Integrative Effects of Vine Water Relations and Grape Ripeness Level of *Vitis vinifera* L. cv. Shiraz/Richter 99. I. Physiological Changes and Vegetative-Reproductive Growth Balances

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The water relations and physiological status of the grapevine are critical for obtaining a quality product and for fully exploring vineyard and grape potential. The objective of this investigation was to determine the effect of grapevine water status (induced by means of two field water capacity-based irrigation levels, 75% and 100%, applied at single and combined vine developmental stages) on morphological and physiological changes in *Vitis vinifera* L. cv. Shiraz/Richter 99 grapevines and grapes (harvested at different soluble solid levels) under field conditions. The integrative effects of vine water relations and grape ripeness level, specifically in a Mediterranean high winter rainfall area, have not yet been investigated systematically. The terroir affected the reaction of the vines to treatments. The soil displayed high water-holding capacity and a buffer against favourable evapotranspiration conditions, even with a western aspect and being subjected to long and relatively dry seasons, with frequent occurrence of high temperatures and grapevines with fully developed canopies. The vines did not seem overly stressed – in line with the relatively high base soil water fractions of mostly more than 50% of field water capacity. Primary and secondary leaf water potential and stem water potential displayed similar patterns and the water potential of the primary and secondary leaves was similar. Despite relatively high base soil water contents that prevented excessively low plant water potential and classic leaf and berry behaviour to surface, the vines still responded in a noticeable way to volume and timing of irrigation in relation to the grape ripeness level status. Water relations, ripeness level and terroir conditions showed an integrated, steering impact on physiological, vegetative and reproductive behaviour. Post-véraison irrigated vines were expected to maintain relatively high water potential during the last weeks of the ripening period, but this seemed not to be the case. All vines seemed to have recuperated/stabilised during this time, maintaining their water balances. Physical, physiological and compositional changes in the berry during late ripening under field conditions were clarified further. New information was obtained on the relationships between the behaviour of the root system, canopy and grapes and the changing terroir conditions during the ripening period.

INTRODUCTION

The physiological functioning of the grapevine, growth balances and the capacity to endure stressful conditions over seasons are an integrated response of the variety-rootstock combination to the terroir conditions that are experienced and the vineyard practices that are applied (Smart & Coombe, 1983; Hunter & Myburgh, 2001; Hunter & Bonnardot, 2002; Vaudour, 2003; Deloire *et al.*, 2005a, 2005b; Hunter *et al.*, 2010; Hunter & Bonnardot, 2011). Healthy vines and continued fertility are required for sustainability, whereas uniform shoot growth and grape development per surface area would favour lower production costs as well as grape and wine quality (Hunter *et al.*, 2010, 2011).

Together with temperature, plant water status is generally recognised as one of the most critical factors affecting the growth balances of the grapevine (Smart & Coombe, 1983; Coombe, 1987; Hunter & Myburgh, 2001; Hunter & Bonnardot, 2011). The gradual depletion of soil water during the growth season normally results in increasing water deficits experienced by the grapevine, which have a direct

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impact on its capacity to buffer the potentially deleterious effects of adverse environmental conditions. Considering the vegetative and reproductive growth patterns of the grapevine and concomitant water requirements, it follows logically that the volume of accessible water at specific developmental stages during the season may have differential effects on growth as well as the eventual grape and wine quality. This is complicated by the difference in sensitivity of the various physiological processes and plant organs to water deficits (Mohr & Schopfer, 1995). Optimum irrigation strategies that would allow timely water deficits in order to curb vegetative growth, but at the same time maintain a canopy capacity and microclimate that would benefit the required berry size, grape composition and wine quality (as depicted by different wine styles), directly or indirectly, are still pursued for different terroirs. In view of a changing climate that would leave resources and environmental conditions for grape production more and more marginal in the future, water management will gain even greater importance (Schultz, 2000; Cyr & Shaw, 2010; Hunter *et al.*, 2010; Schultz & Stoll, 2010; Hunter & Bonnardot, 2011).

The supply (*via* photosynthesis) and loading (into the phloem) of sugar (sucrose) in plant sources, sink hierarchy/ priority, phloem transport and unloading in sinks (such as the grape berry) after partitioning, as well as metabolism of sugar in sinks, are critical events in the grapevine growth cycle (Hunter *et al.*, 1994; Hunter, 2000; Hunter & Ruffner, 2001). Despite many attempts based on, *e.g.*, berry dimension responses (after transport disruption by means of girdling and heat treatment) (Lang & Thorpe, 1989; Greenspan *et al.*, 1994, 1996), flow of water-soluble dyes (Düring *et al.*, 1987; Findlay *et al.*, 1987; Creasy *et al.*, 1993; Rogiers *et al.*, 2001), monitoring of xylem and phloem mobile mineral transport (Creasy *et al.*, 1993; Rogiers *et al.*, 2000; Etchebarne *et al.*, 2009), hydraulic conductance measurements (Tyerman *et al.*, 2004), the measuring of berry turgor and hydraulic dynamics (Greer & Rogiers, 2009) and xylem tracheary element analyses (Chatelet *et al.*, 2008a), the mechanisms involved in the triggering and regulation of sugar and water import, as well as berry shrinkage at a specific ripeness level (particularly for a highly expressive cultivar like Shiraz), are not yet resolved and even still controversial. Most of the mechanisms, hypotheses and disputes are summarised in Fig. 1.

Essentially, the softening and deformability of fruit are due to the breakdown of cortex parenchyma cell walls, the latter which are composed mainly of polysaccharides, classified into pectin, cellulose and hemi-cellulose; significant depolymerisation occurs in grapes (Goulao & Oliveira, 2008). The cell wall composition, dynamics and flexibility are extremely complex and still far from fully understood (Harholt *et al.*, 2010). During grape ripening and the processing of the grapes (e.g. during skin contact and pressing), the berry cell walls are barriers to the diffusion and integration of many components essential to wine quality. Using transcriptomic tools to unravel berry softening processes, Glissant *et al.* (2008) found several structural and regulatory genes with expression profiles correlating to different ripening phases (middle-ripe, harvest-ripe and over-ripe) and which may be involved in cell wall modification. Co-expression, which suggests potential functional relationships between genes, and the concomitant action of isoforms, were also highlighted as playing significant roles. In a comprehensive study on the transcriptional network involved in the regulation of berry development, Deluc *et al.* (2007) profiled metabolites as well as mRNA expression in parallel. The results showed a magnitude of expressions at various stages of berry development, and

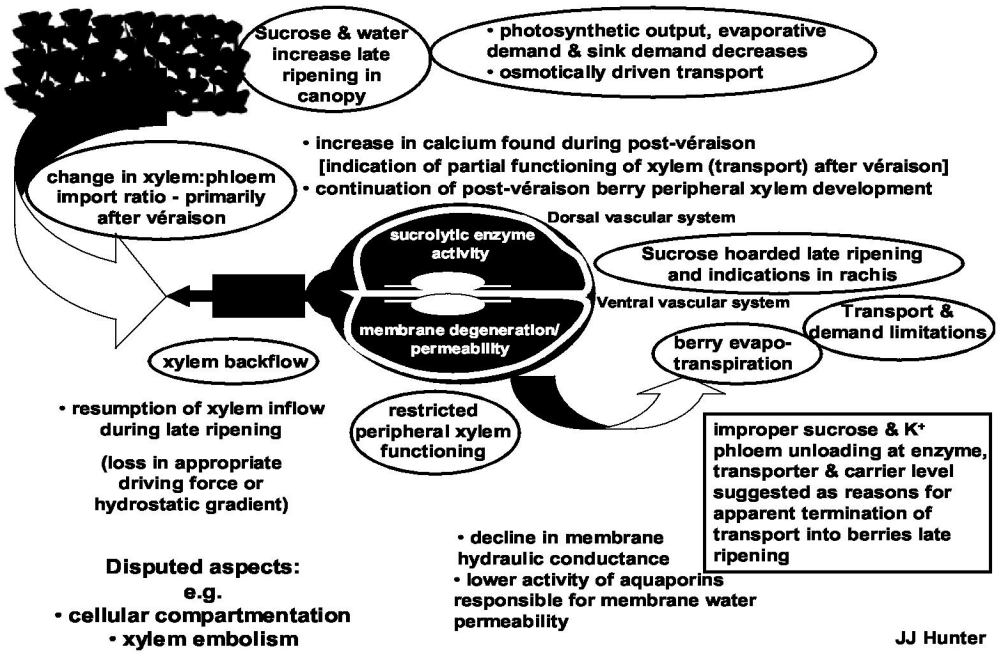

FIGURE 1
Regulatory processes between canopy and grapes during ripening (Hunter *et al.*, 2010).

clearly illustrated the dynamic nature of berry development, both metabolic and structural. Amongst the array of known (and obscure) transcripts related to practically all generally known physiological processes involved in the taste, flavour and protective behaviour of berries, multiple genes that may play key functional roles in cell wall structure, metabolism and softening were also identified. It clearly revealed the complex orchestration of metabolic, transport and control processes during the whole cycle of the developing berry. This study is complemented by another thorough study revealing the phenotypic plasticity of the grapevine under field conditions, showing clearly the environment-sensitivity of the expression and function of berry transcripts related to secondary metabolism (Dal Santo *et al.*, 2013).

Indications are that sucrose and water transport to the grapes is regulated by a combination of photosynthetic activity, canopy and berry microclimate, osmotically driven transport, berry evapotranspiration, sucrolytic enzyme activity, membrane degeneration/permeability and a change in the ratio of xylem:phloem import, primarily after véraison (Lang & Düring, 1991; Greenspan *et al.*, 1994; Rebucci *et al.*, 1997; Dreier *et al.*, 1998, 2000; Greer & Rogiers, 2009; Hunter *et al.*, 2010). Many aspects, such as cellular compartmentation and xylem embolism, have been disputed (Chatelet *et al.*, 2008a, 2008b; Fontes *et al.*, 2011). According to Bondada *et al.* (2005), a loss in appropriate driving force or hydrostatic gradient may be involved in the (partial) loss of active xylem function after véraison. Furthermore, xylem backflow from the berries back to the parent vine during the late ripening stages has been proposed by many (Lang & Thorpe, 1989; Schaller *et al.*, 1992; Greenspan *et al.*, 1996; McCarthy & Coombe, 1999; Rogiers *et al.*, 2006; Tilbrook & Tyerman, 2009). A resumption of xylem inflow during the late ripening stages has also been suggested, presumably to match the diminishing phloem flow (Schaller *et al.*, 1992; Rogiers *et al.*, 2006). Etchebarne *et al.* (2009) argued that the increase in calcium found during post-véraison under sufficient water supply may be taken as an indication that there is partial functioning of the xylem (transport) after véraison. A migration of calcium and potassium between berry compartments (from seeds/flesh to skins) also seems to occur during this time. This may have been a normal migration along with the osmotic gradients/balances created by the loss of water from the berry, and according to changes in cellular compartmentation integrity (Dreier *et al.*, 1998, 2000). A continuation of post-véraison berry peripheral xylem development seems evident (Chatelet *et al.*, 2008b), but whether this is involved (or at least significantly) in post-véraison water translocation into the berry (Greer & Rogiers, 2009) is still debatable. Improper sucrose and potassium phloem unloading at enzyme, transporter and carrier level was suggested as a reason for the apparent termination of transport into the berries (Davies *et al.*, 1999; Fillion *et al.*, 1999; Pratelli *et al.*, 2002). A decline in membrane hydraulic conductance seemed evident and significant during ripening (Tyerman *et al.*, 2004), and lower activity of the aquaporins responsible for membrane water permeability and the control of water stress (Lovisolo *et al.*, 2010) has been implicated (Delrot *et al.*, 2001; Tyerman *et al.*, 2002, 2004). It stands to reason that hormone activity, *e.g.* abscisic acid, may have a bigger role than mere association, particularly with regard to the triggering of berry ripening and, more often, its activity-steering involvement in osmotic balances and stress recognition and mediating the physiological state of the berry (Coombe, 1992; Hiratsuka *et al.*, 2001; Yu *et al.*, 2006; Wheeler *et al.*, 2009).

