Leaf:Fruit Ratio and Vine Water Status Effects on Grenache Noir (*Vitis vinifera* L.) Berry Composition: Water, Sugar, Organic Acids and Cations

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Seasonal variation in the water, sugar, organic acid and cation contents of developing grape berries (*Vitis vinifera* L. 'Grenache noir') under different levels of water supply (with and without deficit irrigation) and leaf:fruit ratios (18, 10 and five leaves per primary shoot, with one bunch per shoot), were investigated over two successive years in Mediterranean conditions (South of France). Fourteen shoots per vine were left for each leaf:fruit ratio level, and each vine was considered as having homogeneous primary shoots. The growth rate of the berries was increased with irrigation. Total dry matter content of the berry was not affected by leaf:fruit ratios, but the sugar loading decreased during berry development with a lower leaf:fruit ratio (five leaves per bunch). Treatments had little effect on organic acid contents and pH. Berry cation accumulation depended on vine water status and not on the total leaf area of the vine. Under irrigated conditions, calcium continued to accumulate in the berries after véraison. This confirms a partial functioning of the berry xylem during the post-véraison period. The seasonal variation in berry composition was less dependent on the leaf:fruit ratio than on the water status of the vine (mainly cations and sugar). This study provides evidence for the importance of plant water status effects on berry composition, irrespective of the leaf:fruit ratio. Vine water status is a major regulating factor for source-sink relationships.

INTRODUCTION

The grapevine is a species particularly well suited to an understanding of the mechanisms that govern the vegetative: reproductive growth relationship, partly due to its great architectural and physiological plasticity (Champagnol, 1984). Environmental conditions and some plant management practices affect fruit growth by changing the assimilate supply and water availability within the competitive framework of the whole plant (Dai *et al*., 2010). Source-sink ratios play an important role in the seasonal variation of water and solute transport and accumulation in the grape berry. In this regard, the reaction of the vine depends largely on the level and timing of manipulation (Koblet 1987; Kliewer *et al*., 1988; Hunter *et al*., 1991; Poni *et al*., 1994; Kliewer & Dokoozlian 2005). As in most fleshy fruits, water and carbon transport into and out of grape berries is essential for volumetric growth and the accumulation of primary and secondary compounds, which determine the final fruit composition and quality (Coombe & McCarthy, 2000). This transport varies with fruit development and environmental conditions (Hunter & Visser, 1988a; Ollat *et al*., 2002). The flow rate of imported carbon changes at véraison due to a shift in phloem sugar unloading from the symplastic to the apoplastic pathway, which allows high levels of soluble hexoses to accumulate (Zhang *et al*., 2006). At the same time, the water influx pathway to the berry shifts from a combination of xylem and phloem water supply to predominantly phloem supply (Düring *et al*., 1987; Coombe 1992; Greenspan *et al*., 1994, 1996; Bondada *et al*., 2005; Rogiers *et al*., 2006). Although berry growth is dependent on both xylem and phloem flow through the pedicel (Lang & Thorpe, 1989; Greenspan *et al*., 1994, 1996), the relative contributions from véraison until late ripening remain unclear (Rogiers *et al*., 2001). An understanding of the patterns of mineral element accumulation in the berry would provide further information about vascular flow to the berry during development (Rogiers *et al*., 2006).

Berry growth and chemical composition can be regulated by source-sink relationships (Hunter & Visser, 1988b; Kliewer and Dokoozlian, 2005). Assimilate supply from a source may be increased by increasing the leaf:fruit ratio, which generally leads to a larger fruit size in grapes (Petrie *et al*., 2000), mango (Chacko *et al*., 1982; Léchaudel *et al*., 2005) and peach (Souty *et al*., 1999). However, abiotic stress, such as drought, can reduce the leaf area and photosynthesis of the vine (Smart *et al*., 1974; Hardie & Considine, 1976; Van Zyl, 1987; Matthews & Anderson, 1988), thus limiting leaf function and changing the source-sink balance. If the grapevine is near the critical leaf area:fruit ratio, the vine may be expected to be more sensitive to additional drought stress due to heavy crop loads (Poni *et al*., 1994). Excessive crop loads cause a delay in ripening and may reduce fruit and wine

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quality (Jackson & Lombard 1993), but the boundary between an adequate and excessive crop load is not obvious (Keller *et al*., 2008). Many studies have dealt with the response of vines to water deficit, comparing different leaf areas and crop loads, but there is little quantitative information available to determine the relationship between different leaf area:fruit ratios and the water status of the grapevine during the growing season.

The present study was conducted to determine the relative importance of both irrigation supply and leaf:fruit ratio on grape composition (water, sugar, organic acids and cations) in order to better understand the ability of grape berries to accumulate substantial amounts of solutes under certain restrictive conditions.

MATERIALS AND METHODS

Experimental conditions and treatments

Experiments were conducted on young Grenache noir vines (clone 134), grafted onto R110. The vines were planted in 2002 and grown on a clay limestone soil (dry Mediterranean limestone marl) at the INRA Pech Rouge experimental station grounds in Gruissan, France (latitude 43° 08' 35''N; longitude 3° 7' 59''E). The vines were spaced 1 m apart within rows and 3 m between North-South-orientated rows. They were trained to a lyre trellising system, spur pruned, and drip irrigated. During both growing seasons of the experiment (2006 and 2007), two irrigation treatments were imposed: irrigated (I) and non-irrigated (NI). During the 2006 growing season, two leaf:fruit ratio sub-treatments, i.e. 10 and 18 leaves per primary shoot, with one bunch per shoot, were applied for each of the irrigation treatments. During the 2007 growing season, the sub-treatment of 10 leaves per shoot was repeated and a sub-treatment of five leaves per shoot, with one bunch per shoot, was applied for both of the irrigation treatments. In all cases, 14 shoots per plant were left and the secondary shoots, as well as the tendrils, were removed as they appeared. Each sub-treatment comprised four identical blocks. In each block, seven uniform vines (according to cane mass and trunk circumference) were used for sampling.

