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# Influence of water deficits on grape berry growth

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## Summary

**The effects of early and late water deficits on pericarp cell division and enlargement of Syrah berries (*Vitis vinifera* L.) was determined by DNA extraction and quantification. Different periods and different levels of water deficit were applied between anthesis and maturity to grapevines growing under controlled water supply in two consecutive years. DNA extraction profiles showed that water deficit did not affect cell division. Reduction of berry size and berry weight was caused exclusively by a decrease of pericarp volume, independent of the intensity of the water deficit or the stage of berry development. Decreased cell volume as a result of an early water deficit from flowering to veraison was irreversible. These results support the hypothesis that early water deficits modify the structural properties of the cell components and consequently cell wall extensibility, thereby limiting the subsequent enlargement of pericarp cells.**

K e y w o r d s : *Vitis vinifera*, water deficit, DNA, fruit growth, cell division, cell enlargement.

## Introduction

Final berry size is an important factor which determines grape quality via the ratio skin area: juice volume (Champagnol 1998); besides other factors, the grapevine water status strongly affects berry size.

Water deficits between anthesis and veraison decrease berry size and this is often irreversible even if there is no water shortage after the beginning of ripening (Hardie and Considine 1976; Matthews *et al.* 1987; McCarthy 1997).

Final berry size is more influenced by water deficits of similar intensity between flowering and veraison than between veraison and maturity. During the ripening period (phase III) the size of stressed berries recovers partially or totally, if water is avaible (Van Zyl 1984; Naor *et al.* 1993; Poni *et al.* 1994; McCarthy 1997).

Cell division of the pericarp occurs only during the first growth phase (phase I) (Considine and Knox 1981; Jona and Botta 1988; Ojeda *et al.* 1999; Coombe and McCarthy 2000). A widely accepted hypothesis is that early water deficits reduce the rate of cell division, which would explain the inability of berries to recover in size after an early water deficit. After veraison berry size reduction due to water deficits is thought to be a consequence of a limitation of cell enlargement. However, this hypothesis has not been verified. The effects of water deficits on cell division and enlargement in the grape pericarp are not clearly understood.

Ojeda *et al.* (1999) have presented a method to quantify indirectly the size and division of pericarp cells by extraction and quantification of DNA. This technique was applied to study the influence of early and late water deficits on the dynamics of cell division and enlargement of the pericarp of grape berry, cv. Syrah.

## Material and Methods

P l a n t m a t e r i a l a n d c u l t i v a t i o n : Berries were harvested from 5- and 6-year-old grapevines (*Vitis vinifera* L. cv. Syrah), grafted on Fercal, trained as a Lyre system, spur- pruned and grown in 70-l pots in the field (Carbonneau and de Loth 1985). The substrate was a mixture of perlite and sand (90:10 v/v). Water and the mineral solution were applied by drip irrigation, the supply was controlled according to light absorption ("Ecotron").

W a t e r t r e a t m e n t s : In 1997 and 1998 plots of 10 plants were irrigated. The water supply to each regime was determined daily as the percentage of total water of the control. Evapotranspiration of control vines was estimated by daily measuring water consumption of each pot by water replacement. In 1997 three irrigation programs were applied: the control treatment ($C_I$) received daily the equivalent of 100 % of the evapotranspiration throughout the season; the early water deficit ($S_A$) treatment received 30 % of $C_I$ between anthesis and veraison; and the late water deficit ($S_B$) received 30 % of $C_I$ water between veraison and maturity. In 1998, 4 irrigation regimes were applied: the control, $C_{II}$ (100 %); two levels of early water deficit applied between anthesis and veraison, $S_1$ (30 %) and $S_2$ (50 %); and a late water deficit applied between veraison and maturity, $S_3$ (30 %).

P l a n t w a t e r s t a t u s : The water status was determined by the predawn leaf water potential ($\Psi$) measured by the pressure chamber technique (Scholander *et al.* 1965). For each measurement, 6 fully expanded leaves were chosen in the centre of the canopy from 6 different plants per treatment. Measurements were carried out at 4-d intervals during the experiment .

S a m p l e p r e p a r a t i o n : In 1997, three clusters per plant were harvested, giving 30 randomised clusters per sample. Two samples were harvested: The first one week

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before the onset of veraison, as determined by softening of 10 % of the berries, 47 d after anthesis (day 47; 2.7.97), and the second at maturity (day 116; 5.9.97).