Within the above perspective, the challenge at a practical and technological level lies in finding the relation between the cultivation-required aspects and complex physiological and developmental changes in canopy, conduits and berries, and the readiness of the grapes for harvesting and potential wine style/s that can be expected. A better understanding of the inter-relationships is required. To our knowledge, vegetative and reproductive growth that is subjected to various vine water status levels (with consideration of both volume of water and stage of application) has not been monitored systematically during ripening (specifically at different berry ripeness levels) under field conditions and in a challenging winter-rainfall region with occasional summer showers.

The focus of this study was on quantifying changes in vegetative and reproductive growth during the berry ripening period under different vine water status levels, introduced by means of irrigation at different stages and levels (volumes) during the growth season, as single or multiple applications. This study was followed by another study detailing the impact of vine water relations and grape ripeness levels on grape composition and wine quality/style.

MATERIALS AND METHODS

Vineyard

A seven-year-old *Vitis vinifera* L. cv. Shiraz (clone SH1A) vineyard, grafted onto Richter 99 (*Vitis Berlandieri* x *Vitis rupestris*) (clone RY2A), was used. The vineyard was located on the Experiment Farm of ARC Infruitec-Nietvoorbij in Stellenbosch, Western Cape, South Africa. The area is under the influence of a Mediterranean climate with winter rainfall (Fig. 2) (Hunter & Bonnardot, 2011). The vines were spaced 2.75 m x 1.5 m on a Glenrosa soil with western aspect (26° slope) and trained onto a seven-wire (cordon wire and three sets of movable wires, laterally spaced 15 cm) lengthened Perold (VSP) trellising system with cordon wire at 60 cm. Vines were pruned to two-bud spurs with a spur spacing of approximately 15 cm (~10 spurs/vine). Canopies were suckered (judicious removal of non-allocated infertile shoots on the cordon before the growth stage of approximately 30 cm primary shoot length), shoot positioned (shoots orientated to a vertical position by means of movable wires and then positioned by hand in line with their corresponding spurs – practice repeated as required with further canopy development) and tipped/topped [tipping (removal of primary shoot tips) and topping (removal of primary shoot apical parts to 30 cm above the top wire) were done as required during the period berry set to pea size/véraison]. No leaf thinning was done in the canopies. Lateral shoots were tucked into the canopy and positioned vertically between the wires. Rye was sowed (at a density of 80 kg/ha) between the rows in autumn to serve as cover crop during the winter. The cover crop was killed in spring before bud break and left as mulch on the ground during the summer.

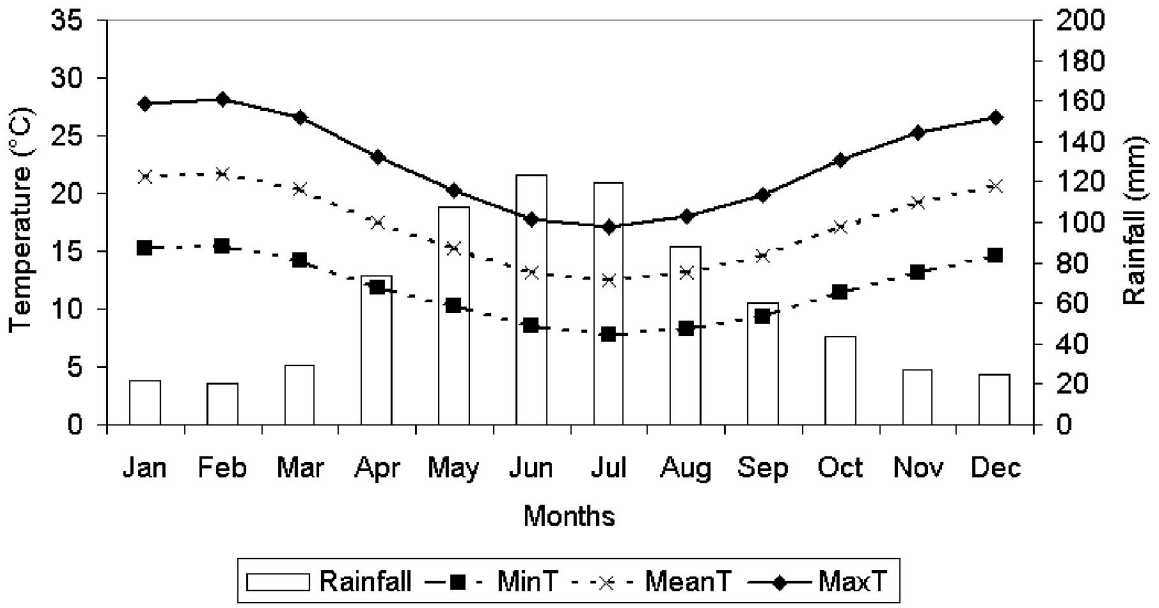

FIGURE 2
Mean monthly rainfall and temperature for Stellenbosch (33°9'S/18°9'E) (1967-2002) (Hunter & Bonnardot, 2011).

Treatments and layout

Fifteen treatments, comprising single and combined micro-sprinkler irrigations that differed in volume of water supplied and stage/s of application, were applied, as indicated in Table 1. Three water levels were implemented, i.e. no irrigation (0% NI), and soil volume filled to 75% and 100% of field water capacity, respectively. Each irrigation treatment comprised either a single or different combinations of irrigations at different growth stages [berry set (BS), pea size berry (PS), véraison (V), and post-véraison (PV)]. Véraison represented at least 75% of grape colouring. Post-véraison refers to three weeks after V. The treatments were completely randomised in two blocks (representing two replications), with a buffer row on each side of a treatment row and one buffer vine on each side of a treatment plot within the row. Thirty vines per replicate were used. Measurements were done at BS, PS, V, PV and at three ripeness levels. Soluble solid contents were used as indicator of ripeness level, *i.e.* 23°B, 25°B and 27°B (approximately 14 days between ripeness levels, corresponding to the beginning, middle and end of March). The treatments were applied in summer for four years in a row. The first two years were judged as calibrating years under field conditions. Mean values of the last two years of the experiment (2006/2007 and 2007/2008) are presented.

Measurements and analyses

Field water capacity (FWC) and bulk density of undisturbed soil cores (at 15 cm, 45 cm and 75 cm) were determined by standard methods between the vineyard rows in six locations distributed at random in the experiment block. Both these soil parameters were used to calculate the volume of water needed to adjust the soil water content to either 75% or 100% of field water capacity at the different stages of irrigation application. Soil water (Sw) contents were determined gravimetrically, as well as by neutron probe at 10 to 30 cm, 30 to 60 cm and 60 to 90 cm depth respectively, at each measurement stage. Samples for soil particle size distribution and chemical characteristics (Soil Classification Working Group, 1991) were taken at the same depths.

Seven shoots (including bunches) per vine were sampled in order to determine total leaf area, primary and secondary leaf areas, number of primary leaves, number of secondary leaves and shoots, primary and secondary shoot lengths, bunch mass, and berry mass and volume. Berry skins were separated from the pulp, the fresh and dry mass was determined, and the water content was calculated. Leaf area was determined by means of a LICOR Model 3100 area meter.

Light intensity in the bunch zone of the canopy was measured during mid-morning by means of a LICOR Line Quantum Sensor (inserted at random into three separate canopies) and expressed as a percentage of ambient light level determined in the vine row at maximum canopy height at regular intervals during the measurement period. Photosynthetic activity (Pn) (together with transpiration) of three randomly selected exposed leaves on primary and secondary shoots in the basal and apical parts of the canopy was measured during mid-morning, using an open system ADC portable photosynthesis meter (The Analytical Development Co., Ltd., England), as specified in Hunter and Visser (1988, 1989). Leaf (Ψ_L) and stem (Ψ_S) (bagged for at least 30 min. before measurement) water potential was determined on three randomly selected exposed mature leaves on primary and secondary shoots from early to mid-afternoon, using a pressure chamber as described by Scholander *et al.* (1965).

Soluble solids (°Balling) of the grape must per replicate were determined by standard methods after crushing of the grapes for winemaking purposes. Soluble solids per berry were calculated. Individual sugars in the leaves, rachis, whole berry, skin and pulp (obtained from two random sub-samples of the respective parts of the sampled shoots mentioned

TABLE 1
Irrigation treatments applied to the Shiraz/Richter 99 vineyard.

Irrigation treatment	Berry set	Pea size	Véraison	Post-véraison
1. No irrigation (NI)	O	O	O	O
2. 75% all stages (75% all stages)	¾X	¾X	¾X	¾X
3. 100% all stages (all stages)	X	X	X	X
4. 75% pea size (75% PS)	O	¾X	O	O
5. 100% pea size (PS)	O	X	O	O
6. 75% véraison (75% V)	O	O	¾X	O
7. 100% véraison (V)	O	O	X	O
8. 75% post-véraison (75% PV)	O	O	O	¾X
9. 100% post-véraison (PV)	O	O	O	X
10. 75% pea size & véraison (75% PS+V)	O	¾X	¾X	O
11. 100% pea size & véraison (PS+V)	O	X	X	O
12. 75% pea size & post-véraison (75% PS+PV)	O	¾X	O	¾X
13. 100% pea size & post-véraison (PS+PV)	O	X	O	X
14. 75% véraison & post-véraison (75% V+PV)	O	O	¾X	¾X
15. 100% véraison & post-véraison (V+PV)	O	O	X	X

Véraison = at least 75% colouring of grapes
Post-véraison = three weeks after véraison
O = No irrigation, ¾X = irrigation to 75% field water capacity, X = irrigation to 100% field water capacity

above) were extracted and analysed at all measurement stages by gas liquid chromatography (GLC) (after silylation), as described by Hunter and Ruffner (2001).

At any given stage, soil water determinations, physiological measurements and vegetative and reproductive growth sampling were completed during the course of two days, after which irrigation was applied as required for application of the different treatments.

Statistical analyses

The experiment design was a randomised block with two replications and thirty vines per replicate. Treatment design was a split-plot. The main plot was a factorial with treatments and stages as factors. According to Little and Hills (1978) a split-plot principle can be applied to experiments in which successive observations are made on the whole units over time (years). Analysis of variance was performed using SAS version 9.2 (SAS, 2012). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference was calculated at the 5% significance level to compare treatment means (Ott, 1998).

RESULTS AND DISCUSSION

Soil structural, chemical and water conditions

The Glenrosa soil used is classified as a predominantly sand-clay-loam soil (Table 2) and has an average FWC of approximately 17% (dry mass basis). The FWC of the different soil layers was similar. The soil compaction index (measured as bulk density) of the different soil layers slightly exceeded the critical value of 1.5 g/cm^3, beyond which root penetration is believed to decline (Richards, 1983). Resistance of the soil increased with increasing depth, whereas P, K and Ca decreased (Tables 3a & 3b). Except for Fe, contents of the micro-elements and carbon, as well as the texture of the soil, decreased with depth.