Irrigation

All plants of the irrigated treatment (I) were drip-irrigated by means of irrigation lines installed on the soil surface and with drippers spaced 0.5 m apart with a flow rate of 2.5 L/h. Irrigations were scheduled to keep the values of pre-dawn leaf water potential (Ψ_{pd}) higher than -0.3 MPa. Irrigation started from bunch closure.

Determination of grapevine water status

The evolution of the pre-dawn leaf water potential (Ψ_{pd}) was monitored from berry set to ripening using a pressure chamber (Scholander *et al*., 1965). The first measurement was carried out on every plant in the experimental plot. Thereafter, measurements were carried out on six plants; six young, fully expanded leaves on the outer rim of the canopy were measured every seven to 15 days according to climatic conditions and the phenological stage of the plant.

Meteorological data

Meteorological data were obtained from an automatic weather station (CIMEL 516i), located at the INRA Pech Rouge experimental station at Gruissan, France (latitude 43° 08' 35''N; longitude 3° 7' 59''E). Climatic data for the vineyard are shown in Table 1.

Leaf area estimation

Leaf area was determined by measuring the length of the main vein of the leaf, using the quadratic relationship (r^2 = 0.971) defined for Grenache noir, at the end of vegetative growth: $y = 0.0134L^2 - 0.0762L$, where 'L' is the total length of the main vein of the leaf at the time of measurement (Lebon *et al*., 2006). Total leaf area per vine was calculated by multiplying "y" by the number of leaves per treatment (5, 10 and 18), and by the number of shoots per plant (14).

Flowering period, bunch and shoot numbers

The full bloom stage corresponded to the date when more than 50% of the caps were open or had dropped. The error margin due to the heterogeneity of the flowering period of the vine is about two days. Shoot number per vine and bunches per shoot were determined at flowering. The bunch number was reduced to one bunch per shoot. Cane mass was determined at pruning.

Berry sampling and sample preparation

At each sampling date, four bunches were taken for each treatment during the early morning (08:00–08:30) and transported to the laboratory. Each replicate comprised all the berries of one bunch, with care being taken to retain the pedicels on the berries. In 2006, berry sampling was carried out at regular time intervals from bunch closure until harvest ripeness. In the 2007 season, berry sampling began at pea-size berry (7 mm diameter) and continued until the over-ripe stage.

TABLE 1

Meteorological data from the INRA Pech Rouge weather station (1 km south of the vineyard site) and total seasonal application of irrigation water.

Year	GDD[1] (ºC)	Rainfall (mm) Annual	Rainfall (mm) Seasonal[2]	ET[3] (mm) Seasonal[2]	Irrigation supplied (mm)
2006	1762	414	73	838	74
2007	1568	495	254	758	94

[1] *Cumulative growing degree days (>10°C) from 24 March to 10 Sep. (budbreak to harvest).*
[2] *Seasonal values are from 24 March to 10 Sep. (budbreak to harvest).*
[3] *Penman method modified by Doorenbos & Pruitt (1977).*

TABLE 2
Values for vegetative growth for the different leaf:fruit ratios and irrigation treatments over the two-year study.

Year	Treatments	Pruning weight (g/vine)	Leaves no./vine	Leaf area m^2/vine	Leaf area m^2/shoot
2006	10 I	349a	140a	1.99a	0.142a
	18 I	660b	252b	3.56b	0.254b
	10 NI	379a	140a	1.96a	0.140a
	18 NI	600b	252b	3.49b	0.249b
2007	10 I	382a	140a	2.01a	0.144a
	5 I	192b	70b	0.98b	0.070b
	10 NI	386a	140a	1.94a	0.138a
	5 NI	180b	70b	0.95b	0.068b
2006-2007	10 I	ns	ns	ns	ns
	10 NI	ns	ns	ns	ns

*The different letters indicate significant differences between means of treatments analysed for each year at $p \leq 0.05$. For 10 leaves treatment values compared between two years, ns: non significant and *: significant at $p \leq 0.05$.*

TABLE 3
Values for reproductive growth for the different leaf:fruit ratios and irrigation treatments over the two-year study.

Year	Treatments	Bunch weight (g)	Berries no./bunch	Berry weight (g)	Yield (Kg/vine)	Leaf to fruit ratio (cm^2/g)
2006	10 I	498 a	215	2.28 a	6.98 a	2.85 d
	18 I	448 ab	225	2.16 a	6.28 ab	5.67 b
	10 NI	376 c	223	1.70 b	5.26 c	3.72 c
	18 NI	397 bc	218	1.67 b	5.55 bc	6.28 a
2007	10 I	470 a	201	2.36 a	6.58 a	3.06 ab
	5 I	386 ab	208	2.15 b	5.41 ab	1.81 b
	10 NI	360 b	191	1.96 b	5.04 b	3.83 a
	5 NI	373 ab	198	2.03 b	5.23 ab	1.82 b
2006-2007	10 I	ns	*	ns	ns	ns
	10 NI	ns	*	ns	ns	ns

*The different letters indicate significant differences between means of treatments analysed for each year at $p \leq 0.05$. For 10 leaves treatment values compared between two years, ns: non significant and *: significant at $p \leq 0.05$.*

From each of the four replicates of each treatment, all the berries of a bunch were cut at the pedicel base, counted, and classified according to volume using a Dyostem$^{®}$ instrument (Vivelys Society, France). Each class was characterised by its frequency and the mean volume of berries. Ten berries were selected from the major class (the class containing the highest number of berries, according to volume), rinsed in ultra-pure water, dried with absorbent paper, and frozen at -20°C until further use. Before cation analyses, the berries were dried in an oven at 80°C to constant weight, for periods varying between 48 and 168 hours, depending on the phenological stage of the berry. For the remaining berries of each sample, the juice was centrifuged at 8 000 rpm for 5 minutes (Centrifuge 5810, Eppendorf). The fresh juice was used for the determination of soluble solids, pH, titratable acidity, and tartaric and malic acids.