In 1998, for $C_{II}$, $S_1$ and $S_2$ treatments, measurements started at anthesis (day 0; 30.5.98) and for $S_3$ at veraison (day 49; 18.7.98). Samples were harvested every 10 d, from anthesis to maturity (day 110; 17.9.98). For each determination, 8 clusters from two plants per treatment were sampled. Samples were taken from each plant only once during the experiment.

Classification and characterization of berry populations: All berries of a cluster were cut at the distal end of the pedicel, counted, weighed and classified according to their diameter by sieving. Each class was characterized by its frequency (number of berries per class) and by the mean berry weight. The mean diameter and the weight of the berry population were determined. The mean berry density of the major class, *i.e.* the class with the highest frequency, was measured by floating berries in solutions of sucrose with different concentrations (ROMIEU, pers. comm.). The mean berry volume of the major class was determined by water displacement.

The use of major classes allowed to work with representative and homogeneous berries since major classes were strongly correlated with the mean berry weight ($R^2$=0.97) and the diameter ($R^2$=0.95) of the whole berry population. Therefore results are expected to be more accurate and representative of the total population.

Sampling of berries and estimation of DNA: Sampling and preparation of berries for DNA analyses, extraction and purification were carried out by the method described by OJEDA *et al.* (1999). Triplicate extractions were made for each sample.

Cell division and enlargement: Cell division and enlargement were determined indirectly by quantifying total DNA per berry pericarp. Variation of cellular volume was estimated by a cell enlargement index (CEI) (OJEDA *et al.* 1999). The CEI (ml $\mu g^{-1}$) represents the pericarp volume per unit weight of DNA.

Statistical analysis: Analysis of variance was performed using the PROC GLM of SAS (SAS Institute Inc., Cary, NC) statistics program. Differences between means of treatments were compared using Tukey's test for significant differences at the $p \leq 0.05$ level.

## Results and Discussion

Plant water status: In 1997, predawn leaf water potential ($\Psi$) of $C_I$ remained >-0.2 MPa during the entire experimental (Fig. 1A). In this first year variations of $\Psi$ of the water deficit treatments were irregular but drought symptoms (yellowing of the leaves and/or partial leaf fall at the shoot basis) were observed at $S_A$ and $S_B$ plants.

In 1998, $\Psi$ of the control ($C_{II}$) remained close to -0.2 MPa and was always >-0.4 MPa throughout the experiment (Fig. 1 B). In vines exposed to two levels of early water deficit, between day 2 and day 40 after anthesis, $\Psi$ varied between -0.6 and -1.0 MPa for $S_1$ and between -0.5 and -0.8 MPa for $S_2$. $\Psi$ for these two treatments remained close to the $\Psi$ of $C_{II}$ during the later part of the experiment when the plants were watered at veraison. For $S_3$, the water deficit period started one week after the beginning of veraison and $\Psi$ remained below -0.4 MPa during the major part of maturation, showing a peak of -1.0 MPa in the middle of the water deficit period. Drought symptoms (yellowing of leaves

