ANALYZING THE FUNCTIONAL ASSOCIATION AMONG SEED TRAITS, BERRY GROWTH AND CHEMICAL COMPOSITION IN CABERNET-SAUVIGNON BERRY (*VITIS VINIFERA* L.) USING A MATHEMATICAL GROWTH FUNCTION

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Abstract

Aims: This study aimed at assessing the functional linkage among seed traits (including seed number, seed weight), berry growth and berry sugar and acid concentration by adapting a mathematical growth function with parameters having biological importance.

Methods and results: The evolution of berry diameter of Cabernet-Sauvignon was satisfactorily fitted to a bi-phasic growth function with six parameters. Correlations between the parameters and berry characteristics, including time of skin color change, seed number, seed weight, final berry size and chemical composition, were analyzed. Results showed that berry growth within a bunch deviated according to seed number, which positively related to parameters describing diameter increment (D_1) and initial growth rate (GR_{ini}) during the first rapid growth phase, but not related to any parameters involved in the second growth phase. The time of skin color change was negatively associated with mean seed weight, and coincided with growth parameter DAB_{max} that denotes the time when growth rate is maximum during the second rapid growth phase. Sugar concentration was negatively correlated to final berry fresh weight (BFW), seed fresh weight (SFW), GR_{ini} and diameter increment during the second rapid growth phase (D_2). Path analysis further revealed that the negative effects of SFW and D_2 were indirectly mediated via BFW and GR_{ini}.

Conclusions: We thus conclude that seed affects berry growth and exerts its influence only during the first growth phase via modifying the parameters D_1 and GR_{ini}. The time of colour change is also affected by seed, and might not be a good indicator of growth restoration after the lag phase. Sugar concentration is linked to final berry size, specific berry growth parameters and seed weight, for the last mainly in an indirect manner.

Significance of the study: This study, integrating the analysis of berry growth and composition with a mathematical growth function, provides a valuable approach for deeper understanding of the functional associations among seed traits, berry growth and berry sugar and acid concentration at the berry level.

Keywords: fruit growth analysis, seed, sugar, *Vitis*

Résumé

Objectifs : Cette étude a pour objectif de déterminer par une analyse mathématique des paramètres des courbes de croissance de baies de raisin, les effets du nombre de pépins au sein d'une baie sur sa taille et ses concentrations en sucres et acides à maturité.

Méthodes et résultats : L'évolution du diamètre de baies de Cabernet-Sauvignon a été correctement ajustée par une courbe de croissance bi-phasique à six paramètres. Les corrélations entre les paramètres et les caractéristiques de chaque baie, comme la date de changement de couleur de la pellicule, le nombre et le poids individuel des pépins, la taille finale de la baie et sa composition biochimique ont été analysées. Les résultats ont montré que la croissance d'une baie au sein d'une grappe variait significativement en fonction de son nombre de pépins, lequel s'est révélé être corrélé positivement aux paramètres décrivant l'augmentation de diamètre (D_1) et la vitesse initiale de croissance (GR_{ini}) de la baie durant la première phase de croissance, mais pas aux paramètres impliqués dans la deuxième phase de croissance. La date de changement de couleur de la pellicule a été négativement corrélée au poids moyen des pépins, et coïncidait avec le paramètre DAB_{max} qui représentait la date durant la deuxième phase de croissance où la vitesse de croissance de la baie est maximale. La concentration en sucres a été négativement corrélée au poids frais final de la baie (BFW) et des pépins (SFW), à GR_{ini} et à l'augmentation de diamètre durant la deuxième phase de croissance (D_2). Enfin, une analyse statistique des chemins a révélé que les effets négatifs de SFW et D_2 étaient contrôlés indirectement par BFW et GR_{ini}.

Conclusions : Nous concluons ainsi que les pépins affectent la croissance d'une baie et exercent leur influence uniquement durant la première phase de croissance en modifiant les paramètres D1 et GR_{ini}. La date de changement de couleur de la pellicule a été également affectée par les pépins, et pourrait ne pas être un bon indicateur de la reprise de croissance après la phase de transition observée à veraison. La concentration en sucres a été corrélée à la taille finale de la baie, à des paramètres spécifiques de sa croissance et au poids des pépins, de manière indirecte cependant pour cette derniere variable.

Signification de l'étude : L'approche mathématique développée dans cette étude a permis de mieux expliciter l'impact du nombre de pépins par baie sur la croissance et la composition biochimique finale d'une baie de raisin.

