences at a 5% error level. We used nonlinear regression to define empirical relationships between data (**Figures 2** and **5**).

RESULTS AND DISCUSSION

Mean Values of Sampling Times. We investigated berries from two cultivars (Bacchus and Silvaner) at four sampling times (T1−T4). Data from each of these eight groups of berries are represented as mean values with 95% confidence intervals in **Figures 1**, **3**, and **4**. **Figure 1A** shows that concentrations of total sugars ranged between 5 and 240 g L^{-1}. These data agree well with minimum and maximum sugar contents in other cultivars (*2*, *5*, *6*, *30*), and we, therefore, conclude that our investigation covers most of the ripening process of berries.

While fructose and glucose were the principal sugars, minor amounts of sucrose were also detected (**Figure 1A−D**): at T1, glucose and fructose represented 88 and 94% (w/w) of total sugars in Bacchus and Silvaner berries, respectively, but these two hexoses always accounted for more than 95% in the remaining groups of berries. We observed marked accumulation of all sugars in Bacchus berries at T2, but Silvaner berries
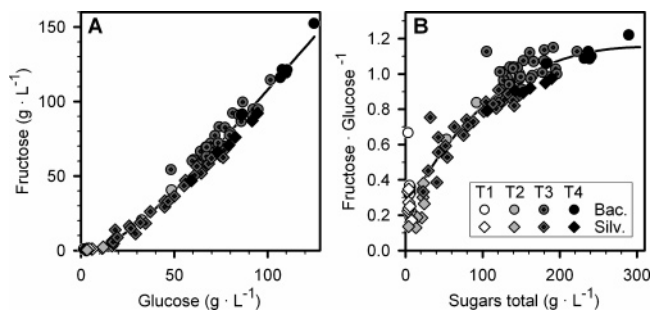

Figure 2. Relation between fructose and glucose concentrations (A) and between fructose/glucose ratios and total sugar concentration (B) in Bacchus and Silvaner berries. The grapevine cultivar and sampling time of individual data points are identified by the legend in panel B. Solid curves in panels A and B result from fitting the sum of an exponential and a linear function to data points. Equations of solid curves and coefficients of determination (R^2) are panel A, $f(x) = 37 + 0.041x + 1.4e^{37x}$ ($R^2 = 0.983$) and panel B, $f(x) = 2.0 - 0.002x - 1.8e^{-0.006x}$ ($R^2 = 0.915$).

exhibited no substantial increase in sugars until T3 (**Figure 1A−D**). Further, from T2 to T4, all sugar data of each cultivar increased steadily but, within each sampling period, concentrations were noticeably higher in Bacchus than in Silvaner berries, which is consistent with the fact that Bacchus berries ripen earlier than Silvaner berries (personal communication, H. Hofmann and J. V. Herrmann, Bayerische Landesanstalt für Weinbau und Gartenbau, Germany).

A closer examination of **Figure 1C**,**D** reveals that, in both cultivars, glucose accumulation preceded that of fructose, thus resulting in lower fructose/glucose ratios at the earlier sampling times than at later ones (**Figure 1E**). These data are consistent with the observed increase in fructose/glucose ratios during the ripening process, which is due to the conversion of imported sucrose into hexoses by invertase (*30−32*). Remarkably, when individual fructose versus glucose concentrations are plotted, data points from both cultivars seem to follow the same curvilinear trend line (**Figure 2A**). Similarly, one function was sufficient to describe the relationship between fructose/glucose ratios and total sugar (**Figure 2B**). Therefore, similar stoichiometrical relationships exist between sugars in both cultivars, although they differ in both time course and amount of sugar accumulated.

When data at T1 and T4 are compared (**Figure 3**), berries within each cultivar always increased in fresh weight, diameter, and density in a statistically significant manner: of these three characteristics, fresh weights exhibited the largest relative increase that was, in both cultivars, about 3.5-fold higher at T4 than at T1 (**Figure 3A**). However, by comparison, relative increases of sugar concentrations were clearly higher and ranged between 8-fold for sucrose in Silvaner berries (**Figure 1B**) and 100-fold for fructose in both cultivars (**Figure 1D**). Only the relative changes in fructose/glucose ratios were comparable to variations in fresh weight (**Figures 1E** and **3A**). Notably, the marked differences between cultivars observed in sugar measurements (**Figure 1**) were not obvious in **Figure 3**. Overall, sugar concentrations seem to be better suited to report ripening than weight, diameter, and density.