Chemical analyses

Analyses were performed on grape juice and dry berry samples.

Total soluble sugars, pH and titratable acidity

Fresh grape juice extracts were analysed for the determination of total reducing sugars using the Fehling method until just after véraison, and a digital refractometer (Euromex) thereafter for determining total soluble solids (°Brix). The pH of the juice was measured with a pH meter (InoLab WTW 720 series) using a glass electrode. Titratable acidity (TA) was measured by titration to pH 7 (20°C) and the results were expressed in g/L sulphuric acid.

Malic and tartaric acids

Malic and tartaric acids were measured by ion chromatography (Dionex DX-100). For this analysis, the fresh juice was diluted to obtain concentrations appropriate for analysis – from 1/25th to 1/10th of the initial berry concentrations.

Potassium, calcium, magnesium and sodium

In 2006, the cations were measured from the end of the vegetative growth period (lag phase) to harvest ripeness of the berries. In 2007, measurements were taken from the pea-size stage to harvest, but also until the point when berries were considered to be over-ripe. The mineral elements were extracted from the berry dry matter by agitation with 10 mL 0.1N HCl for seven days before véraison, and with 20 mL HCl after véraison (concentrations of mineral elements were higher during the latter period). The extracts were allowed to precipitate naturally and the supernatants were diluted

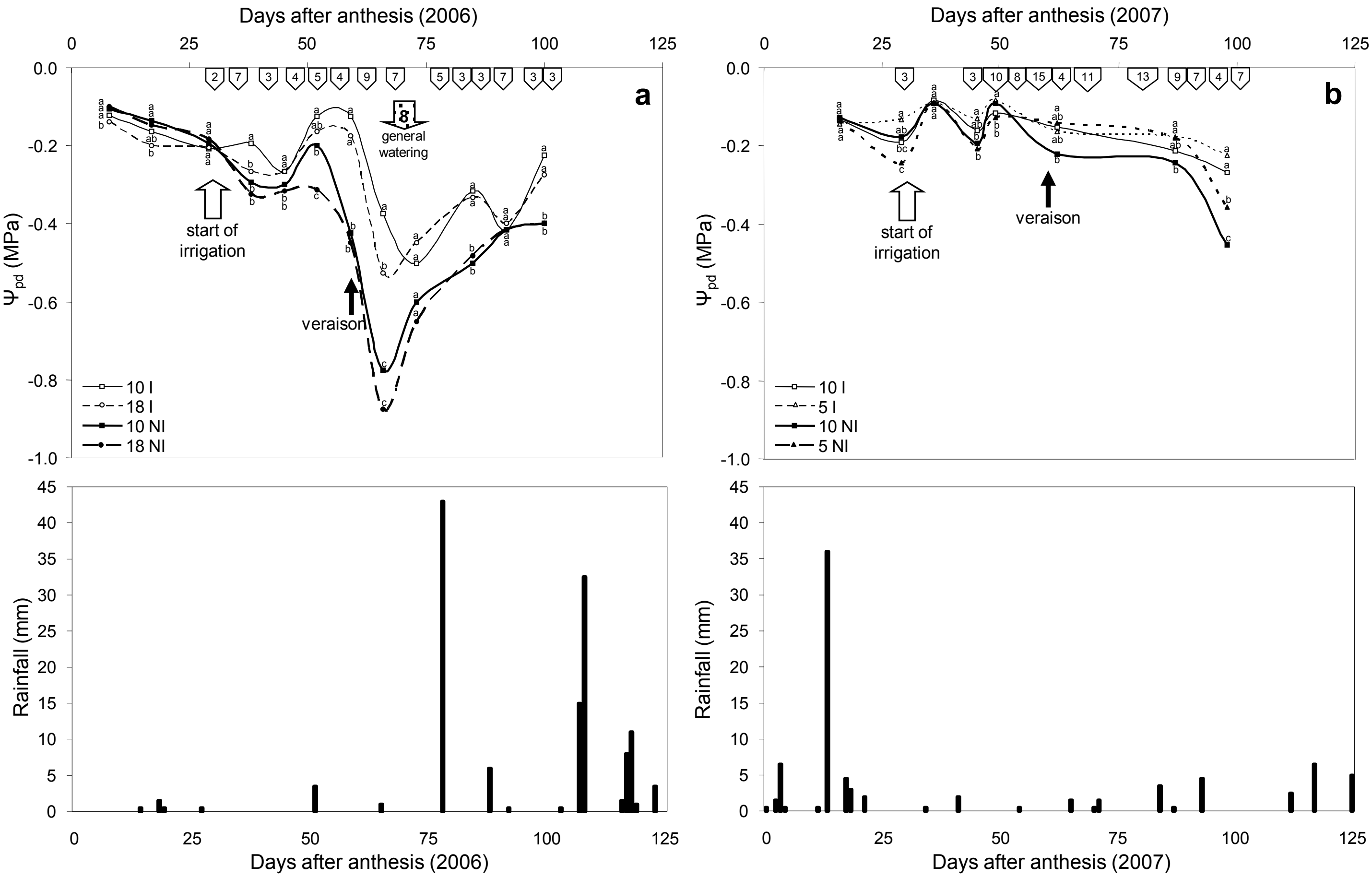

FIGURE 1

Pre-dawn leaf water potential (Ψ_{pd}) of non-irrigated and irrigated grapevines cv. Grenache noir under field conditions, and daily rainfall (mm) over the two-year experimental period (2006–2007). Labels 5, 10 and 18 refer to different number of leaves per primary shoot. I and NI indicate the irrigated and non-irrigated treatments. Arrows on the y-axis indicate the timing and the amount of water supplied by irrigation. Arrow indicating 'general watering' indicates the amount of water supplied to improve the general water status of the vineyard. Different letters indicate significant differences between the mean of treatments analysed for each year at $p \leq 0.05$.