Keywords: analyse de la croissance de la baies, pépin, sucre, *Vitis*

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INTRODUCTION

Grape (*Vitis vinifera* L.) quality, especially the chemical composition at harvest, is pivotal to shape wine quality (CONDE *et al.*, 2007). The berry chemical composition such as sugar concentration can be influenced by berry growth, but itself also affects berry growth through feedback adjusting of berry water status (MATTHEWS and SHACKEL, 2005). In this context, factors that affect berry growth might have a potential role in modifying berry composition, and the final berry size and composition may be well represented by growth properties of the same berry. During its post-flowering development, berry growth follows a typical double-sigmoid curve, which consists of two rapid growth phases separated by a lag phase (COOMBE, 1980; COOMBE, 1992). The final berry size at maturity is due to cell division and some extent of cell enlargement during the first rapid growth phase plus cell enlargement during the second rapid growth phase (HARDIE *et al.*, 1996; OJEDA *et al.*, 1999). Berry growth can be affected by many inherent and external factors, among which seed plays an important role through a functional linkage to cell division during the first growth phase (RISTIC and ILAND, 2005). At maturity, berry size is often positively related to seed number and seed weight (EBADI *et al.*, 1996; OLLAT and GAUDILLÈRE, 2000; OLLAT *et al.*, 2002; ROBY and MATTHEWS, 2004; WALKER *et al.*, 2005). In addition, berry size is considered as an indicator of berry chemical composition, among which acids are mainly accumulated during the first rapid growth phase while sugars are largely accumulated during the second rapid growth phase (RUFFNER, 1982; COOMBE *et al.*, 1987). For example, a negative correlation between berry fresh weight and its total soluble solids was observed in Cabernet-Sauvignon grapes (ROBY *et al.*, 2004). Because seed impacts berry size, it might in turn also affect the final berry composition. Moreover, the onset of the skin color change is reported to be associated with the seed maturity in seeded red grape cultivars (CAWTHON and MORRIS, 1982; RISTIC and ILAND, 2005). These results indicate the complexity of the nature that seed functions as a factor affecting berry growth and composition. However, the functional associations among seed traits, berry growth and chemical composition in grape berry, especially on a single berry basis, are not well documented. Accordingly, experiments on the single berry basis and considering the dynamic growth process are of value to improve our knowledge of controlling of berry quality build-up.

Fruit growth analysis assisted with mathematical growth function can quantify and summarize the growth characteristics among different growth phases of an individual fruit through biological meaningful parameters (BUCHWALD, 2007). This approach, therefore, makes it possible to assess the relationships between characteristics of fruit growth and final fruit size and composition. This kind of analysis has already been successfully applied in grapes and other fruits, especially in assessing the effects of carbohydrate availability (DEJONG and GOUDRIAAN, 1989; OLLAT and GAUDILLÈRE, 1998) and genotype (FANIZZA and COLONNA, 1996; GÉNARD *et al.*, 1999) on fruit growth or composition. In this study, a growth function formerly proposed by Genard and Bruchou (1993) was applied to individual berries in order to gain further insights into the functional associations among seed traits, growth and chemical composition of grape berry.

MATERIALS AND METHODS

Plant materials

Fruiting cuttings of *Vitis vinifera* L. cv. Cabernet-Sauvignon were prepared according to the method of Ollat and Gaudillère (1998). Grape cuttings were planted in small pots (0.5 L) containing a mixture of perlite, sand, and vermiculite (1:1:1). They were placed in an environment-controlled greenhouse with mean temperature of 24.4 °C, relative humidity 65.7 % and natural light supply (maximum720 °mol m^{-2} s^{-1}) from 20 February to 12 July, 2007. A complete nutrient solution (1.20 mM N, 0.57 mM P, 1.75 mM K, 1.23 mM Ca, 0.69 mM Mg, 1.27 mM Cl and 0.99 mM S, plus standard micronutrients) was supplied to each pot with a drip irrigation system 3-5 times a day to avoid any water stress throughout the experimental period. One shoot and one bunch were retained for each plant. The shoot was topped when the total leaf number reached 16, giving a leaf-to-fruit ratio that minimizes carbon limitation (KLIEWER and DOKOOZLIAN, 2005). Lateral shoots were removed as soon as they appeared throughout the course of the experiment.

Experimental measurements

Nine days after bloom (50 % of the bunches flowering), 5 uniformly growing plants were chosen, and 4 outer berries of similar size from the middle part of each bunch were tagged. This date (hereafter termed DAB0) was chosen because at that time the berries were just big and hard enough for easily measuring with electronic calliper. Since the specific flowering date for every single berry was not determine due to technical difficulties, we tried to choose berries with similar initial diameters within a bunch to minimize their potential differences in physiological age. The diameters of the 20 tagged berries were measured at equatorial points from DAB_0 to berry maturity at a one-or two-day interval using an electronic calliper (± 0.01 mm precision). Specifically, the berry diameter measured on DAB_0 was termed IniDia

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hereafter. The date the berries showed visible color change in their skin was recorded.