F_0 fluorescence (**Figure 4A**) appeared to be negatively associated with sugars (**Figure 1**) but not with our other ripening data (**Figure 3**). A substantial decline of the F_0 signal was apparent already at T2 in Bacchus berries, but reduction of F_0 started only after T2 in Silvaner berries. By comparison, except for sucrose, concentrations of sugars and fructose/glucose ratios were much higher at T2 as compared to T1 in Bacchus berries,
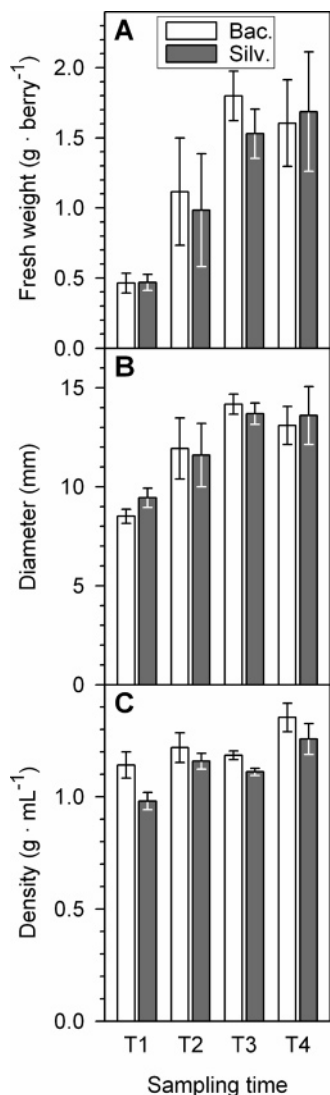
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Figure 3. Apparent growth and density of Bacchus and Silvaner berries. For comments on abscissa, see the legend to Figure 1. Also, the number of measurements, *n*, was as reported for Figure 1 except at T1 where *n* was <8. Panels A and B depict fresh weights in grams per berry and berry diameters, respectively. Densities were calculated for individual berries from fresh weights and diameters and subsequently averaged (panel C).
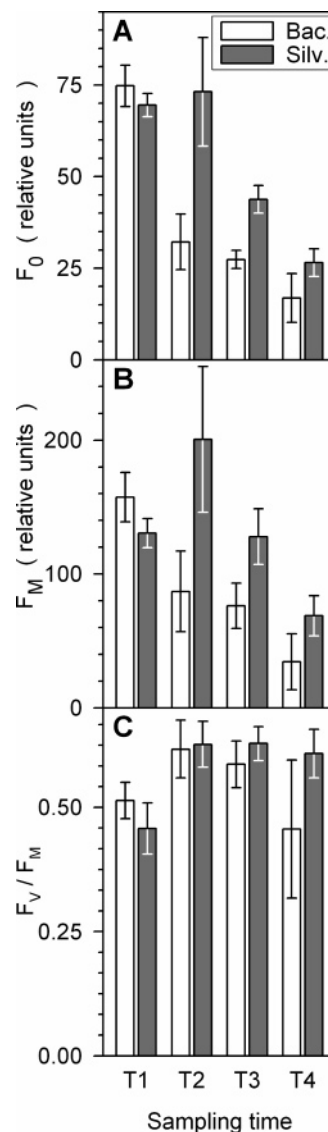

Figure 4. Fluorescence data of Bacchus and Silvaner berries. See legend to Figure 1 for comments on abscissa data and the number of measurements. Panels A and B depict relative values for F_0 fluorescence intensities and F_M fluorescence intensities. The F_V/F_M values (where $F_V = F_M - F_0$) were calculated for individual berries and then averaged (panel C).

but these data increased markedly only after T2 in Silvaner berries (**Figure 1**). Moreover, a stepwise decrease in F_0 took place from T2−T4 in both cultivars, and F_0 levels within a particular sampling time were lower in Bacchus than in Silvaner berries (**Figure 4A**). At the same time, major sugars and fructose/glucose ratios increased from T2−T4 in both cultivars, and within a sampling time, these data were higher in Bacchus than in Silvaner berries (**Figure 1**).

The pattern of F_M fluorescence resembled that of F_0 fluorescence (**Figure 3A,B**), suggesting that the two fluorescence parameters are similarly associated with sugars. However, the F_M of each of our eight berry populations exhibited greater variations than the corresponding F_0: on average, the coefficient of variation of F_M data was 1.7-fold higher that that of F_0 data. The higher variability might be explained by different exposure histories of individual berries resulting in various degrees of sustained photoinhibition, which affects the F_M fluorescence much more than the F_0 level (*17, 18, 33*). Still, the relatively invariant mean values of F_V/F_M ratios suggests the absence of

large differences of mean photochemical efficiencies of PS II between either sampling times or cultivars. Most likely, the decreases from T1−T4 of mean fluorescence intensities (F_0 and F_M) result from decreasing chlorophyll concentrations during ripening (*17, 18*).