to obtain concentrations appropriate for analyses – 1:2 - Na^+, 1:50 - Ca^{++} and Mg^{++}, and 1:100 - K^+. Concentrations of K^+, Ca^{++}, Mg^{++} and Na^+ in berry dry matter extracts were measured with an Atomic Absorption Spectrophotometer (Thermo S4 AA system). The K^+ and Na^+ were analysed by atomic emission spectroscopy, and Ca^{++} and Mg^{++} were determined by atomic absorption spectroscopy. The results were expressed as mg/berry.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). Mean comparisons were performed using Fisher's least significant difference (LSD) test; significance was set at $p \leq 0.05$. Principal component analysis (PCA) was performed to identify any factors differentiating the treatments that were imposed. All statistical analyses were performed using the software STATGRAPHICS Plus 4 (StatPoint Inc., Northern Virginia, USA).

RESULTS

Grapevine water status

In 2006, leaf water potential (Ψ_{pd}) measurements during plant growth showed clear differences in vine water status between the two main treatments, i.e. irrigated (I) and non-irrigated (NI). Leaf:fruit ratio treatments had a significant effect on the evolution of vine water status for some dates during the season (Fig. 1a), with a weak predisposition to more water stress being observed in the high-ratio leaf:fruit treatments. The I treatment maintained a water status of close to -0.2 MPa until 60 days after anthesis (DAA), after which it dropped to -0.3 to -0.5 MPa as a result of a period of severe drought during August (corresponding to the ripening period). Parallel to this, the Ψ_{pd} values for the NI treatment dropped to approximately -0.9 MPa at 66 DAA. Consequently, the plot was irrigated with 8 mm at 70 DAA to improve the general water status of the vines. This watering and the 43 mm of rainfall (78 DAA) rehydrated the plants, particularly for the NI treatment.

In 2007, the seasonal evolution of Ψ_{pd} showed significant differences among the I and NI treatments earlier in the season, but more substantially during ripening (Fig. 1b). In contrast, the Ψ_{pd} values were higher than in the 2006 experiments. Both treatments maintained high Ψ_{pd} values until véraison (about -0.2 MPa), and then the values of the NI treatment became progressively lower towards the end of ripening (about -0.4 MPa at 100 DAA). In this case, the effect of the leaf:fruit ratio treatments was particularly pronounced around the end of ripening (Fig. 1b).

This observed year-to-year variation is principally due to an overall rainfall surplus of 81 mm and a reference evapotranspiration

(ET) rate that was much lower in 2007 relative to 2006. In 2007, the rainfall accumulated from budbreak to harvest was greater (growing season 2006 = 73 mm versus 2007 = 254 mm), while ET was less pronounced (758 mm) than that during the 2006 growing season (838 mm) (Table 1).

Canopy components

The increased leaf area/shoot or leaf area/vine due to different leaf:fruit ratio treatments resulted in higher pruning weights (Table 2). However, no irrigation effect was found on leaf area and pruning weights, mainly because the secondary shoots were removed as they appeared and no compensatory growth seemed to occur for the primary shoot.

Yield components

In 2006, the yield components were affected by the irrigation treatments but not by the leaf:fruit ratio treatments (Table 3). In 2007, the irrigation treatment had a significant effect on all the yield components only between the 10I and 10NI treatments. Moreover, the difference from one year to the next in berry number per bunch was most probably a consequence of a difference in fruit set due to the weather conditions in each year, and not as a result of the treatments (applied after berry set).