The Sw contents are given only at the different harvest times (rest of data not shown) (Fig. 3). Although general trends are recognisable, the gravimetric and neutron probe Sw results, for the purpose of calibration, showed an extremely poor relationship, and gravimetric measurements were therefore used to determine Sw contents and calculate irrigation volumes. This aspect has serious implications for producers relying solely on neutron probe results for irrigation scheduling; re-calibration of the measuring device from soil to soil and with soil depth in complex, layered soils (with relatively high stone content) is essential. Although the familiar trend of increasing Sw content with increasing soil depth was evident, it is interesting that similar treatment effects occurred in the different soil layers. The drainage, holding and withdrawal dynamics of water in the different soil layers were similar. With a few exceptions, the Sw content (on a dry mass basis) stayed above 50% FWC. However, a decreasing trend was noticeable from the first to the third harvest stage, with the water loss between the first two stages being the most noticeable (Fig. 3), irrespective of rainfall just prior to the second harvest stage in both seasons (Figs 4a & 4b). Despite the relatively regular rainfall during the active growth period and the ripening period (Figs 4a & 4b), at all stages (control), and noticeably at PV, and at PV and earlier stages combined, treatments with Sw adjusted to 100% FWC showed elevated Sw contents in all soil layers and at the different harvest stages. The treatments therefore were successful in reaching deeper soil layers, despite the increase in clay content with depth and the soil compaction that may have affected soil porosity, particularly in the top soil layer (Table 2). It seems that the reduction in Sw stemmed mostly from a loss of supplemented water and

TABLE 2
Physical properties of the soil.

Depth (cm)	Clay (%)	Silt (%)	Stone (vol%)	Sand (%)					Bulk density (g/cm³)	Field water capacity (dry mass basis) (%)
				Fine	Medium	Coarse	Total	Classification*	Measured at 15, 45 & 75 cm depth	
0-30	21.60	12.44	**16.0**	49.35	8.99	7.62	65.96	Sa-Cl-Lm	1.62	17.20
30-60	25.58	10.60	**19.5**	48.67	8.69	6.46	63.82	Sa-Cl-Lm	1.60	16.92
60-90	28.56	10.64	**12.0**	45.95	8.44	6.42	60.80	Sa-Cl-Lm	1.60	17.37

*Sa-Cl-Lm = Sand-clay-loam soil

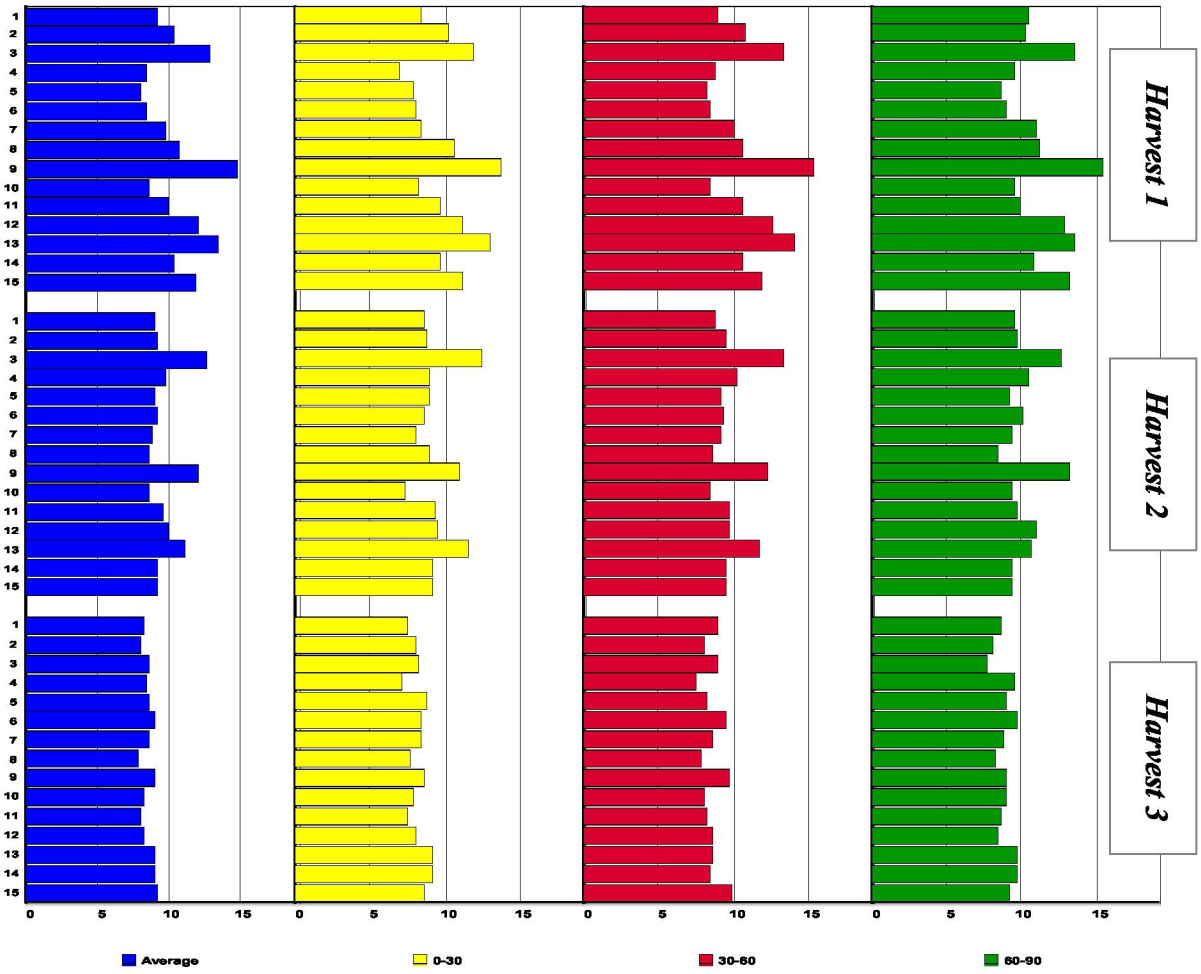

FIGURE 3
Effect of level and stage of irrigation (number refers to the treatment as depicted in Table 1) on soil water content, measured in three layers and at three ripeness stages in the Shiraz/Richter 99 vineyard.

that a base Sw fraction stayed largely intact (Fig. 3). This is also evident from the relatively high Sw content of the non-irrigated treatment, particularly in the deepest soil layer and at the last harvest stage. The treatment effects on Sw content progressively diminished from the first to the third ripeness stage. It can be accepted that the water tension in the soil increased during the later stages of the growth season, but that the impact of plant transpiration on this would have been reduced because of less demanding climatic conditions, a senescing canopy, and reduced phloem water gradient between the berry and the parent plant during this time (Lang & Thorpe, 1989; Schaller *et al.*, 1992; Greenspan *et al.*, 1996; McCarthy & Coombe, 1999; Dreier *et al.*, 2000; Hunter *et al.*, 2004; Tyerman *et al.*, 2004; Rogiers *et al.*, 2006).

The soil clearly has a high water-holding capacity and

TABLE 3a
Chemical analyses of the soil.

				P Bray II	K	Exchangeable cations [cmol(+)/kg]			
Depth (cm)	pH (KCl)	Resistance(Ohm)	H (cmol/kg)	(mg/kg)		Na	K	Ca	Mg
0-30	5.8	2160	0.46	6.0	131.0	0.12	0.34	2.62	0.92
30-60	5.7	2520	0.39	3.0	62.0	0.10	0.16	2.25	0.54
60-90	5.5	2960	0.42	2.5	51.5	0.11	0.13	1.85	0.82

TABLE 3b
Chemical analyses of the soil.

	Cu	Zn	Mn	B	Fe	C	Na	K	Ca	Mg	T-value
Depth (cm)	(mg/kg)					(%)					(cmol/kg)
0-30	1.17	2.25	19.00	0.15	3.11	0.94	2.55	4.44	59.06	20.72	4.44
30-60	0.97	0.60	9.90	0.08	4.44	0.41	2.91	3.44	65.43	15.67	3.44
60-90	0.70	0.30	6.45	0.07	4.55	0.17	3.31	3.32	56.24	23.22	3.32

a buffer capacity against favourable evapotranspiration conditions, even with its western aspect and being subjected to long and relatively dry seasons with frequent occurrence of high temperatures (Figs 4a & 4b), and having grapevines with canopies fully developed on the trellising system. Although a root study was not part of the investigation, it is unlikely that the composition of the root system (in terms of root thickness) and distribution in the different soil layers could have been similar. It may be assumed that the root system would have been grossly distributed in the top 0 to 80 cm layer and that fine root presence would have been higher in at least the top soil layers, mostly the 0 to 30 cm layer, similar to what has been found for different cultivars in different soils (Archer & Strauss, 1985; Swanepoel & Southey, 1989; Hunter *et al.*, 1995; Hunter, 1998a, and references therein).

Vine water relations

The vine Ψ data showed clear trends (Table 4). Both level of irrigation and stage of irrigation effects were evident at all stages. The water status of the vines, irrespective of treatment, generally followed Sw content patterns. In general, an increasing water deficit seemed evident from the BS stage until the PV stage. Although the Sw contents showed a decreasing trend from the first to the second harvest stage (Fig. 3), the water relations of the vines mostly appeared to increase during this time (Table 4), most likely as a result of the rainfall just before the second harvest stage (Figs. 4a & 4b). The general impression is that vines were not overly stressed. A well-developed root system, promoted by efficient soil preparation, may have contributed to the plant water status (Hunter & Myburgh, 2001; Myburgh, 2005); this was however not determined in this study. Nonetheless, the reasonably well-maintained plant water relations are in line with relatively high base Sw fractions of mostly more than 50% of FWC (Fig. 3). Primary and secondary Ψ_L and the internal hydraulic conductivity in the trunk and shoot (as reflected by Ψ_S) displayed similar patterns. The Ψ of primary and secondary leaves was not very different. The Ψ_S was generally higher than Ψ_L. This is found commonly (Choné *et al.*, 2001; Di Lorenzo *et al.*, 2005). It also indicates that Ψ_L over mid-day is more sensitive to environmental changes in *e.g.* light, temperature and wind. However, the drier the conditions, the less the difference seemed to be. This tendency was also noticed under extremely hot conditions during the course of the experiment (data not shown), indicating stomatal closure. Although differences in Ψ_S between treatments were more noticeable, Ψ_L as well as Ψ_S indicated that, in line with their higher Sw contents (Fig. 3), fully irrigated control vines (75% and 100%) and single and combined PV irrigated vines (75% and 100%) were largely irresponsive to the rainfall just before the second harvest stage (Table 4). All other treatments responded positively. From the second to the third ripeness level, a general reduction in vine Ψ occurred.