At maturity, only 12 berries (8 one-seeded, 4 two-seeded) were harvested, and the other 8 berries were dropped during the experiment period. Among the 8 dropped berries, there were 3 seedless berries which exhibited only one rapid growth phase and failed to fully mature. These 3 seedless berries were just cut in half to verify seed number without measuring chemical composition, because their final volumes were not big enough to perform such analyses. The 4 two-seeded berries distributed on 4 of the 5 bunches, and each bunch has two or three measured berries (see figure 2). Berry diameters and fresh weights were measured, and then carefully separated into skin, pulp and seed with a sharp blade. Seed number and its fresh weight were subsequently recorded after blotted dry with paper towels. Berry pulp was homogenized using a ball-crusher (Retsch MM200, Fisher Bioblok Scientific, France) for 5 min and was centrifuged at 5,000 rpm for 5 min. An aliquot of the supernatant was used for total soluble solids (°Brix) measurement using a hand refractometer. The extracted juice was diluted four times with distilled water and was stored at -40 °C for later sugar, acid and titratable acid analysis. Sugars, including glucose, fructose and sucrose, were measured enzymatically with an automated micro-plate reader (Elx800UV, Bio-tek Instruments Inc., France) as described by Gomez *et al.* (2007). Tartaric acid and malic acid were measured using an automated colorimetric method with the continuous-flow analyzer TRAACS800 (Bran+Luebbe GmbH, Germany), which followed the method described by Blouin (1992). Titratable acid at pH 7 was determined using an automated compact titrator equipped with a pH meter (Crison Instrument, Italy) with NaOH (0.005 N).

Data analysis and growth function fitting

The change in diameter of an individual berry, was fitted to a growth function, formerly proposed by Génard *et al.* (1991) and Génard and Bruchou (1993) and successfully applied in the analysis of peach fruit growth that exhibits a double sigmoid growth curve (GENARD *et al.*, 1991; GENARD *et al.*, 1999). This function, termed« bi-phasic growth function » hereafter, is composed of two components. The first part is a monomolecular function, which describes the berry growth during the first rapid growth phase, including cell division and cell enlargement. The second part is a logistic component, which describes berry diameter dynamics during the second growth phase. The lag phase is a combination of these two growth components. The complete growth function is defined as:

$$D = \overbrace{D_0 + D_1\left(1 - e^{-GR_{ini}(DAB-DAB_0)/D_1}\right)}^{\text{The first growth phase}} + \overbrace{D_2/\left(1 + e^{-4GR_{2max}(DAB-DAB_{max})/D_2}\right)}^{\text{The second growth phase}}$$

where D (mm) denotes berry diameter, DAB is the number of days after bloom, DAB_0 is the date of the first measurement (an input), and D_0, D_1, D_2, GR_{ini}, GR_{2max}, and DAB_{max} are parameters. Parameter Do (mm) refers to the diameter that a berry has reached at the initial time of simulation (here, 9 DAB), while parameters D_1 (mm) and D_2 (mm) are the increments in diameter during the first and second growth phase, respectively. GR_{ini} (mm day^{-1}) corresponds to the initial diameter growth rate at the beginning of the first diameter measurement. GR_{2max} (mm day^{-1}) is the maximum diameter growth rate in the second growth phase, and DAB_{max} is the time when GR_{2max} occurs.

For each individual berry, the six parameters were estimated with a non-linear least square method. The goodness-of-fit was verified through root mean squared error (RMSE) and relative root mean squared error (RRMSE), which are commonly used to assess the mean difference between measured and fitted values (KOBAYASHI and SALAM, 2000).

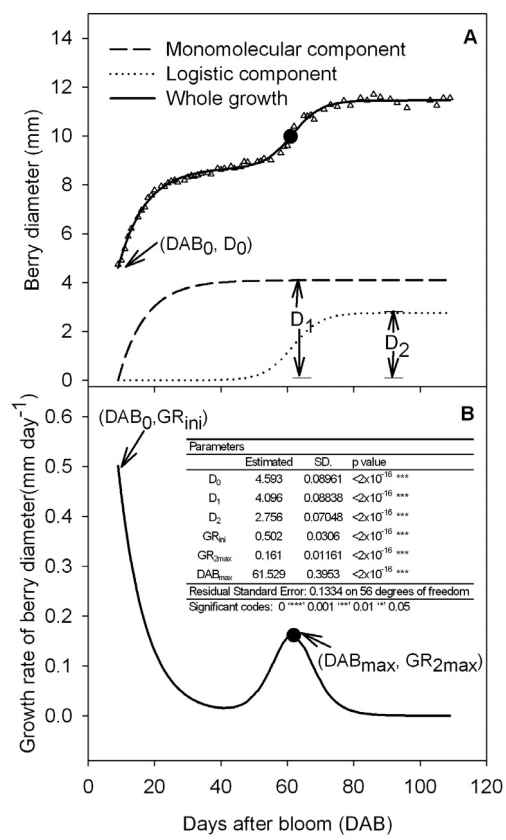


Figure 1 - Changes in diameter (A) and rate of increase in diameter (B) of a single berry.
Points (uptriangles) are measured values, and the line is a fitted curve by a bi-phasic growth function. Parameter meanings in the growth function were indicated in the figure. Their estimated values and contributions to the function were also inserted in (B) as an example. The filled circle indicates the date of change in skin color.