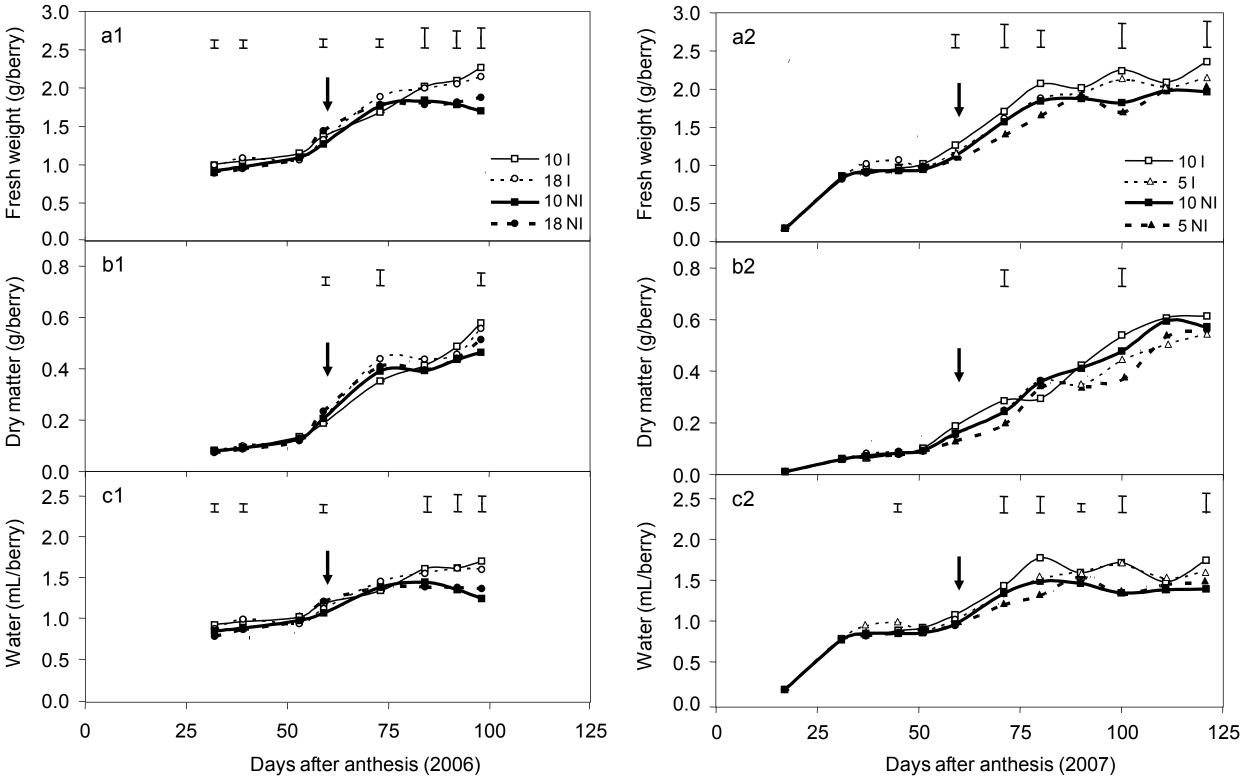

FIGURE 2

Seasonal changes in grape berry fresh growth (a), total dry matter content (b) and total water content (c) of Grenache noir from post-fruit set to ripening in 2006 (left) and 2007 (right). Labels 10, 18 and 5 refer to different number of leaves per primary shoot. I and NI indicate the well-irrigated and non-irrigated treatments. Arrows indicate véraison. Bars represent least significant differences (p ≤ 0.05) between treatment means for each sampling date; n = 4.

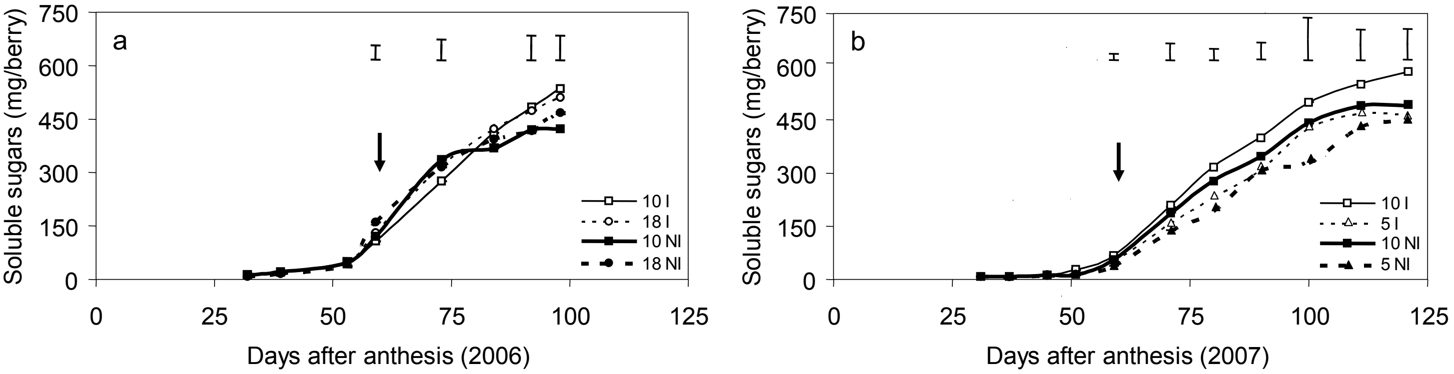

FIGURE 3

Seasonal changes in grape berry soluble sugars of Grenache noir, expressed in mg/berry, during the post-fruit set to ripening period in 2006 (a) and 2007 (b). Labels 10, 18 and 5 refer to different number of leaves per primary shoot in irrigated (I) and non-irrigated (NI) conditions. Arrows indicate véraison. Bars represent least significant differences (p ≤ 0.05) between treatments for each sampling date; n = 4.

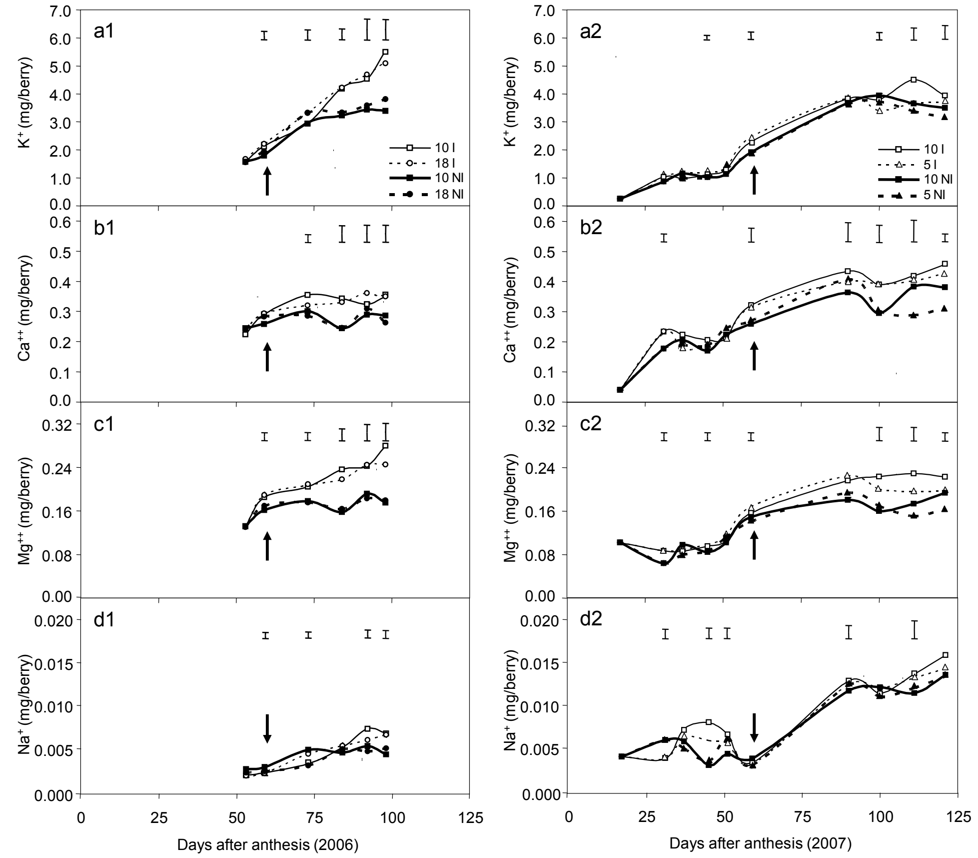

FIGURE 4

Seasonal changes in Grenache noir grape berry K^+, Ca^{++}, Mg^{++} and Na^+, expressed in mg/berry, during the post-fruit set to ripening period in 2006 (left) and 2007 (right). Labels 10, 18 and 5 refer to different numbers of leaves per primary shoot. I and NI indicate the irrigated and non-irrigated treatments. Arrows indicate véraison. Bars represent least significant differences ($p \leq 0.05$) between means of the leaf:fruit ratio and between the irrigation (I) treatments on each sampling date; n = 4.