Fully irrigated vines and vines irrigated at PV, and in combination with earlier stages, also clearly responded to more (100%) or less (75%) water. Continuously irrigated vines maintained reasonably high Ψ during the season. The Ψ_S results showed that fully irrigated vines and vines irrigated to 100% at PS+V best maintained water relations until the last harvest stage, whereas the highest deficits at the last harvest stages occurred with combinations of 75% irrigation at PS+V and 75% irrigation at PS+PV. Interestingly, all V treatments that were combined with earlier (PS) water applications, as well as all PV treatments, whether single or in combination with any earlier (PS and V) treatment, responded noticeably to more or less water. It further seems that, despite a general tendency towards lower values, NI vines and vines irrigated only pre-véraison (at PS) developed an adaptive behaviour towards lower Sw contents and diurnal environmental stress; these vines reached their lowest water contents at the PV stage, after which they recuperated towards the second harvest stage and then largely stabilised after that. In contrast, the water relations of V- and PV-irrigated vines seemed more unstable. According to Patakas & Noitsakis

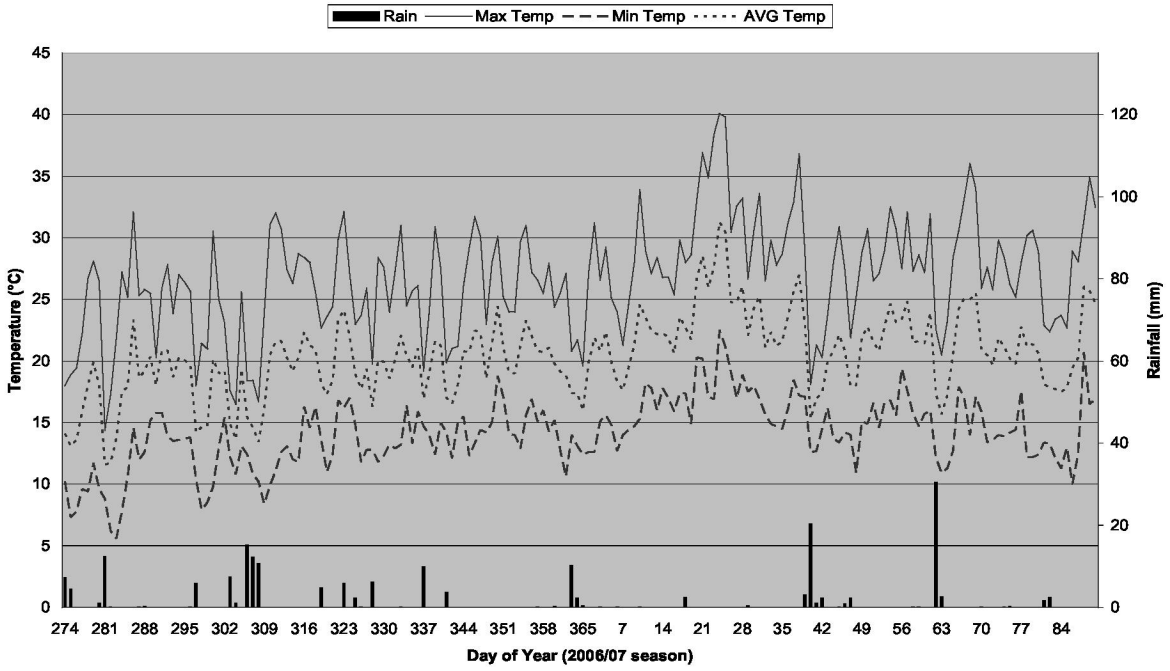

FIGURE 4a
Temperature and rainfall patterns for the 2006/2007 season at the experiment location.

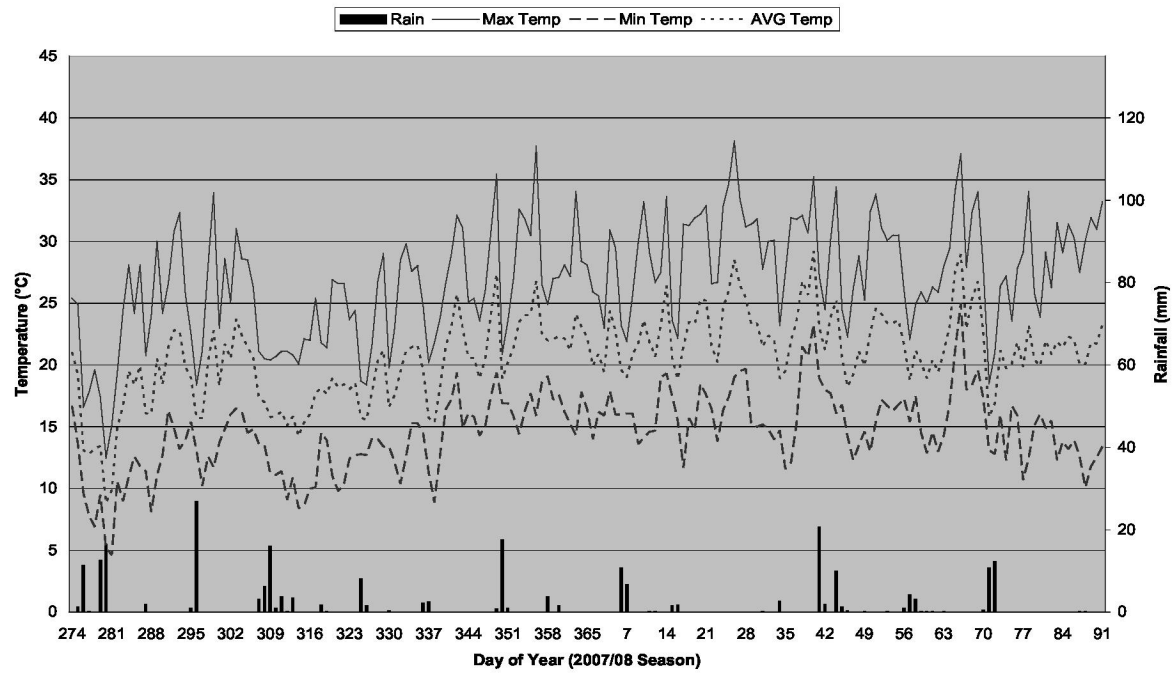

FIGURE 4b
Temperature and rainfall patterns for the 2007/2008 season at the experiment location.

(1999), an active osmotic adjustment may occur during the day under dry conditions. This would enable vines to maintain turgor. The most pronounced decrease in Ψ from the first to the last harvest stage occurred for PV irrigations, both single and in combination with water applications at earlier stages. Although this behaviour coincided with the Sw loss trends shown in Fig. 3, the decrease in plant water was too excessive to judge as being solely resulting from a decrease in Sw when comparing this to the trends of the other treatments. In comparison to the other treatments, any PV-irrigated vine was expected to maintain relatively high Ψ during the last weeks of the ripening period, but this seemed not to be the case.

Photosynthetic activity

Differences amongst treatments were not consistent and highlighting any would be speculative (Table 5). However, seasonal trends are evident. From after véraison until the

TABLE 4
Effect of level and stage of irrigation on Ψ_L and Ψ_S, measured in the bunch zone of the canopy of Shiraz/Richter 99.

Stage	Irrigation treatment (% of field capacity and stage)														
	NI	75 All	100 All	75 PS	100 PS	75 V	100 V	75 PV	100 PV	75 PS+V	100 PS+V	75 PS+PV	100 PS+PV	75 V+PV	100 V+PV
							ΨL (- kPa)								
							Primary leaves								
							At BS stage (before irrigation) ψ = 683.3 *[LSD(p=0.05)=207.35]*								
PS	956	744	669												
V	1 081	950	969	1 188	944										
PV	1 269	1 106	931	1 363	1 425	1 275	1 088			1 338	1 050				
H1	1 188	888	825	1 175	1 288	1 138	1 088	938	788	1 313	1 025	1 025	825	1 038	850
H2	950	863	863	1 050	1 088	1 013	1 038	1 038	838	1 088	863	1 050	925	1 050	900
H3	1 038	913	963	1 013	1 075	1 175	1 275	1 325	1 100	1 275	950	1 388	1 138	1 225	1 013
							Secondary leaves								
							At BS stage (before irrigation) ψ = 687.5 [LSD(p=0.05)=230.42]								
PS	869	825	813												
V	800	931	938	1 125	944										
PV	1 331	1 219	1 125	1 400	1 419	1 356	1 113			1 488	969				
H1	1 075	950	825	1 238	1 275	1 238	1 113	900	813	1 338	913	1 038	800	1 038	788
H2	913	975	838	1 025	1 038	1 075	963	1 025	963	1 063	900	1 113	825	1 075	1 013
H3	1 113	925	950	1 100	1 025	1 275	1 238	1 225	1 175	1 188	925	1 313	1 063	1 138	1 025
							ΨS (- kPa)								
							At BS stage (before irrigation) ψ = 587.5 [LSD(p=0.05)=169.77]								
PS	775	706	594												
V	956	747	688	1 025	838										
PV	1 088	1 038	869	1 300	1 300	1 063	913			1 206	831				
H1	1 088	788	688	1 175	1 213	1 138	975	775	555	1 250	850	913	750	1 000	738
H2	925	850	750	950	988	925	938	938	650	1 013	813	1 000	763	913	750
H3	1 063	888	850	1 038	975	1 125	1 125	1 163	1 025	1 250	850	1 363	1 025	1 188	1 025
							Primary ΨL / ΨS [LSD(p=0.05)=0.2584]								
H1	1.09	1.13	1.2	1	1.06	1	1.12	1.21	1.42	1.05	1.21	1.12	1.1	1.04	1.15
H2	1.03	1.02	1.15	1.11	1.1	1.1	1.11	1.11	1.29	1.07	1.06	1.05	1.21	1.15	1.2
H3	0.98	1.03	1.13	0.98	1.1	1.04	1.13	1.14	1.07	1.02	1.12	1.02	1.11	1.03	0.99
							Secondary ΨL / ΨS [LSD(p=0.05)=0.2584]								
H1	0.99	1.21	1.2	1.05	1.05	1.09	1.14	1.16	1.46	1.07	1.07	1.14	1.07	1.04	1.07
H2	0.99	1.15	1.12	1.08	1.05	1.16	1.03	1.09	1.48	1.05	1.11	1.11	1.08	1.18	1.35
H3	1.05	1.04	1.12	1.06	1.05	1.13	1.1	1.05	1.15	0.95	1.09	0.96	1.04	0.96	1

BS = Berry set; PS = Pea size; V = Véraison; PV = Post-véraison; 75 = irrigation to 75% field water capacity; 100 = irrigation to 100% field water capacity; NI = No irrigation; Trm. av. = Treatment average; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3; Before (I) irrigation = Means of measurements at berry set

third harvest stage, apical primary leaves and secondary leaves generally displayed higher Pn than primary basal leaves. Apical leaves generally outperformed basal leaves on either primary or secondary shoots. Being younger, apical primary leaves and secondary leaves generally responded better to decreasing photosynthetic active radiation during ripening, and would be more involved in metabolic processes to satisfy sucrose and osmotic balance demands during this time (Hunter *et al.*, 1994; Hunter & Ruffner, 2001; Hunter *et al.*, 2004). They would also have been less sensitive to abiotic influences compared to the primary leaves. According to Patakas *et al.* (1997), the capability for osmo-regulation was almost the same in mature and immature leaves, but decreased with age. Immature leaves have more elastic cell walls, rendering them able to maintain positive cell turgor under lower leaf water conditions. Although not necessarily immature, the younger secondary leaves thus may have a better ability to buffer the impact of unfavourable environmental conditions, *e.g.* high temperatures, on grape development and ripening (Hunter, 2000; Hunter *et al.*, 2004; Novello & Hunter, 2004). In addition to water management (and appropriate fertilisation programmes), it is important that the initiation and development of secondary leaves are stimulated pre-véraison by judicious canopy management in order to maintain canopy capacity and increase the potential of the canopy to support the grapes when adverse environmental conditions are experienced during ripening (Hunter, 2000).