Data of Individual Berries. We suggested previously that a common relationship exists in both cultivars between fluorescence intensities and sugar measurements. Therefore, both cultivars are considered collectively when associations between data of individual berries were analyzed using the Pearson product moment correlation (**Table 1**).

Generally, statistics with individual berries were consistent with the behavior of mean values. Among the three types of fluorescence data, the best correlations were between the F_0 and the F_M: that the correlation between the two fluorescence intensities was not perfect (correlation coefficient: $r = 0.794$) agrees with the higher variability of the latter as compared to the former (**Figure 4A,B**). The correlation between F_M and F_V/F_M was comparably weaker but statistically significant: this

Table 1. Pearson Product Moment Correlation[a]

	F_0 (r.u.)			F_M (r.u.)			F_V/F_M		
	r	P	N	r	P	n	R	P	n
F_M (mV)	0.794	<0.01	107						
F_V/F_M	−0.097	0.321	107	0.445	<0.01	107			
density (mg μL^{-1})	−0.552	<0.01	97	−0.411	<0.01	97	−0.018	0.860	97
diameter (mm)	−0.672	<0.01	97	−0.351	<0.01	97	0.299	<0.01	97
fresh weight (g berry^{-1})	−0.723	<0.01	107	−0.416	<0.01	107	0.325	<0.01	107
fructose (g L^{-1})	−0.891	<0.01	97	−0.729	<0.01	97	−0.113	0.269	97
glucose (g L^{-1})	−0.899	<0.01	97	−0.689	<0.01	97	−0.012	0.910	97
sucrose (g L^{-1})	−0.714	<0.01	97	−0.606	<0.01	97	−0.219	0.031	97
sugar total (g L^{-1})	−0.896	<0.01	97	−0.713	<0.01	97	−0.071	0.488	97
fructose·glucose^{-1}	−0.919	<0.01	107	−0.691	<0.01	107	0.147	0.131	107

[a] Strength of association among the three parameters of fluorescence recorded and between fluorescence and other variables was tested for all Bacchus and Silvaner berries. The table lists correlation coefficients (*r*), *P* values (*P*), and the number of data pairs involved in the computation (*n*).

is consistent with the notion that variations in F_V/F_M result mainly from variations in F_M and are largely independent from F_0, which was not correlated with F_V/F_M. Apparently, F_M integrates both chlorophyll concentration and PS II function, but F_0 fluorescence depends predominantly on chlorophyll concentration as has been suggested for other fruits by Smillie et al. (*18*).

F_0 fluorescence was always better correlated to all our ripening-associated data than F_M (**Table 1**), which agrees with the higher variability of F_M than F_0 (**Table 1**). As is expected from the mean values for the F_V/F_M ratio (**Figure 4C**), this ratio was weakly or statistically insignificantly correlated to all ripening-associated data (**Table 1**). However, high correlation coefficients of around 0.9 were observed (**Table 1**) for the relationships between F_0 fluorescence and concentrations of fructose, glucose, or total sugar but, also, when F_0 fluorescence is compared with fructose/glucose ratios. Clearly, these high associations suggest that the F_0 fluorescence is particularly suited to noninvasively report changes in sugar contents in berries during ripening.

The actual data points of these four best correlations are shown in **Figure 5**. In each of these plots, data of the two cultivars follow the same trend and, thus, demonstrate that a common relationship exists between fluorescence and sugars, although berries of different cultivars exhibited different patterns of sugar accumulation (**Figure 1**). Presupposing that F_0 reports chlorophyll concentrations, one might speculate about similar regulatory mechanisms coordinating chlorophyll decay and sugar synthesis in both cultivars; however, such a causal relationship clearly cannot be proven to exist by the data correlations provided here.

We used the sum of a linear and an exponential function (**Figure 5B**, legend) to establish trend-lines for the three plots of F_0 versus sugar concentrations (**Figure 5A**−**C**), but a linear function was sufficient to describe the relationship between F_0 and fructose/glucose ratios (**Figure 5D**). Coefficients of determination observed for the four trend-lines ranged between 0.828 and 0.861. Therefore, F_0 fluorescence measurements appear well-suited to noninvasively measure the accumulation of sugars in grape berries.