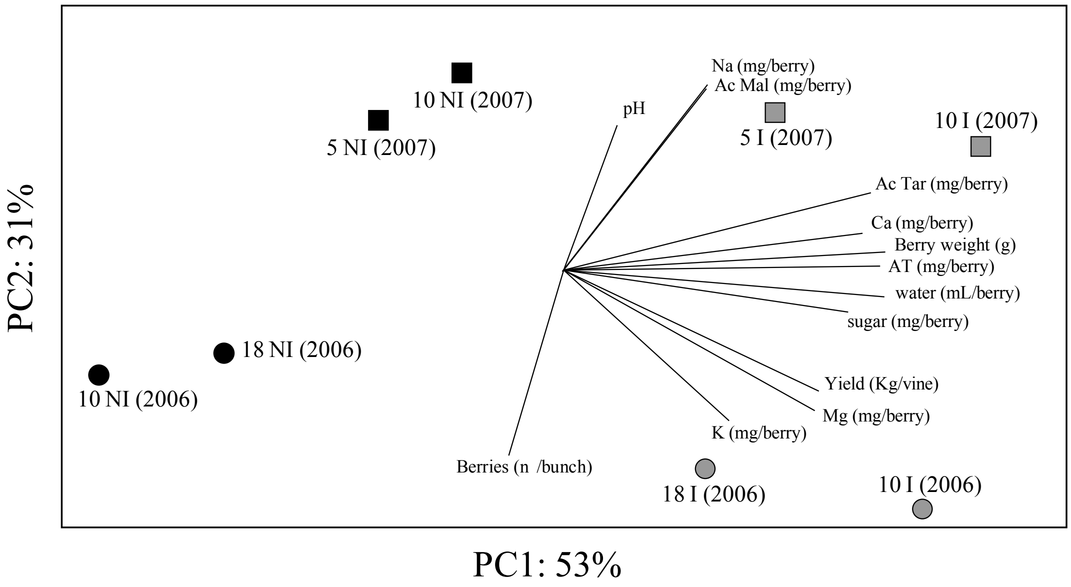

FIGURE 5

Principal component analysis of values for reproductive growth and berry composition to ripeness in the different leaf:fruit ratio and irrigation treatments over the two-year study. Labels 10, 18 and 5 refer to different number of leaves per primary shoot. I and NI indicate the irrigated and non-irrigated treatments. The sense and size of the vectors indicate the way and weight of each variable respectively to separate the different treatments studied.

Berry growth

The increase in berry fresh weight followed the typical double sigmoid curve in both years (Fig. 2). Maximum berry weight occurred at approximately 80 DAA in both years and for all treatments. Total berry dry matter and water content followed similar patterns during berry development (Figs. 2 and 3). Effects of the leaf:fruit ratio treatments on berry dry matter and water content were not significant in either year, but irrigation treatments had a significant effect on these two parameters in both years. For the I treatments, berry fresh weight and water content increased, whereas these parameters became stable or decreased progressively for berries from the NI treatments, as was evident in 2006 (Figs. 2a*1*, 2c*1*). In the latter year, dry matter per berry was significantly higher in the I treatments, but only on the last date (Fig. 2b*1*). In 2007, under low levels of water deficit (> -0.3 MPa), differences between the treatments were less evident during practically the whole period of berry development (Figs. 2a*2*, 2b*2*, 2c*2*).

Berry juice composition

Total soluble solid accumulation per berry was stable and low until approximately 50 DAA. The onset of rapid sugar accumulation in the berry varied between the seasons (Fig. 3). A progressive increase in the accumulation of sugars was observed in the berries over the study period, with sugars being most intense after véraison. In 2006, sugar accumulation continued until harvest (100 DAA) for all treatments, except for the 10NI treatment during the latter phase of ripening. From 75 DAA, sugar accumulation was more intense in the I than in the NI treatments, irrespective of the number of leaves (Fig. 3a). In 2007, the total soluble solids per berry increased progressively during fruit ripening, decreasing in rate from approximately 100 DAA for the I and NI treatments (Fig. 3b); during the last phase of ripening, only the 10I treatment continued to accumulate soluble solids. At maturity, the amount of sugar per berry ranged from 447 to 586 mg/berry across all the treatments. The lower leaf:fruit ratio, i.e. five leaves for one bunch for both the I and the NI treatments, showed less sugar per berry in comparison to the higher leaf:fruit ratio, i.e. ten leaves for one bunch (Fig. 3b). Except for the 10 I treatment on the last date (121 DAA), these results were not significantly different

Titratable acidity (TA) of grape juice increased until 39 and 45 DAA, respectively, for 2006 and 2007, and varied between 18 and 20 mg/berry under NI as well as I conditions, with a lag period of a few days until 53 and 59 DAA depending on the year. After véraison, TA decreased rapidly during a first period of 13 to 15 days, and less intensely during the rest of the ripening period of the berry. There was no effect of leaf:fruit ratio treatments on berry TA, but the TA of the berry at harvest was higher under I conditions (6.2 mg/berry) than under NI conditions (5.1 mg/berry) in both years of this study.