After the second harvest stage (and despite the rainfall just prior to that – Figs 4a & 4b), a general drop in Pn occurred (Table 5). This may be indicative of senescence of the whole canopy, but also may be evidence that sucrose build-up in the leaves as a result of reduced demand from the rest of the vine, including the berries (Hunter *et al.*, 1994, 2004). Noticeably, the vines were not highly stressed and non-irrigated vines maintained comparatively high Pn. This may be a result of isohydric behaviour of the grapevine, which involves an active stomatal regulation of transpiration in order to prevent low Ψ-induced leaf damage (Schultz & Matthews, 1988; Naor & Wample, 1994; Escalona *et al.*, 2002). The general decrease in photosynthetic water-use efficiency (indicated by the Pn:T ratio) from after véraison was reversed by the second harvest stage (Table 5); this corresponded with the increase in water relations during this time (Table 4) and is also indicative of a still active canopy. Given the relatively high Sw contents (Fig. 3), decreasing Ψ occurring for irrigated vines during late grape ripening would probably be imposed mainly by a lack of continued high water absorption by the roots, as is particularly noticeable for vines that received irrigation during the ripening period. This may still have triggered so-called stress hormones, *e.g.* abscisic acid (Hunter, 1998b; Lovisolo *et al.*, 2002; Patakas *et al.*, 2005), and may have been favoured by a senescing canopy and prevailing cooler day and night temperatures, which would influence Ψ gradients and source:sink relationships. High levels of abscisic acid in the leaves (Patakas *et al.*, 2005) may trigger and increase the sensitivity of stomata to vapour pressure deficit (VPD) (Lovisolo *et al.*, 2002). The general impression, however, was that vines had recuperated/stabilised during this time, maintaining water balances to support reserve-accumulating compartments, amongst others. This is supported by the slightly decreasing

TABLE 5
Effect of level and stage of irrigation on photosynthesis (Pn), transpiration (T) and photosynthetic water use efficiency (Pn:T) of leaves of Shiraz/Richter 99.

		Pn (μmol/m²/s)/T (mmol/m²/s)/Pn:T (X10⁻³)											
		Primary leaves						Secondary leaves					
	Irrigation treatment	Basal			Apical			Basal			Apical		
Stage	(stage + level)	Pn	T	Pn:T	Pn	T	Pn:T	Pn	T	Pn:T	Pn	T	Pn:T
PS	NI	14.3	5.2	2.8	9.9	3.7	2.6	12.6	4.41	2.9	8.9	3.9	2.3
	75All stages	13.7	5.1	2.7	11.2	4.2	2.6	10.0	4.14	2.4	9.6	3.9	2.5
	100All stages	15.0	5.4	2.8	8.9	3.7	2.4	11.5	4.61	2.5	8.8	3.9	2.3
V	NI	10.1	4.6	2.2	8.7	5.4	1.6	10.9	4.69	2.3	12.8	5.5	2.3
	75All stages	6.6	3.8	1.8	9.6	5.0	1.9	9.8	4.94	2.0	10.9	6.7	1.6
	100All stages	6.0	5.0	1.2	4.3	4.5	2.3	5.7	4.71	1.2	12.5	5.9	2.1
	75PS	7.6	3.6	2.1	4.2	2.4	1.7	6.6	3.22	2.0	7.7	3.6	2.2
	100PS	8.4	3.9	2.1	9.1	5.6	1.6	6.1	5.81	1.0	11.7	5.4	2.2
PV	NI	7.0	3.3	2.1	10.9	4.6	2.4	7.3	3.89	1.9	8.2	4.1	2.0
	75All stages	4.9	3.9	1.3	11.7	6.0	1.9	9.0	4.99	1.8	12.0	5.5	2.2
	100All stages	9.6	5.8	1.7	12.0	6.1	2.0	9.8	5.51	1.8	12.8	6.4	2.0
	75PS	8.3	3.8	2.2	7.9	4.6	1.7	6.0	3.47	1.7	10.8	5.2	2.1
	100PS	4.3	2.8	1.5	9.1	4.5	2.0	6.2	3.75	1.7	6.9	4.4	1.6
	75V	9.7	5.5	1.7	11.4	6.1	1.9	9.6	5.00	1.9	10.8	6.0	1.8
	100V	4.8	5.1	0.9	10.3	5.6	1.8	5.2	5.33	1.0	10.9	6.2	1.8
	75PS+V	6.1	4.3	1.4	6.6	4.9	1.3	9.7	4.30	2.3	7.5	4.9	1.5
	100PS+V	8.1	4.8	1.7	11.3	6.0	1.9	6.5	5.53	1.2	7.1	5.0	1.4

TABLE 5 (CONTINUED)

Stage	Irrigation treatment (stage + level)	Pn (µmol/m²/s)/T (mmol/m²/s)/Pn:T (X10⁻³)											
		Primary leaves						Secondary leaves					
		Basal			Apical			Basal			Apical		
		Pn	T	Pn:T	Pn	T	Pn:T	Pn	T	Pn:T	Pn	T	Pn:T
H1	**NI**	8.4	6.7	1.3	9.4	6.6	1.4	5.5	6.27	0.9	5.6	7.1	0.8
	75All stages	2.8	4.6	0.6	6.2	7.1	0.9	9.1	6.55	1.4	9.9	7.0	1.4
	100All stages	7.7	6.4	1.2	12.4	7.5	1.7	7.7	7.40	1.0	6.2	6.8	0.9
	75PS	3.6	4.3	0.8	8.7	5.2	1.7	9.0	5.40	1.7	10.6	5.6	1.9
	100PS	5.5	4.3	1.3	5.0	3.9	1.3	5.2	3.98	1.3	6.7	4.3	1.6
	75V	4.0	4.3	0.9	8.6	5.0	1.7	7.5	4.81	1.6	8.9	5.9	1.5
	100V	7.0	6.8	1.0	6.4	6.3	1.0	6.9	6.46	1.1	10.9	6.7	1.6
	75PV	6.3	6.3	1.0	9.3	6.4	1.5	10.2	6.58	1.5	9.4	6.5	1.4
	100PV	9.0	5.4	1.7	9.9	5.5	1.8	5.2	6.06	0.9	3.1	6.8	0.5
	75PS+V	2.9	3.2	0.9	6.3	4.4	1.4	4.4	3.33	1.3	5.5	4.0	1.4
	100PS+V	9.2	6.2	1.5	9.2	6.7	1.4	8.3	5.81	1.4	10.0	6.0	1.7
	75PS+PV	6.6	5.4	1.2	7.5	6.2	1.2	2.7	6.32	0.4	4.6	6.5	0.7
	100PS+PV	5.9	5.6	1.1	9.4	7.4	1.3	8.7	6.42	1.4	10.9	6.7	1.6
	75V+PV	9.1	6.3	1.5	10.9	6.4	1.7	12.0	7.11	1.7	12.3	6.9	1.8
	100V+PV	9.5	6.4	1.5	11.7	7.0	1.7	8.0	6.27	1.3	9.4	6.6	1.4
H2	**NI**	8.5	4.8	1.8	9.5	5.4	1.7	9.7	5.37	1.8	11.9	5.7	2.1
	75All stages	6.8	4.8	1.4	7.1	4.0	1.8	8.1	4.05	2.0	12.6	6.0	2.1
	100All stages	7.3	4.8	1.5	9.8	5.8	1.7	12.6	5.87	2.1	10.4	6.0	1.7
	75PS	4.1	2.6	1.6	7.1	4.4	1.6	7.6	3.81	2.0	8.4	4.0	2.1
	100PS	4.9	3.0	1.6	8.2	4.3	1.9	3.6	2.85	1.3	6.9	3.8	1.8
	75V	3.6	2.4	1.5	8.0	3.7	2.2	10.6	4.85	2.2	10.1	4.3	2.3
	100V	4.5	3.9	1.2	10.8	5.0	2.2	8.0	4.44	1.8	5.6	5.4	1.0
	75PV	9.1	5.1	1.8	7.3	4.8	1.5	11.5	5.32	2.2	11.4	5.1	2.2
	100PV	7.7	4.2	1.8	9.2	4.6	2.0	8.9	4.24	2.1	13.5	5.0	2.7
	75PS+V	4.5	2.8	1.6	5.3	3.1	1.7	4.4	2.85	1.5	8.0	4.5	1.8
	100PS+V	6.4	4.6	1.4	6.5	4.3	1.5	8.5	4.60	1.8	13.8	6.2	2.2
	75PS+PV	5.6	3.4	1.6	10.7	4.8	2.2	12.7	5.57	2.3	12.7	5.7	2.2
	100PS+PV	5.6	3.7	1.5	10.3	5.3	1.9	11.1	5.29	2.1	9.7	5.5	1.8
	75V+PV	7.2	3.9	1.8	8.7	4.6	1.9	11.5	5.28	2.2	10.7	5.1	2.1
	100V+PV	5.8	3.8	1.5	10.9	5.2	2.1	8.3	4.62	1.8	12.1	5.7	2.1
H3	**NI**	4.6	3.0	1.5	5.3	3.1	1.7	4.8	3.20	1.5	7.2	4.2	1.7
	75All stages	4.7	4.0	1.2	7.6	5.0	1.5	2.7	2.93	0.9	4.4	4.5	1.0
	100All stages	5.4	4.0	1.3	6.4	4.5	1.4	4.1	3.31	1.2	8.8	5.1	1.7
	75PS	3.7	2.5	1.5	5.9	3.4	1.7	4.6	2.65	1.7	3.6	3.5	1.0
	100PS	3.7	2.6	1.4	6.4	4.1	1.5	2.5	2.09	1.2	6.0	3.5	1.7
	75V	5.6	3.2	1.7	6.1	3.6	1.7	3.3	2.12	1.6	7.2	3.6	2.0
	100V	3.7	3.1	1.2	5.3	3.3	1.6	5.6	3.40	1.7	6.1	3.5	1.8
	75PV	2.5	2.4	1.1	7.6	4.1	1.8	2.8	2.02	1.4	6.5	4.0	1.6
	100PV	8.0	3.8	2.1	10.7	4.4	2.4	7.9	3.76	2.1	9.8	4.3	2.3
	75PS+V	2.8	1.7	1.6	6.0	4.3	1.4	4.4	2.53	1.7	3.5	2.2	1.6
	100PS+V	2.3	2.6	0.9	6.3	4.2	1.5	5.2	3.77	1.4	5.4	4.0	1.4
	75PS+PV	3.4	2.0	1.7	8.9	3.8	2.3	5.0	2.44	2.0	8.1	3.6	2.2
	100PS+PV	3.8	3.0	1.3	7.1	4.1	1.7	6.6	4.27	1.5	7.8	4.8	1.6
	75V+PV	2.4	1.8	1.3	3.7	3.4	1.1	5.5	3.19	1.7	6.7	3.4	2.0
	100V+PV	4.5	3.6	1.2	6.8	4.6	1.5	6.2	4.27	1.5	6.1	4.7	1.3
	LSD (p=0.05)	***4.93***	***1.60***	***0.95***	***6.35***	***1.52***	***1.04***	***4.90***	***1.70***	***0.81***	***5.39***	***1.23***	***0.91***

BS = Berry set; PS = Pea size; V = Véraison; PV = Post-véraison; 75 = irrigated to 75% field water capacity; 100 = irrigated to 100% field water capacity; NI = No irrigation; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3

or stable "Ψ_L (primary & secondary)":"Ψ_S" ratio from the first to the third harvest (Table 4).