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An analysis of variance was conducted to assess the effect of seed number on the 6 parameters, with consideration of bunch variability by regarding bunch as a block. Spearman's rank correlation and linear regression were also undertaken when needed. Moreover, a path analysis was conducted to evaluate the direct and indirect effects of seed fresh weight, berry fresh weight, GR_{ini} and D_2 on berry total soluble solids. Path analysis is a statistical method that can decompose the correlation between any independent variable and the dependent variable into direct and indirect effects. The direct effect is quantified by the standardized partial regression coefficient between the dependent variable and a given independent variable. The indirect effect results from the correlations between multiple independent variables. Because of these features, path analysis is appropriate to explore complex causal links between variables where one independent variable affects a second independent variable, which in turn influences the dependent variable (QUINN and KEOUGH, 2002). All data analyses were performed with software R (R Development Core Team, http://www.R-project.org).

RESULTS

Reliability of the approach

An illustration of the bi-phasic growth function, its fit to the diameter evolution and the deduced growth rate of a representative berry are shown in figure 1. The increase in diameter was fairly well fitted using the bi-phasic growth function (RMSE=0.13 mm, RRMSE = 1.4 %). All six parameters were well estimated with relatively low standard deviations and significant contributions to the curve as revealed by the summary inserted in figure 1B. Although the berry growth exhibited a large berry-to-berry variation, they were all well fitted by the growth function (figure 2) as revealed by the RMSE and RRMSE values. These values were always smaller than 0.24 mm and 2.9 %, and had the mean values of 0.17 mm and 2.1 %, respectively.

Effect of seed on berry growth within and between bunches

Berry growth showed large variations within a bunch (figure 2A-E) and between bunches (figure 2F), resulting in considerable differences in berry diameter (from 8.6 to 12.3 mm) and fresh weight (from 0.5 to 1.2 g) at maturity (table 1). Growth parameter D_0, which is approximately equal to the measured initial berry diameter (IniDia, table 1), was not affected by seed number within a bunch (table 2). However, the values of D_0 were significantly different between bunches (table 2). Despite the variation in berry growth, however, there were clear divergences in berry diameter according to seed number within a bunch from about 20 days after bloom to maturity (figure 2A-E). The growth divergences associated with seed number became less clear but still distinguishable

Table 1 - Maximum, minimal, mean, standard deviation (SD) and coefficient of variance (CV) of measured berry diameter at 9 days after bloom (IniDia) and at harvest (HarDia), fresh weight of berry (BerFW) and seed (SeedFW) and total soluble solids (TTS), glucose (Glc), fructose (Fru), malic acid (Mal), tartratic acid (Tar) and titratable acidity (TA) at harvest.

	IniDia mm	HarDia mm	BerFW g	SeedFW mg	TSS °Brix	Glc g L^{-1}	Fru g L^{-1}	Mal meq L^{-1}	Tar meq L^{-1}	TA meq L^{-1}
Max	4,6	12,3	1,2	56,3	26,6	138,2	128,2	102,9	107	181,6
Min	2,7	8,6	0,5	13,8	20,3	101,3	87,7	58,4	68,1	89,2
Mean	3,4	10,5	0,8	31,1	22,7	115,2	104,0	80,0	92,0	116,3
SD	0,7	1,0	0,2	13,5	1,8	10,4	11,0	13,2	11,9	25,0
CV%	20,6	9,5	25	43,4	7,9	9	10,6	16,5	12,9	21,5

Table 2 - Analysis of variance for the six parameters of the bi-phasic growth function with seed number as factor and bunch as block.

	D_0 mm	D_1 mm	GR_{ini} mm day^{-1}	D_2 mm	GR_{2max} mm day^{-1}	DAB_{max} day
Seedless	3.16±0.35	2.24±0.39	0.47±0.02	--	--	--
One-seeded	3.56±0.74	4.62±0.81	0.51±0.116	2.11±0.65	0.16±0.088	60.6±5.72
Two-seeded	3.11±0.69	6.04±1.44	0.65±0.067	2.56±0.88	0.15±0.069	61.8±3.23
Seed Number	ns	***	*	ns	ns	ns
Block (bunch)	***	***	ns	ns	**	**

ns=not significant; *=significant at $P<0.05$, **=significant at $P<0.01$; ***=significant at $P<0.001$.
Values are means ± SD, Fisher test was used.