Upward curvature of trend-lines, established for relationships between F_0 and sugar concentrations, signify that F_0 fluorescence reports accumulation of sugars with higher sensitivities at the beginning of the ripening process, when sugar contents are low, as compared to later ripening stages with higher sugar contents (**Figure 5A**−**C**). The fructose/glucose ratios varied more during initial sugar accumulation as compared to sugar build-up at later ripening stages (**Figure 2B**). This explains why F_0 fluorescence was linearly related to fructose/glucose ratios.
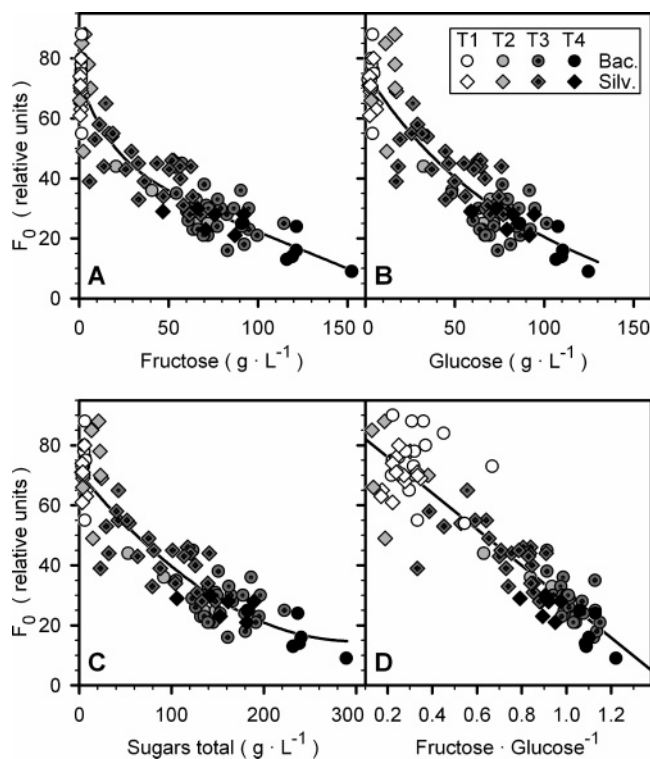

Figure 5. Relationships between F_0 fluorescence intensities and sugar concentrations or ratios in Bacchus and Silvaner berries. In panels A–D, F_0 fluorescence of individual berries is plotted against concentrations of fructose, glucose, total sugars, and fructose/glucose ratios, respectively ($n = 97$). The grapevine cultivar and sampling time of individual data points are identified by the legend in panel B. Solid curves in A–C result from fitting the sum of an exponential and a linear function to data points. Equations of solid curves and coefficients of determination (R^2) are panel A, $f(x) = 46 − 0.24x + 27e^{-0.06x}$ ($R^2 = 0.861$); panel B, $f(x) = 24 − 0.14x + 51e^{-0.02x}$ ($R^2 = 0.828$); panel C, $f(x) = 36 − 0.09x + 39e^{-0.01x}$ ($R^2 = 0.847$); and panel D, $f(x) = 88 − 60x$ ($R^2 = 0.845$).

To our knowledge, we show for the first time that high associations between F_0 fluorescence intensities and sugar concentrations in white grape berries exist. Consequently, the process of sugar accumulation during ripening in white berries can be assessed noninvasively using F_0 fluorescence. Most likely, the method can be extended to red grapes, which accumulate anthocyanins in their skin as these compounds absorb maximally between 500 and 600 nm (*34*−*36*) with minor absorption at longer wavelengths, where we excited and detected chlorophyll fluorescence (see Materials and Methods).

The method is suitable for use in laboratories where homogeneous material is required for subsequent physiological and molecular biological investigations or for selecting grapes after harvest. In the field, fluorescence measurements must be confined to periods of low natural radiation to measure F_0. Furthermore, for measurements carried out at varying distances from berries (see the Introduction), a fluorescence standard placed close to the sample is required to calibrate the fluorometer.

Earlier, fluorometry has been established to estimate the content of flavonols in white grape berries (*37*), and the fluorometrical determination of anthocyanins has been established for olive fruits (*38*). It seems promising, therefore, to develop fluorometers that are able to concomitantly measure sugars, flavonols, and anthocyanins in grape berries. The fast information gained noninvasively by such instruments will certainly advance our understanding of the dynamics of the ripening process and assist grape growers to determine the optimum time for harvesting.

ABBREVIATIONS USED

F_0, minimal fluorescence intensity observed when PS II reaction centers are open and photosynthetic membranes are in the nonenergized state; F_M, maximal fluorescence intensity observed when PS II reaction centers are closed and photosynthetic membranes are in the nonenergized state; PS II, photosystem II.

ACKNOWLEDGMENT

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