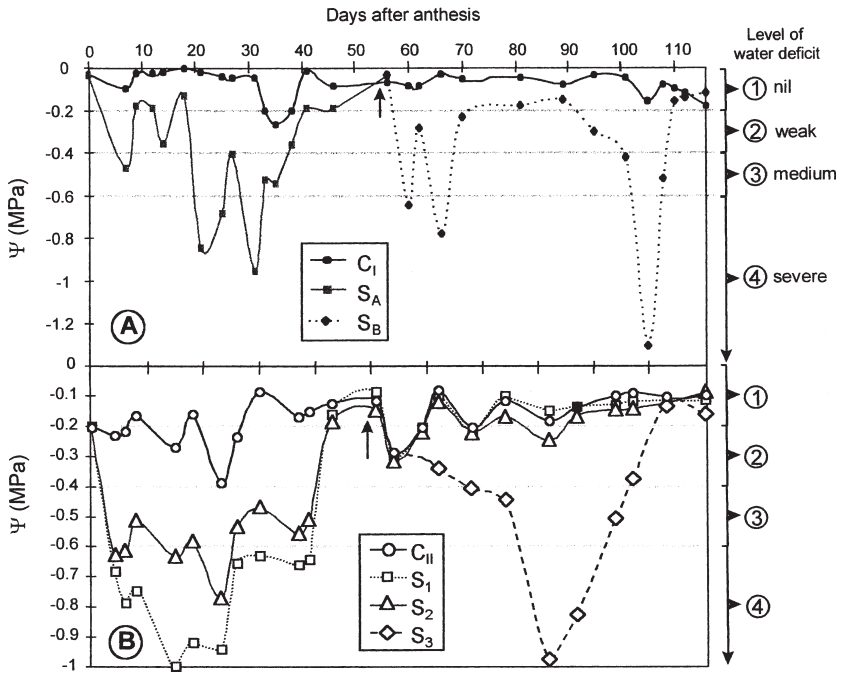


Fig. 1: Predawn leaf water potential ($\Psi$) of Syrah grapevines. **A**) 1997: $C_I$= control, $S_A$ = water deficit applied between anthesis and veraison, $S_B$ = water deficit applied between veraison and maturity. **B**) 1998: $C_{II}$= control. $S_1$ and $S_2$ = two levels of early water deficit between anthesis and veraison ($S_1$ = severe, $S_2$ = medium). $S_3$ = severe late water deficit between veraison and maturity. Arrows indicate the onset of veraison. Dotted lines represent means of 6 leaves. ⓫, ¶, ① and ↘ indicate different levels of water deficit (CARBONNEAU 1998).

and/or partial leaf fall at the shoot basis) were observed for plants of the $S_1$, $S_2$ and $S_3$ treatments.

B e r r y g r o w t h : In 1997, berry weight and diameter (Tab. 1) of the major classes were affected only if water deficits occurred between anthesis and veraison (treatments $S_A$), while late deficits ($S_B$) did not significantly modify berry weight and diameter. It is important to note, however, that late water deficits ($S_B$) were not consistent and that the most severe drought took place briefly at around day 105 (Fig. 1 A).

In 1998, berry weight (Fig. 2 A) and diameter (Fig. 2 B) of the major class berries were considerably reduced for all treatments. The reduction of the final berry size was more significant for $S_1$ and $S_2$ than for $S_3$. Final berry weight for $S_1$, $S_2$ and $S_3$ samples reached 47.5, 67.8 and 85.0 % of the control. Berry size reduction depended on the intensity of water deficit from anthesis to veraison ($S_1$ and $S_2$).

T a b l e 1

Fresh weight and diameter of berries as affected by water deficit in 1997. $C_I$ = control, $S_A$ = water deficit applied between anthesis and veraison, $S_B$ = water deficit applied between veraison and maturity. FW = fresh weight. Values with the same letter are not significantly different ($p \leq 0.05$)

| Treatments | Berry weight (g FW) Days after anthesis | | Diameter (mm) Days after anthesis | |
|---|---|---|---|---|
| | 47 | 116 | 47 | 116 |
| $C_I$ | 0.86 a | 1.96 a | 11.0 a | 14.1 a |
| $S_A$ | 0.48 b | 1.12 b | 9.1 b | 11.9 b |
| $S_B$ | 0.88 a | 1.95 a | 11.1 a | 13.8 a |

In all cases, the growth curve of berries was double sigmoid (Fig. 2 A, B) but water deficits modified both, the onset and duration of the individual phases. For the control, the first growth phase (phase I) ended 35 d after anthesis, when the sum of the daily mean temperature above 10 °C reached 426 °C. For $S_1$ and $S_2$ berries, phase I stopped 3-4 d earlier. Phase II (the lag phase) was shortened by water deficits and lasted 14, 10 and 6 d for $C_{II}$, $S_2$ and $S_1$, respectively. For $S_1$ and $S_2$, the second growth period (phase III) started at about day 41 or 8 d before the beginning of veraison. In other words, for $S_1$ and $S_2$, phase III started when water was supplied again. The onset of veraison occurred at about day 49 (630 °C·d) for all treatments. Growth resumption was significantly slower for grapevines subjected to water deficit.

Harris *et al.* (1968) found that in Sultana phase II was displaced in time and its duration was variable, depending on growth conditions. They suggested that environmental factors and/or cultural conditions were responsible for this effect and that final berry size would be affected by the same variables. In this work, the start and the length of phase II were modified by early water deficits. Plant water status is consequently one of those variables.