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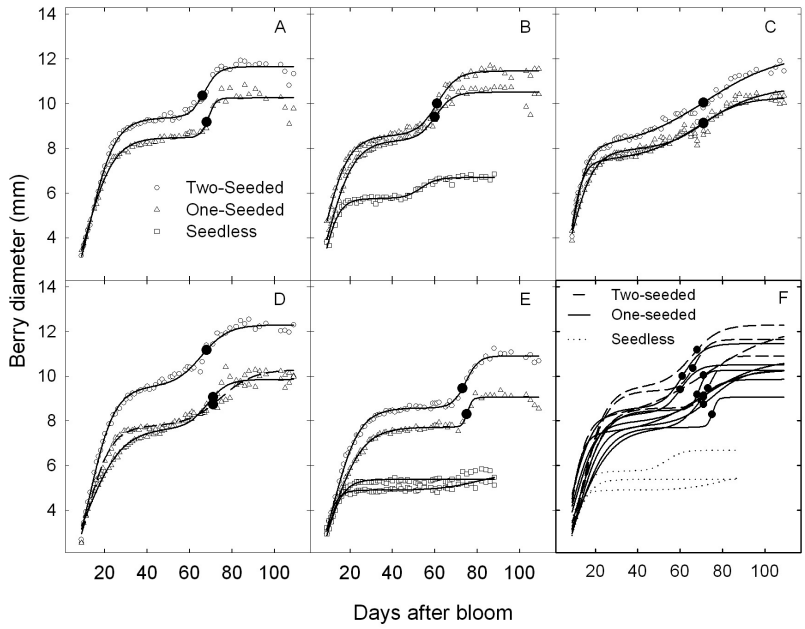


Figure 2 - Measured (open symbols) and fitted (solid lines) changes in diameter of all observed berries showing separately for each bunch (A-E) or pooling all curves together (F).

Filled circles indicate the time of change in skin color for each observed single berry. Only the monomolecular component of the bi-phasic growth function was used to fit the changes in diameter of the seedless berries (E).

when pool all the growth curves together (figure 2F). Generally, the more seeds a berry had, the larger its diameter is and the faster it increased. To identify during which growth phase the seed influences berry growth, the two growth components in the growth function, namely, the monomolecular and logistic part, were separately shown in figure 3 so that the « pure » influence of events taking place in each single phase could be quantified. As revealed, the diameter increments were about two times higher during the first growth phase than those of the second one. The plateau of the first growth phase exhibited a similar divergence according to seed number as observed in the whole growth curves (figure 3A). In contrast, no clear divergence according to seed number was observed in the plateau of the second growth phase (figure 3B). Further analysis revealed that the seed had no effect on the growth parameters describing the second growth phase (table 2). Seed mainly exerted influence on growth through altering the parameters D_1 and GR_{ini} (table 2), which were both positively related to total seed weight (figures 4A and B) and to the number of seeds (figures 4D and E). A positive correlation between berry fresh weight and total seed fresh weight was also observed (figure 4C).

Effect of seed on berry color change

Consistent with the variation in berry growth, the date when individual berries changed their color also exhibited large variations between bunches (from 60 to 75 DAB). This date was not presented at the beginning of the second rapid growth phase (figure 2). In fact, it coincided with the time when the maximum growth rate reached during the second rapid growth phase, that is, the parameter DAB_{max} (figures 2 and 5A). No significant correlation between berry seed number and the time of change in color was observed (table 2). In contrast, the occurrence of the change in skin color was accelerated by the increased mean seed weight (figure 5B).

Effects of seed, berry size, and berry growth parameters on chemical composition

Concentrations of sugars and acids showed large variations among berries at harvest (table 1). Berry sugar (i.e. total soluble solids (TSS), glucose, and fructose) concentration was negatively correlated with seed fresh weight, berry fresh weight, as well as with the parameters of GR_{ini} and D_2 (table 3). Path analysis revealed that the effects of berry fresh weight and GR_{ini} on berry TSS, were primarily direct effects. In contrast, berry seed weight and berry growth during the second growth phase (D_2) exhibited a moderate direct positive effect on TSS and a large indirect negative effect via berry fresh weight and the parameter GR_{ini} (table 4). Considering the acids, only the concentration of malic acid was positively related to the parameter DAB_{max} (table 3).