Vegetative growth

Since the vines were topped, vigour would have been channelled mainly into secondary shoot growth. A natural decrease in primary and secondary leaf area occurred during berry ripening from at least the PV stage (Fig. 5, Tables 6a & 6b) [see also Hunter & Visser (1990) and Hunter *et al.* (2004)]. Although the later timing of irrigation treatments resulted in generally lower secondary leaf area, development at large showed varying responses to the volume of irrigation. Even with growth stimulation it would have been unlikely that primary or secondary leaves initiated in the late season would have reached sucrose export status and still significantly contributed to the grapes (Hunter & Visser, 1988). On the other hand, re-growth after late-season irrigation may also be an indication of a still-active canopy, which may extend the harvesting window by continued translocation/contribution to grapes by existing exporting leaves. Sustaining early season-initiated secondary leaf area may not only increase photosynthetic capacity and support grape development, but also contribute to reserve accumulation after harvest (Hunter *et al.*, 1994; Hunter, 2000; Vasconcelos & Castagnoli, 2000). Non-irrigated vines maintained surprisingly high leaf area (Fig. 5), especially secondary leaf area, most probably because of the relatively high base Sw content (Fig. 3). This led to what were generally the lowest primary:secondary leaf area ratios. Pre-véraison irrigation seemed to result in the lowest ratios at the three harvest stages, which may be because of early stimulation of secondary leaf area development during a period in which tipping/topping was also done (Hunter, 2000).

Reproductive growth

Berries reached their highest mass approximately three weeks after véraison (PV) (Table 7). This is in agreement with earlier findings (Hunter *et al.*, 2004). High base soil water under the conditions of the study may largely have prevented the surfacing of treatment as well as classic water-deficit berry-size reduction effects (Williams & Matthews, 1990; McCarthy, 1999; Ojeda *et al.*, 2002; Roby & Matthews, 2004; Myburgh, 2005). Bunch mass and volume started to decrease from PV already, whereas the rachis generally reached its highest mass only at the first harvest stage, after which it decreased (Fig. 6). Bunch, berry and rachis mass and volume continued to decrease during all ripening stages. The appearance of the rachis may not be an indication of berry condition (Hunter *et al.*, 2004). The results seemed to indicate independent development and/or senescence for the berry and rachis, particularly during late ripening. The Sw contents (Fig. 3), Ψ_L and Ψ_S (Table 4), Pn (Table 5) and bunch and berry mass (Table 7) seemed concerted during the last ripening stages. Sucrose concentrations in the leaves reached peak values between PV and the first harvest stage, after which a general decline occurred (Fig. 7), in line with a senescing canopy (Fig. 5, Tables 6a & 6b). It previously was found that sucrose built up in the primary and secondary leaves during late ripening (Hunter *et al.*, 1994, 2004). This was also evident in this study (Fig. 7), particularly in the secondary leaves, and coincided with a general decrease in Ψ (Table 4). This build-up of sucrose in the leaves, *i.e.* an over-supply of sucrose (be it because of concentration and/or a decrease in demand and/or phloem loading and/or phloem transport), may also nullify potential negative effects that the re-growth of secondary shoots or even tertiary shoot initiation after late-season irrigation may have on grape ripening. Parallel to that in the leaves, sucrose concentrations

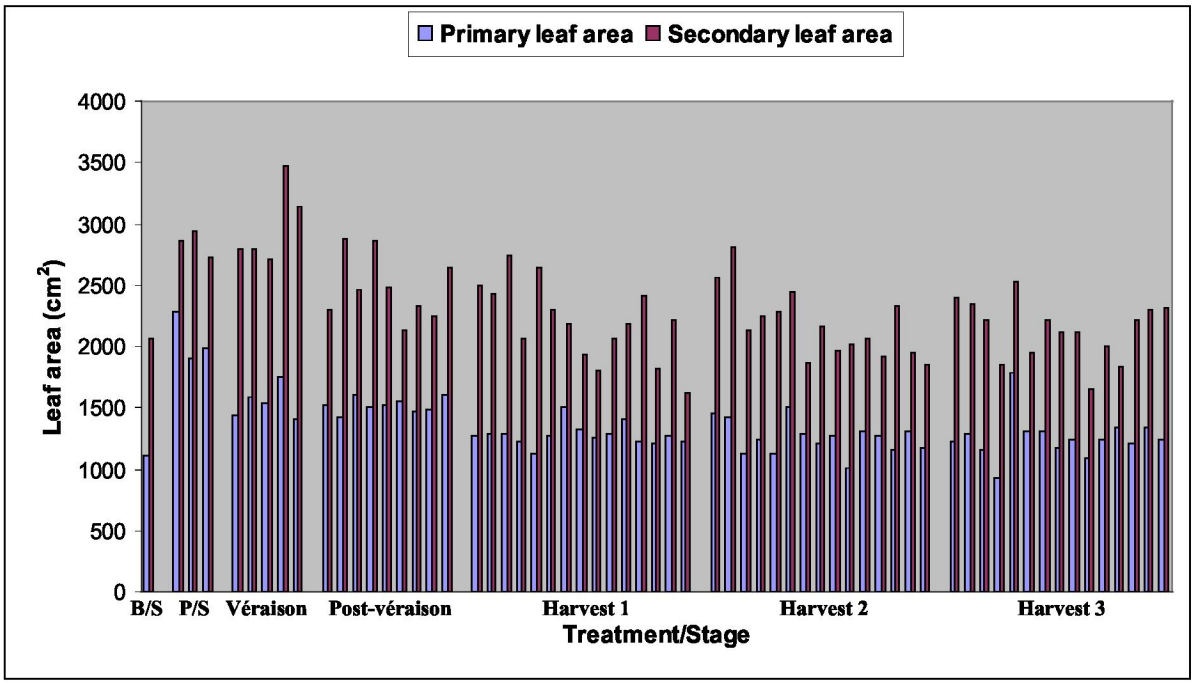

FIGURE 5
Trends in the evolution of vegetative growth of Shiraz/Richter 99 over stages.

in the berry skin also seemed to peak between PV and the first harvest stage (Fig. 8). In contrast, the lowest sucrose concentrations in the pulp seemed to occur around the first harvest stage, after which it increased and then remained stable. This increase coincided with the lowest concentrations in the rachis, which then increased again, almost as if a resumption of transport from the rachis to the pulp occurred momentarily with the rainfall just before the second harvest stage. Sucrose concentrations in the skin and pulp therefore followed similar patterns up to PV, after which it increased in the skin and decreased in the pulp up to the second harvest stage; after this, a stable trend was evident in the pulp, but a decreasing trend was seen in the skin. Given the relatively low Ψ in the canopy, the build-up of sucrose in the leaves, a more stable rachis mass, a reduction in berry size and the decreasing sucrose trend in the skin during this time, it is possible that the increase in sucrose in the rachis (Fig. 8) may indicate a point where phloem unloading into the berry is affected by Ψ gradients, as well as by metabolic activity in the berries. The increase in sucrose content of the rachis suggests that transport to berries became restricted during this time, despite a generally and readily favourable sucrose gradient from the rachis to the berry, and a decreasing osmotic potential in the berry. This seems to indicate that rachis and berry behaviour is not concerted during ripening, with the rachis displaying more typical vegetative tissue

TABLE 6a
Effect of level and stage of irrigation on vegetative growth (primary shoots) of Shiraz/Richter 99.

	Irrigation treatment	Leaves/shoot		Leaf mass		Leaf area/shoot		Shoot length	
Stage	(stage + level)	Number	Trm. av.	(g)	Trm. av.	(cm^2)	Trm. av.	(cm)	Trm. av.
BS	**Before irrigation**	10.3	10.3	2.8	2.8	1 112.0	1 112.0	90.1	90.1
PS	**NI**	12.6	12.6	2.9	2.9	2 281.8	2 281.8	103.0	103.0
	75All stages	13.1		2.9		1 907.8		107.3	
	100All stages	13.4	13.3	2.5	2.7	1 978.6	1 943.2	101.3	104.3
V	**NI**	12.0	12.0	2.8		1 439.2	1 439.2	90.8	90.8
	75All stages	13.8		2.6		1 579.8		116.9	
	100All stages	15.5	14.7	2.3	2.4	1 538.4	1 559.1	101.1	109.0
	75PS	14.7		2.8		1 752.3		112.9	
	100PS	12.7	13.7	2.7	2.7	1 400.6	1 576.5	108.0	110.4
PV	**NI**	13.2	13.2	2.6	2.6	1 519.5	1 519.5	112.6	112.6
	75All stages	13.4		2.5		1 416.4		109.5	
	100All stages	16.3	14.9	2.3	2.4	1 606.6	1 511.5	120.4	115.0
	75PS	15.3		2.2		1 501.8		116.4	
	100PS	13.4	14.4	2.7	2.4	1 519.6	1 510.7	109.2	112.8
	75V	15.7		2.3		1 554.0		106.2	
	100V	13.5	14.6	2.6	2.4	1 475.3	1 514.7	103.8	105.0
	75PS+V	14.6		2.3		1 488.3		105.9	
	100PS+V	15.6	15.1	2.6	2.5	1 611.5	1 549.9	110.2	108.0
H1	**NI**	9.5	9.5	3.3	3.3	1 266.8	1 266.8	103.0	103.0
	75All stages	11.0		2.8		1 296.3		110.6	
	100All stages	13.5	12.3	2.3	2.6	1 292.2	1 294.2	105.0	107.8
	75PS	12.0		2.2		1 221.6		109.2	
	100PS	10.1	11.1	2.6	2.4	1 117.7	1 169.7	105.5	107.3
	75V	9.6		3.2		1 273.0		101.6	
	100V	12.0	10.8	2.9	3.1	1 508.3	1 390.7	104.6	103.1
	75PV	11.5		2.6		1 317.0		110.0	
	100PV	11.6	11.6	2.3	2.5	1 263.3	1 290.2	100.2	105.1
	75PS+V	10.9		2.8		1 285.4		106.1	
	100PS+V	12.9	11.9	2.7	2.8	1 403.7	1 344.5	108.8	107.4
	75PS+PV	9.5		3.1		1 228.5		102.3	
	100PS+PV	9.9	9.7	3.0	3.0	1 202.5	1 215.5	96.1	99.2
	75V+PV	9.1		3.4		1 264.5		97.8	
	100V+PV	11.6	10.4	2.6	3.0	1 230.5	1 247.5	93.3	95.6

TABLE 6a (CONTINUED)