In contrast to unstressed berries, in drought-stressed berries the restart of growth occurred as soon as the water deficit was released (day 41), independent of the start of veraison (day 49). Matthews *et al.* (1987) showed that

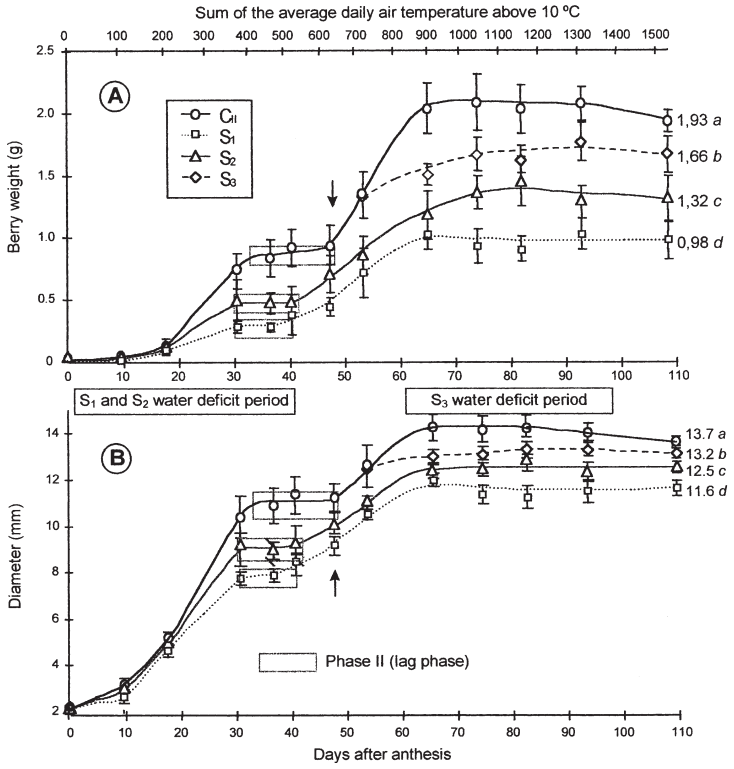


Fig. 2: Fresh weight (**A**) and diameter (**B**) of berries subjected to early and late water deficits in 1998. Vertical bars represent the standard deviation. Values with the same letter are not significantly different ($p \leq 0.05$). For details: Fig. 1.

Cabernet franc berries, subjected to water stress before veraison and normally irrigated again, recover growth 10 d before the control berries, at the moment of water supply, water deficits causing no differences in phenology (budbreak, bloom, veraison and harvest) based upon the accumulation of degree days or time. Our results corroborate this observation and also suggest that in stressed berries, it was not a true phase II but a temporary stop of growth due to the water deficit.

It is interesting to note that the start and the end of berry growth in vines irrigated by Ecotron and in the vineyard are congruent (OJEDA *et al.* 1999), if related to the sum of average daily air temperature (basis 10 °C). In fact, the end of phase I occurred at about 412 °C·d (day 42) for vineyard berries and at about 426 °C·d (day 35) for irrigated berries. The ripening phase of vineyard berries (phase III) started at about 600 °C·d (day 56) and about 630 °C·d (day 49) for potted vines. Starting with anthesis, berry development seems to depend on temperature. This information might be important for mathematically modelling of berry growth. In both years the total weight of seeds per berry was not affected by the treatments (data not presented).

Cell division and enlargement of the berry pericarp: In 1997 total DNA of the pericarp was similar for $C_I$ and $S_A$ treatments (Tab. 2), and between the two dates of measurement total DNA remained almost constant for both treatments. This indicates that cellular division was not affected by water deficit between anthesis and veraison. The CEI indicates that a reduction of the pericarp cell volume was the main cause determining final berry size (Tab. 2).

These results were confirmed in 1998 (Fig. 3). Water deficit treatments did not affect total DNA of the pericarp and cell divisions. Total pericarp DNA increased from anthesis (day 0) until the sum of average daily temperature above 10 °C reached approximately 350 °C·d (day 30), *i.e.* 19 d before the beginning of veraison. From this stage onwards, the total amount of pericarp DNA remained constant (4 µg total DNA per pericarp) until the end of berry growth. Therefore, 350 °C·d indicates the end of the mitotic period in control and stressed vines. That fits with the end of the cell division period of field-grown berries, which occurred close to 340 °C·d (day 35) (OJEDA *et al.* 1999). This boundary in berry development would therefore be valid for a large range of environmental and cultural conditions.

Development of the CEI (Fig. 4) shows that the mean size of the pericarp cells was reduced by water deficit. Compared to the cell volume of the control ($C_{II}$), mean cell volumes of $S_1$, $S_2$ and $S_3$ were reduced to 46.7, 27.8 and 11.4 %, respectively. The increase in cell enlargement after the end of the water deficit (day 42) occurred immediately for $S_1$ and $S_2$, approximately one week before the beginning of veraison. However this growth did not compensate for the differences in the final volume as compared to the control cells.