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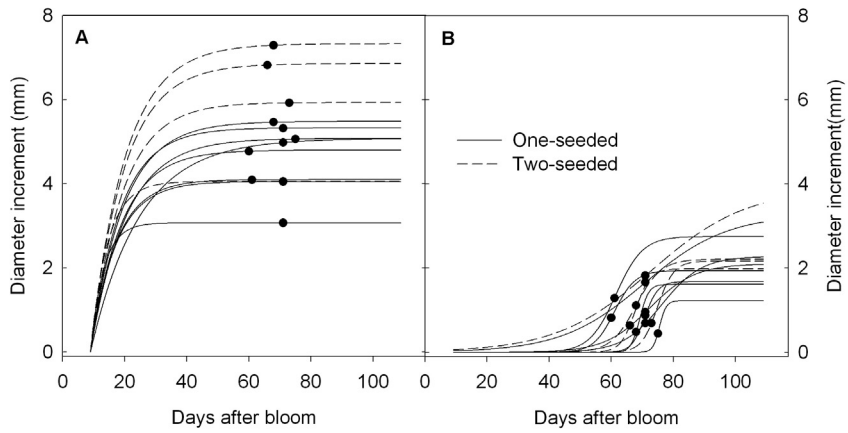


Figure 3 - Diameter increment during the first growth phase (A) and the second growth phase (B).
These curves were obtained by showing separately the monomolecular and the logistic component. Filled circles indicate the time of color change for each berry.

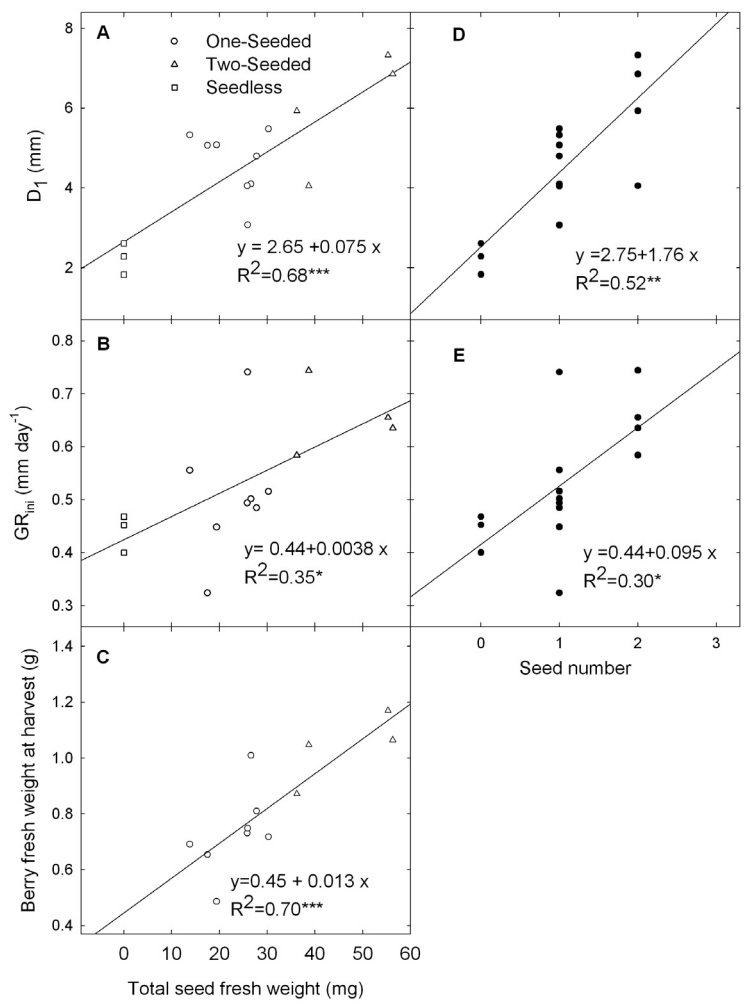


Figure 4 - Diameter increment during the first growth phase (D_1), the initial growth rate at the beginning of measurement (GR_{ini}) and berry fresh weight at harvest in the function of total seed fresh weight (A,B,C) and seed number(D,E).
*: significant at P<0.05, **: significant at P<0.01; ***: significant at P<0.001.

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Table 3 - Correlation between total seed fresh weight (SeedFW), berry fresh weight at harvest (BerFW), and the parameters of the growth function and berry chemical composition (n=12).