Stage	Irrigation treatment (stage + level)	Leaves/shoot Number	Trm. av.	Leaf mass (g)	Trm. av.	Leaf area/shoot (cm^2)	Trm. av.	Shoot length (cm)	Trm. av.
H2	**NI**	12.7	12.7	2.5	2.5	1 452.2	1 452.2	112.1	112.1
	75All stages	12.8		2.6		1 420.2		110.0	
	100All stages	11.5	12.2	2.3	2.5	1 119.5	1 269.9	98.7	104.3
	75PS	13.2		2.3		1 245.3		107.6	
	100PS	10.4	11.8	2.4	2.4	1 129.7	1 187.5	100.6	104.1
	75V	12.2		3.1		1 503.2		116.7	
	100V	11.3	11.8	2.8	2.9	1 288.9	1 396.1	100.0	108.3
	75PV	10.5		2.7		1 211.9		106.4	
	100PV	11.1	10.8	2.7	2.7	1 269.0	1 240.5	108.5	107.4
	75PS+V	9.0		2.6		1 006.5		93.6	
	100PS+V	11.5	10.3	2.6	2.6	1 300.9	1 153.7	103.4	98.5
	75PS+PV	11.5		2.8		1 266.6		109.6	
	100PS+PV	11.4	11.5	2.5	2.6	1 156.3	1 211.5	101.3	105.4
	75V+PV	12.1		2.6		1 309.9		109.1	
	100V+PV	10.8	11.5	2.7	2.6	1 176.8	1 243.3	98.8	104.0
H3	**NI**	10.7	10.7	2.6	2.6	1 217.8	1 217.8	102.5	102.5
	75All stages	11.0		2.7		1 294.7		104.8	
	100All stages	11.3	11.2	2.3	2.5	1 156.9	1 225.8	108.5	106.6
	75PS	10.8		1.9		929.7		102.7	
	100PS	14.4	12.6	2.1	2.0	1 779.1	1 354.4	126.4	114.6
	75V	11.5		2.6		1 313.1		107.7	
	100V	10.9	11.2	2.7	2.6	1 303.6	1 308.4	116.4	112.1
	75PV	9.0		3.0		1 173.0		104.6	
	100PV	11.4	10.2	2.5	2.7	1 236.9	1 204.9	96.8	100.7
	75PS+V	11.3		2.2		1 096.0		109.1	
	100PS+V	10.6	11.0	2.8	2.5	1 246.1	1 171.1	107.5	108.3
	75PS+PV	11.9		2.6		1 346.6		117.8	
	100PS+PV	9.7	10.8	3.1	2.8	1 210.3	1 278.4	107.9	112.8
	75V+PV	9.0		3.1		1 344.9		105.7	
	100V+PV	11.2	10.1	3.0	3.0	1 237.5	1 291.2	99.0	102.4
	LSD (p=0.05)	***3.5900***		***0.5802***		***313.60***		***15.334***	

BS = Berry set; PS = Pea size; V = Véraison; PV = Post-véraison; 75 = irrigated to 75% field water capacity; 100 = irrigated to 100% field water capacity; NI = No irrigation; Trm. av. = Treatment average; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3; Before irrigation = Means of measurements at berry set

TABLE 6b
Effect of level and stage of irrigation on vegetative growth (secondary shoots) and the ratio of primary:secondary leaf area of Shiraz/Richter 99.

Stage	Irrigation treatment (stage + level)	Sec. shoots/prim. shoot Number	Trm. av.	Sec. leaf mass/prim. shoot (g)	Trm. av.	Sec. leaf area/prim. shoot (cm^2)	Trm. av.	Prim. leaf area/sec. leaf area (cm)	Trm. av.
BS	**Before irrigation**	7.5	7.5	33.6	33.6	2 062.1	2 062.1	0.56	0.56
PS	**NI**	9.8	9.8	42.2	42.2	2 854.2	2 854.2	0.80	0.80
	75All stages	10.2		42.4		2 943.6		0.68	
	100All stages	9.2	9.7	31.2	36.8	2 731.8	2 837.7	0.80	0.74

TABLE 6b (CONTINUED)

Stage	Irrigation treatment (stage + level)	Sec. shoots/prim. shoot Number	Trm. av.	Sec. leaf mass/prim. shoot (g)	Trm. av.	Sec. leaf area/prim. shoot (cm^2)	Trm. av.	Prim. leaf area/sec. leaf area (cm)	Trm. av.
V	**NI**	8.9	8.9	48.0	48.0	2 801.3	2 801.3	0.52	0.52
	75All stages	12.4		52.4		2 793.3		0.65	
	100All stages	13.4	12.9	47.0	49.7	2 712.7	2 753.0	0.61	0.63
	75PS	12.8		50.8		3 472.8		0.48	
	100PS	11.7	12.3	317.5	184.2	3 137.5	3 305.2	0.49	0.48
PV	**NI**	10.9	10.9	40.5	40.5	2 299.3	2 299.3	0.67	0.67
	75All stages	10.8		52.0		2 884.1		0.53	
	100All stages	11.4	11.1	53.5	52.7	2 455.4	2 669.7	0.93	0.73
	75PS	12.0		51.9		2 855.7		0.56	
	100PS	10.2	11.1	45.4	48.6	2 478.7	2 667.2	0.67	0.62
	75V	10.5		38.9		2 137.7		0.84	
	100V	11.0	10.8	42.3	40.6	2 323.4	2 230.5	0.78	0.81
	75PS+V	11.0		41.0		2 244.6		0.73	
	100PS+V	11.2	11.1	47.4	44.2	2 645.4	2 445.0	0.61	0.67
H1	**NI**	9.2	9.2	45.6	45.6	2 501.4	2 501.4	0.54	0.54
	75All stages	9.9		44.9		2 422.2		0.55	
	100All stages	13.5	11.7	52.4	48.6	2 741.6	2 581.9	0.51	0.53
	75PS	10.6		38.4		2 065.6		0.62	
	100PS	10.3	10.4	48.9	43.7	2 647.2	2 356.4	0.44	0.53
	75V	8.8		43.8		2 303.3		0.56	
	100V	9.7	9.2	44.8	44.3	2 186.1	2 244.7	0.56	0.56
	75PV	10.0		35.1		1 938.4		0.76	
	100PV	9.7	9.8	32.9	34.0	1 799.0	1 868.7	0.78	0.77
	75PS+V	8.8		39.5		2 065.1		0.66	
	100PS+V	11.7	10.2	40.7	40.1	2 187.5	2 126.3	0.75	0.71
	75PS+PV	9.3		44.5		2 416.5		0.53	
	100PS+PV	8.9	9.1	33.3	38.9	1 822.7	2 119.6	0.67	0.60
	75V+PV	9.9		41.3		2 208.1		0.59	
	100V+PV	9.3	9.6	31.0	36.1	1 614.2	1 911.1	0.86	0.73
H2	**NI**	10.8	10.8	44.4	44.4	2 568.4	2 568.4	0.60	0.60
	75All stages	11.3		52.9		2 816.6		0.67	
	100All stages	11.5	11.4	39.5	46.2	2 124.7	2 470.7	0.62	0.64
	75PS	11.7		43.2		2 242.9		0.59	
	100PS	10.6	11.2	42.7	43.0	2 286.4	2 264.6	0.50	0.54
	75V	11.7		46.1		2 454.5		0.59	
	100V	9.4	10.5	37.2	41.6	1 861.1	2 157.8	0.88	0.73
	75PV	9.3		40.8		2 162.7		0.65	
	100PV	10.8	10.0	39.8	40.3	1 964.9	2 063.8	0.72	0.68
	75PS+V	9.4		38.6		2 016.4		0.62	
	100PS+V	11.1	10.3	35.2	36.9	2 066.7	2 041.6	0.69	0.65
	75PS+PV	9.6		35.4		1 922.3		0.73	
	100PS+PV	10.4	10.0	44.1	39.8	2 335.0	2 128.6	0.60	0.67
	75V+PV	9.6		38.1		1 950.9		0.79	
	100V+PV	9.3	9.5	35.0	36.5	1 859.5	1 905.2	0.90	0.85

TABLE 6b (CONTINUED)

Stage	Irrigation treatment (stage + level)	Sec. shoots/prim. shoot Number	Trm. av.	Sec. leaf mass/prim. shoot (g)	Trm. av.	Sec. leaf area/prim. shoot (cm^2)	Trm. av.	Prim. leaf area/sec. leaf area (cm)	Trm. av.
H3	**NI**	9.1	9.1	44.1	44.1	2 392.1	2 392.1	0.56	0.56
	75All stages	10.0		44.4		2 349.5		0.59	
	100All stages	12.1	11.0	39.3	41.8	2 209.1	2 279.3	0.55	0.57
	75PS	10.0		33.8		1 847.1		0.56	
	100PS	12.3	11.2	44.2	39.0	2 533.4	2 190.3	0.72	0.64
	75V	10.3		33.6		1 949.8		0.77	
	100V	11.4	10.8	40.6	37.1	2 214.0	2 081.9	0.68	0.73
	75PV	8.9		34.3		2 116.0		0.64	
	100PV	10.3	9.6	38.6	36.5	2 112.3	2 114.1	0.68	0.66
	75PS+V	10.3		28.9		1 647.3		0.71	
	100PS+V	9.1	9.7	35.5	32.2	1 993.3	1 820.3	0.72	0.71
	75PS+PV	9.9		33.2		1 832.5		0.83	
	100PS+PV	10.3	10.1	40.4	36.8	2 218.0	2 025.2	0.63	0.73
	75V+PV	9.7		42.2		2 292.5		0.66	
	100V+PV	8.8	9.3	43.6	42.9	2 307.0	2 299.7	0.78	0.72
	LSD (p=0.05)	***2.364***		***14.278***		***815.010***		***0.295***	

BS = Berry set; PS = Pea size; V = Véraison; PV = Post-véraison; Prim. = Primary; 75 = irrigated to 75% field water capacity; 100 = irrigated to 100% field water capacity; NI = No irrigation; Trm. av. = Treatment average; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3; Before irrigation = Means of measurements at berry set

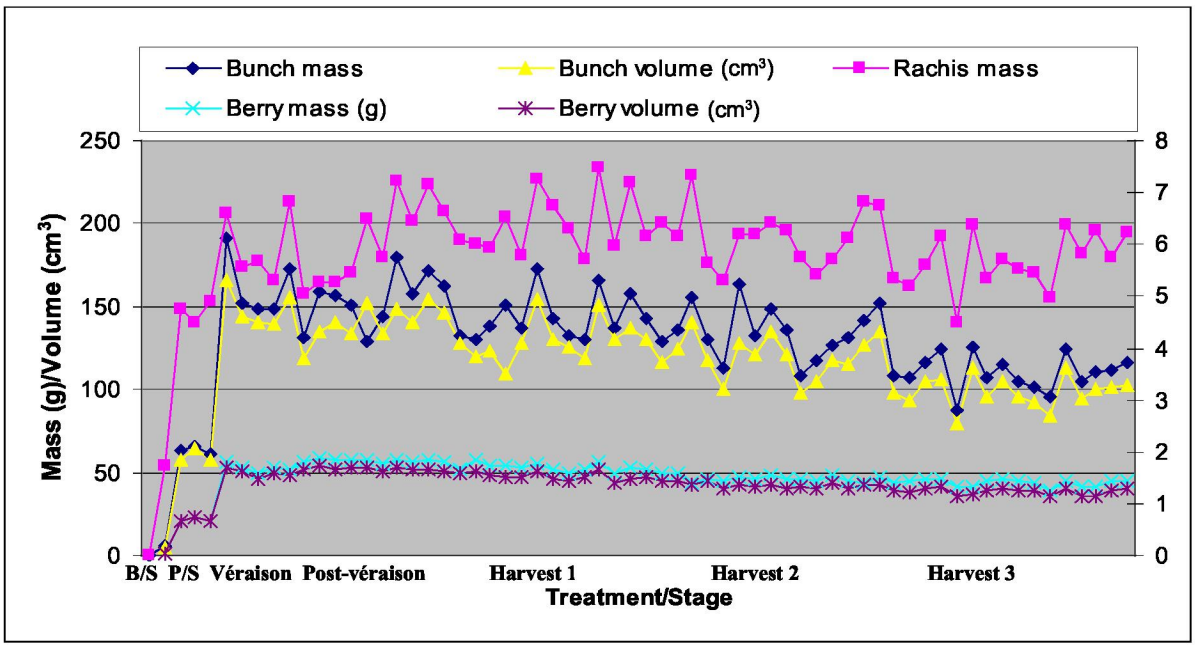

FIGURE 6
Trends in the evolution of bunch, rachis and berry parameters of Shiraz/Richter 99 over stages.

characteristics. An apparent sudden build-up of sucrose in the rachis during ripening certainly has value as a simple indicator of optimal ripeness/berry inactivity and harvest potential of the grapes. This should be investigated further.