The reduction of cell volume as a result of early water deficit was irreversible. This supports the hypothesis that water deficit induced modifications of the composition and physical properties (*e.g.* cell wall extensibility) as was suggested by BOYER (1988).

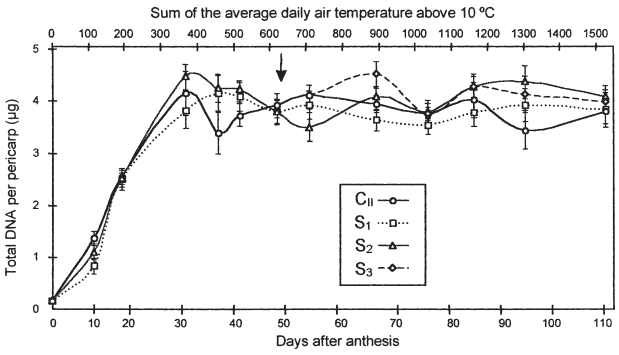


Fig. 3: Variation in the total amount of pericarp DNA of berries subjected to early and late water deficit in 1998. The arrow indicates the onset of veraison. For details: Fig. 1.

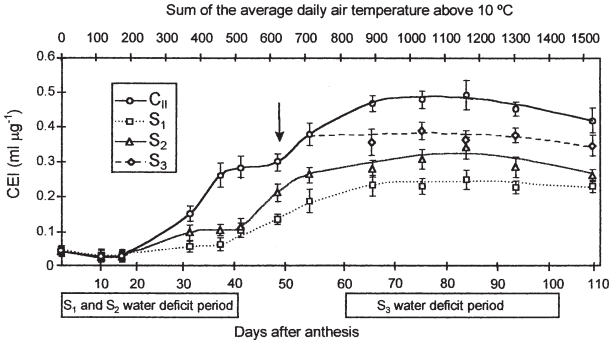


Fig. 4: The cell enlargement index (CEI) during berry development in 1998. For details: Fig. 1.

Table 2

Berry weight, total DNA of the pericarp and cell enlargement index (CEI) of berries. Vines had been irrigated ($C_I$) or were subjected to water stress ($S_A$) during berry development (phases I & II) in 1997. FW = fresh weight. Values with the same letter are not significantly different ($p \leq 0.05$)

| Treatments | Berry weight (g FW) Days after anthesis | | Total DNA of pericarp (µg) Days after anthesis | | CEI (ml µg$^{-1}$) Days after anthesis | |
|---|---|---|---|---|---|---|
| | 47 | 116 | 47 | 116 | 47 | 116 |
| $C_I$ | 0.86 a | 1.96 a | 5.83 a | 5.58 a | 0.16 a | 0.40 a |
| $S_A$ | 0.48 b | 1.12 b | 5.91 a | 5.43 a | 0,07 b | 0.23 b |

Cell wall synthesis seems to be sensitive to water deficit (Sweet *et al*. 1990; Schultz and Matthews 1993). The elastic and plastic properties of the leaf cell walls depend on wall structure. Sweet *et al*. (1990) have shown that the synthesis of grapevine leaf cell wall polysaccharides (particularly cellulose) is highly sensitive to growth inhibiting water deficits. Water deficit decreases the uptake and incorporation of the precursors of cell wall components such as glucose, in a different manner in growing and non-growing tissues. The synthesis of cellulose appeared to be the mechanism most sensitive to environmental conditions such as drought or saline conditions (Iraki *et al*. 1989). Berry wall synthesis in relation to environmentally or developmentally induced changes requires further investigation. In our work, we have clearly shown that medium or severe water deficits, applied to berries in phase I, have significant effects on cell size but not on cell division or on the final berry size.

A decrease in cell volume was observed by the end of maturation, mainly in control berries ($C_{II}$) and in field-grown berries (Ojeda *et al.* 1999); similar observations were made by Davies and Robinson (1996) and McCarthy (1997, 1999). The phenomenon of over-ripening is due to a loss of water and consequently an increase of sugar concentration (McCarthy and Coombe 1999).

Similarities between variations of CEI and berry size in the different treatments confirm that the volume of pericarp cells is most useful in explaining alteration of berry dimension under various water supply conditions.

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