	Berry composition					
Factors	TSS	Glc	Fru	Mal	Tar	TA
SeedFW	-0.674*	-0.658*	-0.657*	-0.546ns	-0.223ns	-0.344ns
BerFW	-0.776**	-0.774**	-0.764**	-0.538ns	-0.118ns	-0.438ns
D_0	-0.36ns	-0.344ns	-0.342ns	-0.35ns	-0.17ns	-0.42ns
D_1	0.03ns	0.014ns	0.022ns	-0.21ns	0.2ns	0.14ns
GR_{ini}	-0.73**	-0.725**	-0.745**	-0.05ns	-0.34ns	-0.48ns
D_2	-0.56*	-0.576*	-0.579*	-0.03ns	-0.09ns	-0.42ns
GR_{2max}	0.36ns	0.35ns	0.367ns	-0.28ns	-0.05ns	0.1ns
DAB_{max}	0.41ns	0.388ns	0.378ns	0.58*	-0.1ns	0.37ns

ns: not significant; *: significant at $P<0.05$; **: significant at $P<0.01$; ***: significant at $P<0.001$.
Values are Spearman's correlation coefficients.

Table 4 - Path analysis assessing the direct and indirect effects of total seed fresh weight (SeedFW), berry fresh weight (BerFW), GR_{ini} and D_2 on berry total soluble solids.

Factors	Direct effect	Indirect effect	Total effect
SeedFW	**0,57**	**-1,246**	**-0.676***
via BerFW		-0,87	
via GR_{ini}		-0,44	
via D_2		0,064	
BerFW	**-1,002**	**0,22**	**-0.782****
via SeedFW		0,5	
via GR_{ini}		-0,46	
via D_2		0,18	
GR_{ini}	**-0,66**	**-0,07**	**-0.73****
via BerFW		-0,69	
via SeedFW		0,38	
via D_2		0,24	
D_2	**0,33**	**-0,89**	**-0.56***
via BerFW		-0,53	
via SeedFW		0,11	
via GR_{ini}		-0,47	

*: significant at $P<0.05$; **: significant at $P<0.01$.

DISCUSSION

Functional association between seed and berry growth

Berry growth was related to seed number and total seed weight (figure2, table 2 and figure 4). This is consistent with the results of many studies that have defined positive relationships between berry weight at harvest and its seed number (HARDIE and AGGENBACH, 1996; WALKER *et al.*, 2005), as well as berry weight and seed weight (EBADI *et al.*, 1996; OLLAT and GAUDILLÈRE, 1998; ROBY and MATTHEWS, 2004; RISTIC and ILAND, 2005). Our approach, which is based on mathematical growth analysis, has further shown that seed number has a strong effect during the first rapid growth phase, but not during the second rapid growth phase (figure 3 and table 2). Moreover the initial growth rate (GR_{ini}) and the increase in diameter (D_1) during the first growth phase were both positively affected by increases in seed number and the total fresh weight of seed (tigure 4), which is in agreement with the results of Coombe (1960) and of Ristic and Iland (2005). On the other hand, the absence of correlation between seed and berry growth during the second rapid growth phase may result from the cessation of seed growth during this stage (RISTIC and ILAND, 2005). However, it should be mentioned that the initiating date of berry diameter measurement has some potential impacts on the curve shape of the first growth phase. If the berry diameter is measured much earlier, for example one day after bloom, the diameter growth of the first growth phase might be better fitted by a logistic function as proposed by FANIZZA and COLONNA (1996), instead of the monomolecular function used here. The observed significant difference in growth parameter D_0 between bunches was not associated with seed number or seed weight. This very early differences in berry size between

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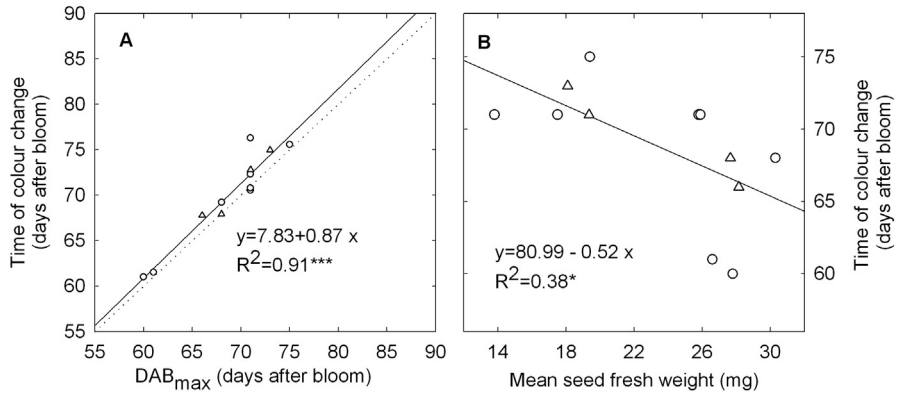


Figure 5 - Relationship between the time of colour change and DAB$_{max}$ (A) and mean seed fresh weight (B). Solid lines are the linear regression lines, and the dotted line is the line of y=x.

*: significant at P<0.05; ***: significant at P<0.001.

bunches might be due to possible difference in flowering time for every single berry (COOMBE, 1980; MAY, 2000).