The malic acid content of the grape juice increased until ~50 DAA to reach a maximum of about 16 mg/berry, and then decreased continuously to 0.8 to 1.3 mg/berry in 2006 and 1.5 to 2.5 mg/berry in 2007. In contrast, tartaric acid reached a maximum at about 30 to 40 DAA, depending on the year, after which it became stable at levels ranging from 11.5 mg/berry under NI conditions to 14.5 mg/berry under I conditions. Although the values for tartaric acid tended to be lower for the NI treatments during the last stages of ripening in both seasons, the tartaric and malic acid contents per berry were not affected significantly by the treatments. The pH of the must nonetheless increased markedly until harvest (100 DAA). The treatments had no effect on pH.

Berry cation accumulation

The berry mineral composition changed during its development (Fig. 4). At harvest (100 DAA) in 2006, K^+ represented 89.4% of the cation pool of the berry, followed by Ca^{++} (6%), Mg^{++} (4.5%) and Na^+ (0.1%). In 2007, these proportions were 86.2%, 9%, 4.5% and 0.3% for K^+, Ca^{++}, Mg^{++} and Na^+ respectively. The mineral accumulation in the berry was affected by irrigation treatments and by the year-to-year variation, and this difference was most important during the post-véraison period. Although the berry mineral composition was generally not affected by leaf:fruit ratio treatments, the 5I and 5NI treatments showed consistently lower values than the corresponding 10I and 10 NI treatments during the last phases of ripening.

In 2006, the K^+ accumulation in the berry increased slightly with fruit development and reached a maximum of 5 mg/berry under irrigated and less than 4 mg/berry under non-irrigated conditions (Fig. 4a*1*). Calcium accumulation tended to increase principally in the pre-véraison period; this accumulation stabilised at véraison for the NI treatments and increased slightly to a higher level for the I treatments during ripening. The Mg^{++} and Na^+ accumulation in the berry increased during ripening, and the vine water deficit reduced the accumulation of these cations in the berry.

The effect of irrigation supply was less pronounced in 2007. In general, the final contents of K^+ and Mg^{++} decreased in comparison to the contents in 2006, but the Ca^{++} and Na^+ concentration increased slightly. The Ca^{++} accumulation increased slowly but constantly during the post-véraison period. During this period, the berry incorporated an average of 25 % of the total amount of Ca^{++} accumulated during the whole growth period, in comparison to 12% in the same period in 2006. Similarly, the total accumulation of Na^+ per berry in 2007 increased by ~100% in comparison to 2006.

Principal component analysis, including all variables concerning berry composition and yield components at harvest, was performed to illustrate graphically the overall behaviour of the experiment (Fig. 5). The first principal component (PC1, 53% of total variance of the experiment) shows clearly the differences between the NI and I treatments. Irrigation supply treatments, located on the right of Fig. 5, were characterised principally by higher contents per berry of sugar, cations, total acidity and water than the NI treatments (to the left of Fig. 5). The second principal component (PC2, 31% of total variance of the experiment) mainly explains the difference between years. In fact, the berries (located at the top in Fig. 5) had higher pH and Na and malic acid contents in 2007 than in 2006, but the berry number per bunch was higher in 2006. For each treatment of water supply and for each year, the different leaf:fruit ratio treatments were placed very close, showing a less significant effect of this factor on the whole experiment in terms of berry composition and yield components.

DISCUSSION

Berry growth

Berry growth and development are supported by the import (from the whole plant) of water, sucrose and mineral nutrients, whereas organic acids are produced inside the berry (Hunter & Ruffner, 2001; Terrier & Romieu, 2001). Variability in berry growth and weight, as a consequence of vine water availability, has been widely reported (Matthews & Anderson, 1988; Poni *et al*., 1993; Esteban *et al*., 1999; Ojeda *et al*., 2002). Research on the comparison of different water stress situations often concludes that vines with post-véraison water deficit may slightly reduce berry size and improve fruit composition (Kennedy *et al*., 2002; Ojeda *et al*., 2002). Our findings are in agreement with these results: the fresh weight and water content of 'Grenache noir' berries were higher in vines that were irrigated during post-véraison. Furthermore, similar to previous research (Santesteban & Royo, 2006), our data have shown clearly that, under the conditions of this experiment, the berry growth rate and final berry size at harvest were not affected by different leaf:fruit ratios for vines with similar fruit load. However, other studies have reported that berry size increases when leaf area increases (Candolfi-Vasconcellos & Koblet, 1990) or when berry number decreases (Dokoozlian & Hirschfelt, 1995).

Sugar, organic acids and pH

The effects of interactions between crop level and different soil water availability on berry composition have been widely reported for *Vitis vinifera* cultivars. These studies have indicated that, although bunch thinning reduces yields, there were, at best, marginal gains in terms of fruit composition (Freeman & Kliewer, 1983; Kliewer *et al*., 1983; Bravdo *et al*., 1985; Poni *et al*., 1993, 1994; Kliewer & Dokoozlian, 2005; Keller *et al*., 2008). A recent study (Keller *et al*., 2008) reported that there were almost no interactions between crop load and irrigation treatments, suggesting that even vines subjected to relatively severe water deficit were able to support and ripen their crop. The increase in soluble solids was not influenced by fruit:pruning weight or leaf area:fruit weight ratios, but depended on the soil water availability, which confirms the findings of Wang *et al*. (2003a, b) and Hunter and Deloire (2005). Santesteban and Royo (2006) suggested that, in climates where light interception is not a limiting factor, photosynthesis depends largely on water status. This may explain discrepancies found among the minimum values of leaf area:yield ratio that have been reported previously as being necessary to reach proper harvest maturity (Winkler, 1930; Buttrose, 1966; Kliewer & Antcliff, 1970). Although the influence on berry soluble solid contents could not be explained only by soil water availability and photosynthetic activity, the interactions between leaf:fruit ratio and plant carbohydrate reserves must also be considered in view of the clear signs that five primary leaves per bunch represented a lower limit and even beyond, under both well-watered and water deficit conditions. In fact, the redistribution of grapevine reserves certainly plays a role in this response, attenuating the effect of leaf area reduction (Hunter & Visser, 1990a, b; Candolfi-Vasconcelos *et al*., 1994). More research on the effect of the leaf:fruit ratio and soil water availability on berry soluble solid accumulation and stored reserves would be necessary for a better understanding of plant carbohydrate balance and distribution.