Color change and renewed berry growth

The change in skin color is often considered as a sign of veraison, which denotes the onset of ripening, the end of the lag phase, and the inception of renewed berry growth (COOMBE, 1992). Despite this change in skin color, however, many other processes including softening, sugaring, specific gene expression, and growth restoration also occur during this specific period. Generally, the softening is considered as the first event (COOMBE, 1980; COOMBE and BISHOP, 1980; TERRIER *et al.*, 2005), then sugar accumulation starts simultaneously or prior to renewed growth (COOMBE, 1960; COOMBE, 1992). However, the relative order between the time of change in color and renewed berry growth is less known, particularly at the individual berry level. Our results precisely anchored the time of this change in color at a specific point in the corresponding growth curve of every individual berry (figure 2). To the best of our knowledge, the current study is the first to investigate the change in berry color in this manner. The time of the change in color for each individual berry was surprisingly found to be consistent with a growth parameter, DAB$_{max}$, which represents the time when the maximum growth rate occurs during the second growth phase (figure 5A). This finding reveals that the change in color is not a good indicator of the beginning of the second growth phase. In addition, the variation in time of change in color could be explained partly by the mean fresh weight of seed (figure 5B). Because the mean fresh weight indicates, at least to some extent, development status and maturity of the seeds, the above observed correlation was therefore in agreement with the previously observed correlation between seed maturity and berry coloring (CAWTHON and MORRIS, 1982).

Implications of berry size and seed in berry composition

Berry size is known as an indicator of berry chemical composition (CAWTHON and MORRIS, 1982; COOMBE *et al.*, 1987; CARBONNEAU, 2004; ROBY *et al.*, 2004; TARTER and KEUTER, 2005). Consistent with the previous results, we also observed a negative correlation between berry diameter and sugar concentration at maturity. However when the final berry diameter was separated into increments from the first and the second rapid growth phases with the bi-phasic growth function, only the diameter increments during the second growth phase (D_2) were found significantly and negatively related to sugar concentration, but not that of the first growth phase (D_0+D_1) (table 3). This indicates that the increase in berry size, which occurred during the first and the second rapid growth phases due respectively to cell number and cell enlargement, may have distinct consequences in relation to sugar concentration. The negative correlation between the initial growth rate during the first rapid growth (GR_{ini}) and sugar concentration further strengthens this argument. Because GR_{ini} reflects, to some extent, the intensity of cell division during the first growth phase, an increase in GR_{ini} can cause an increase in cell number. When the cell number is large, competition for carbon sources among cells might be high (JULLIEN *et al.*, 2001a; JULLIEN *et al.*, 2001b; LESCOURRET and GÉNARD, 2003; QUILOT and GÉNARD, 2008) and could consequently result in a low sugar concentration. Moreover, seed fresh weight was found to exhibit a significant negative effect on sugar concentration. Because seed fresh weight was also related to other berry traits, such as final berry size and GR_{ini}, its

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effects on sugar concentration might be indirectly mediated. Path analysis revealed that seed fresh weight and parameter D_2 exhibited direct positive effects on sugar concentration, although their total effects were negative mainly because of the larger indirect effect via berry fresh weight and parameter GR_{ini}. This implies the possibility that increased seed fresh weight and D_2 might compensate for the negative effect of an increased berry size and GR_{ini} on sugar concentration. With respect to acid concentration, only the variation in malic acid was positively correlated to parameter DAB_{max}, which is consistent with the results in peach (GENARD *et al.*, 1999). However, the results from grape and peach may imply different underling mechanisms because malic acid is the major substrate for respiration in grape berry while it is a storage component in peach fruit. In grape berry, malic acid is mainly accumulated during the first rapid growth phase, and declines after the onset of ripening (RUFFNER, 1982; TERRIER *et al.*, 2001; COOMBE and ILAND, 2004). Suppose all the berries are harvested at the same time, as in our experiment, the later the berry changes in color or the later the onset of ripening, the shorter the time for malic acid to be respired. This may probably be responsible for the observed positive association between malic acid and the parameter DAB_{max}, which coincides with the date of change in color.

CONCLUSION

With the assistance of a bi-phasic growth function, we studied the functional associations among characteristics of berry growth, seed, and berry chemical composition on a single berry basis. Seed number and seed weight can affect berry growth, exerting their impacts mainly during the first growth phase. The time of colour change is located in the middle of the second rapid growth phase parallel with the occurrence of the maximum growth rate, making it a bad indicator for growth restoration. Seed weight and growth parameters both in the first and in the second rapid growth phase have an influence on the final berry composition in terms of sugar and acid. Finally, the proposed approach using mathematical growth function will be useful to improve our understanding of berry growth variability within a bunch in the future research.

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