High or low assimilate supply (leaf:fruit ratio) did not affect the organic acid content under the conditions of this study. Nevertheless, the berries from grapevines with a favourable water status accumulated more organic acids at maturity. Similar results in relation to the effect of irrigation supply on organic acid content and accumulation have been shown by others (Neja *et al*., 1977; Esteban *et al*., 1999).

In general, the response of berry pH to soil moisture status is variable (Williams & Matthews, 1990). Dry *et al*. (1995) found that pH was significantly lower in fruit under partial rootzone drying, possibly due to the reduced canopy density associated with this treatment. However, the pH was not affected by either leaf area:fruit ratio or soil water availability under our experimental conditions. In contrast, Esteban *et al*. (1999) found more differences in pH as distinct differences in soil water availability occurred during different growth seasons. According to Hrazdina *et al*. (1984), changes in the pH of the berry are related to the metabolism of the major acids and the accumulation of cations.

Cations

The hierarchy of mineral nutrients (i.e. $K^+ > Ca^{++} > Mg^{++} > Na^+$) in grape berries is similar to that found in apples (Jones *et al*., 1983), Asian pears (Behboudian & Lawes, 1994) and mango (Léchaudel *et al*., 2005). The relative variation between cations monitored in this study was accentuated during berry growth, with potassium increasing significantly more than the other elements. Similar trends in cation accumulation have previously been reported for various *Vitis vinifera* cultivars (Creasy *et al*., 1993; Ollat & Gaudillère, 1996; Rogiers *et al*., 2006). The effect of higher soil water availability on cation concentration is slightly more complex; despite the fact that it always implies an increase in cation accumulation, cation concentration may sometimes be reduced due to dilution after a significant increase in berry growth. Thus, comparisons under different water availability regimes often show no differences in cation concentration (Stevens & Cole, 1987). However, when cation content per berry is analysed, our findings are in agreement with these results, i.e. less cations accumulate when soil water availability is low (Esteban *et al*., 1999). On the other hand, our data also indicate that the seasonal changes in cations of the berry were affected by soil water availability, but not by leaf:fruit ratio. We are not aware of any previous report evaluating both effects on the cation contents of the berry.

The macronutrients K^+, Mg^{++} and Na^+ are generally considered to be phloem-mobile elements, whereas Ca^{++} is considered to have low phloem mobility (Welch, 1986) and, therefore, is most likely to enter the berry through the xylem. In agreement with earlier research (Creasy *et al*., 1993; Ollat & Gaudillère, 1996; Rogiers *et al*., 2006), K^+ was the most abundant cation, with higher rates of accumulation after véraison, compared to the other cations. However, the results presented here show that vine water availability possibly influences both the K^+ uptake and K^+ translocation to the fruit in the same way. This suggests that the accumulation of K^+ and berry growth processes by water accumulation in the flesh cells may be mechanistically linked. The effect of water availability on Mg^{++} and the reduced effect on Na^+ can be explained by the same argument. The present results confirm those from another study that evaluated irrigation supply under similar climatic conditions, though with higher water supply (Esteban *et al*., 1999). On the other hand, other studies have shown that Ca^{++} accumulation either ceased at véraison (Hrazdina *et al*., 1984) or continued until full ripeness (Ollat & Gaudillère, 1996; Rogiers *et al*., 2000; Cabanne & Donèche, 2003). Our data indicate that the accumulation of Ca^{++} continues during berry ripening under favourable vine water conditions (Etchebarne *et al*., 2009), in agreement with the assertion that berry peripheral xylem continues to be (partly) functional during berry maturation (Bondada *et al*., 2005; Keller *et al*., 2006; Chatelet *et al*., 2008).

Leaf:fruit ratio versus irrigation supply

The principal component analysis with different variables at ripeness shows clearly that soil water availability was the most explanatory factor for the whole behaviour of the experiment (53%), even in 2007, when the water deficit was very mild. The year effects were also important (31%), although less so than water status. Finally, the leaf:fruit ratio level was the least significant factor. These results could provide an explanation for discrepancies in earlier research that evaluated the interactions of crop level and different soil water availability on vine response (Freeman *et al*., 1983; Kliewer *et al*., 1983; Bravdo *et al*., 1985; Poni *et al*., 1993). In fact, very few interactions have recently been reported between irrigation and crop load treatments, which implies that bunch thinning did not affect the response of the grapevine to deficit irrigation (Keller *et al*., 2008).

CONCLUSIONS

The results show that berry composition is less sensitive to leaf:fruit ratio than to grapevine water status, especially when the lower limit in terms of leaf:fruit ratio has not been reached. In this study, five primary leaves per bunch showed clear signs of being a lower limit and even beyond the limit under both well-watered and water deficit conditions. Berries from well-irrigated vines accumulated more sugar, cations and water compared to vines in water deficit situations. Like for other plants, the additional influx of sap may possibly provide an additional influx of cations into the fruit as a natural process in parallel to water supply. The study provides evidence for the importance of plant water status, irrespective of the leaf:fruit ratio, in berry compound accumulation. Vine water status is confirmed as a major factor impacting on source-sink relationships. The role of secondary shoots was not investigated in this study, but would be considered in further studies. Finally, this (field) study supports evidence that there is partial functioning of the berry xylem conduits during post-véraison, at least with reference to Ca^{++} accumulation, for vines under favourable water conditions.

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