Berry composition

A general trend of more water and less concentrated soluble solids occurred (Table 8). The NI vines did not seem able to reach similar soluble solid concentrations than the irrigated vines, especially up to the last harvest stage, but the values compared well to those of the high volume fully irrigated vines. As referred to earlier, the vines seemed to display an increasing independence of Sw as ripening proceeded. At the same time, the senescing canopy apparently produced

less and hoarded more sucrose and the berries lost more water than they could gain from Ψ gradients. Although the soluble solid concentration increased with further ripening, the soluble solid content per berry reached a plateau from approximately 22°B, which was preceded by a very fast import/accumulation from V until three weeks after (PV). This therefore is a critical period in transport to and accumulation in the berry. In a previous study it was found that the linear relationship between sugar transport and accumulation was broken earlier under conditions of soil water reduction (Hunter & Deloire, 2005); this is also evident at the last harvest stage in this study. The berry therefore concentrated (both pulp and skin) during this period, but at the same time lost mass as a result of water loss and reduced phloem sugar and water transport. From Fig. 9 it is clear that the general rachis:pulp+skin sucrose ratio (over all treatments) increased with ripening, especially at the last harvest stage, indicating reduced demand and restricted transport to and unloading into the berry.

General physiological trends

For the Shiraz in this study, shrinkage of the berries continued during (especially late) ripening, irrespective of highly negative Ψ prevailing in the berry during this period (as also found by Rogiers *et al.*, 2006 & Greer & Rogiers, 2009), which may have been expected to facilitate sustained water flow to the berry. The signal of low turgor status of the pericarp cells is apparently not transmitted to the parent vine (including the pedicel), and the berry seems to become at least partly isolated at such time. No clear evidence could be found that even high volumes of water (to 100% FWC) during ripening could sustain berry volume, indicating that not only is the berry less sensitive to water deficit during ripening (as compared to the pre-véraison period – Greenspan *et al.*, 1994, 1996), but it also seems not to be affected by high volumes of water during this time. This is partly in line with the findings of Keller *et al.* (2006). However, they deducted from their studies that watering during ripening would prevent further shrinkage of the berries. In our study, berries continued to lose water, irrespectively. Although it is acknowledged that the field conditions in this comprehensive study may not be considered ideal for studying basic physiological processes, clear trends were found. If it is accepted that the cell can only regulate its water balance (turgor) by actively regulating the osmotic potential, solute accumulation and metabolism are bound to play a significant role. In addition, the Ψ gradients and hydraulic conductivity of the transport pathway are critical in the influx of water and thus the maintenance of turgor (Patrick, 1997; Tilbrook & Tyerman, 2009). Although, by lack of sufficient information, sucrose is considered the main role player, sucrose and K plus accompanying anions represent the major osmotic species translocated in the phloem sap (Patrick, 1997). With K being the most abundant cation, its accumulation certainly also has a role in maintaining berry turgor (Mpelasoka *et al.*, 2003). Co-expression of some aquaporins and sugar transporters involved in sugar and water trans-cellular flux also assumes a link that is functional in unloading and accumulation in berry flesh vacuoles (Delrot *et al.*, 2001; Conde *et al.*, 2006; Fouquet *et al.*, 2008; Zhang *et al.*, 2008). According to Glissant *et al.* (2008), aquaporin expression follows a complex developmental pattern that probably contributes

TABLE 7
Effect of level and stage of irrigation on reproductive growth of Shiraz/Richter 99.

		Bunch	Bunch				Rachis		Berry			Bunch:
Stage	Irrigation treatment	no./ shoot	Mass (g)	Vol. (cm^3)	Length (cm)	Width (cm)	Mass (g)	Vol. (cm^3)	Mass (g)	No./ bunch	Vol. (cm^3)	Rachis mass
BS	**Before I**	1.45	5.35	5.1	11.20	3.41	1.74	1.88	0.05	*	0.05	3.1
PS	**NI**	1.46	63.21	57.9	15.83	6.01	4.75	4.89	0.67	75.0	0.67	13.3
	75All	1.68	65.46	64.0	13.98	6.29	4.50	4.62	0.75	77.3	0.74	14.5
	100All	1.50	61.56	58.0	13.00	5.64	4.89	4.68	0.68	79.7	0.68	12.6
V	**NI**	1.57	191.67	165.9	15.90	8.64	6.61	6.41	1.81	111.5	1.71	29.0
	75All	1.96	151.85	143.6	15.05	7.54	5.58	5.28	1.71	97.0	1.61	27.2
	100All	1.64	148.07	141.1	14.65	7.77	5.66	5.34	1.60	95.0	1.49	26.1
	75PS	1.64	148.76	138.9	14.76	8.08	5.31	5.07	1.68	95.9	1.59	28.0
	100PS	1.68	173.26	155.3	16.28	8.60	6.81	6.66	1.67	115.4	1.56	25.5
PV	**NI**	1.71	131.48	118.6	14.48	6.80	5.05	7.40	1.81	78.0	1.66	26.0
	75All	1.93	158.95	134.5	14.44	7.37	5.28	5.44	1.89	91.9	1.74	30.1
	100All	1.57	151.50	140.2	13.69	6.88	5.28	5.76	1.86	96.3	1.65	28.7
	75PS	1.75	150.90	133.5	16.74	6.90	5.46	5.21	1.84	90.4	1.68	27.6
	100PS	1.86	174.29	152.1	15.04	7.74	6.49	6.41	1.84	101.7	1.68	26.9
	75V	1.54	143.54	133.2	14.23	7.27	5.74	7.33	1.77	90.6	1.63	25.0
	100V	1.57	180.25	149.0	14.85	8.11	7.22	7.24	1.83	104.5	1.68	25.0
	75PS+V	1.64	157.76	140.4	15.42	7.64	6.46	6.64	1.82	97.1	1.65	24.4
	100PS+V	1.86	171.81	154.1	15.22	7.69	7.15	6.71	1.84	102.9	1.67	24.0

TABLE 7 (CONTINUED)

Stage	Irrigation treatment	Bunch no./ shoot	Bunch Mass (g)	Bunch Vol. (cm^3)	Bunch Length (cm)	Bunch Width (cm)	Rachis Mass (g)	Rachis Vol. (cm^3)	Berry Mass (g)	Berry No./ bunch	Berry Vol. (cm^3)	Bunch: Rachis mass
H1	**NI**	1.50	162.27	146.0	15.44	7.60	6.62	5.83	1.79	108.6	1.62	24.5
	75All	1.54	132.59	127.6	14.04	6.83	6.09	4.61	1.67	92.3	1.57	21.8
	100All	1.54	130.76	119.3	14.22	7.12	6.00	5.20	1.83	88.6	1.64	21.8
	75PS	1.61	137.93	123.0	14.93	7.30	5.95	5.41	1.72	95.2	1.53	23.2
	100PS	1.43	150.51	109.0	14.71	7.37	6.54	5.81	1.73	114.7	1.51	23.0
	75V	1.50	136.64	127.4	14.42	7.45	5.80	5.29	1.68	94.4	1.52	23.6
	100V	1.50	172.51	154.8	15.49	8.08	7.25	8.91	1.77	107.8	1.61	23.8
	75PV	1.64	143.36	129.7	14.56	7.20	6.74	5.82	1.65	101.1	1.46	21.3
	100PV	1.57	132.46	125.6	13.99	6.63	6.32	5.36	1.60	92.6	1.43	20.9
	75PS+V	1.68	130.56	118.6	13.77	6.83	5.71	5.32	1.67	94.0	1.50	22.9
	100PS+V	1.64	165.41	150.9	14.96	7.04	7.48	6.68	1.82	101.2	1.66	22.1
	75PS+PV	1.57	136.88	130.6	13.99	7.28	5.97	5.59	1.58	97.0	1.41	22.9
	100PS+PV	1.64	157.51	137.2	14.03	6.70	7.19	6.62	1.71	108.4	1.49	21.9
	75V+PV	1.75	143.08	130.3	13.83	6.78	6.15	5.27	1.67	93.0	1.50	23.3
	100V+PV	1.82	129.40	116.9	14.19	6.71	6.41	5.75	1.58	86.8	1.44	20.2
H2	**NI**	1.79	135.71	124.0	15.77	9.01	6.17	5.73	1.59	99.9	1.43	22.0
	75All	1.64	155.61	140.1	16.34	8.25	7.33	6.27	1.41	103.7	1.38	21.2
	100All	1.43	129.69	117.6	14.57	7.38	5.63	5.01	1.48	96.4	1.45	23.0
	75PS	1.70	112.59	100.7	14.23	7.01	5.32	4.63	1.45	86.2	1.29	21.2
	100PS	1.43	163.20	127.9	15.57	7.61	6.20	5.28	1.52	105.0	1.37	26.3
	75V	1.82	133.06	121.1	16.15	7.49	6.21	5.38	1.49	102.1	1.34	21.4
	100V	1.64	148.63	134.5	16.28	7.85	6.40	6.05	1.55	107.3	1.38	23.2
	75PV	1.57	135.56	120.7	16.02	7.75	6.27	5.74	1.46	108.3	1.30	21.6
	100PV	1.54	108.76	97.6	14.37	7.19	5.74	4.85	1.49	86.4	1.32	18.9
	75PS+V	1.54	117.85	105.0	15.49	7.22	5.41	5.29	1.45	95.7	1.28	21.8
	100PS+V	1.64	126.99	117.7	14.90	7.14	5.72	5.15	1.56	92.2	1.40	22.2
	75PS+PV	1.71	130.99	115.2	15.13	8.05	6.12	5.38	1.45	101.7	1.29	21.4
	100PS+PV	1.68	141.54	126.7	15.63	7.33	6.81	6.43	1.41	99.5	1.36	20.8
	75V+PV	1.64	151.75	135.0	16.47	8.01	6.76	6.10	1.51	113.9	1.36	22.5
	100V+PV	1.29	107.96	97.9	13.78	6.75	5.36	4.72	1.39	87.3	1.24	20.1
H3	**NI**	1.64	107.27	93.6	14.41	6.17	5.20	4.94	1.42	94.3	1.23	20.6
	75All	1.71	115.92	104.7	15.05	7.70	5.62	4.44	1.46	97.2	1.29	20.6
	100All	1.36	124.21	105.5	15.29	6.86	6.15	5.49	1.49	102.6	1.33	20.2
	75PS	1.46	87.32	79.4	12.85	5.90	4.48	3.77	1.32	83.8	1.15	19.5
	100PS	1.79	125.75	113.1	15.48	7.49	6.37	5.40	1.34	105.2	1.18	19.7
	75V	1.64	106.93	96.0	15.14	7.11	5.35	5.18	1.43	94.4	1.26	20.0
	100V	1.71	114.75	105.1	15.29	7.34	5.71	4.85	1.48	98.2	1.29	20.1
	75PV	1.79	105.29	95.3	14.76	7.34	5.54	4.61	1.42	95.0	1.26	19.0
	100PV	1.75	101.89	92.2	13.93	6.78	5.46	4.95	1.40	84.6	1.24	18.6
	75PS+V	1.48	95.88	84.2	14.29	6.50	4.99	4.32	1.26	89.6	1.13	19.2
	100PS+V	1.61	124.18	113.3	14.22	7.20	6.38	5.55	1.45	105.4	1.30	19.5
	75PS+PV	1.86	104.54	94.6	15.02	6.99	5.82	5.58	1.31	86.8	1.16	18.0
	100PS+PV	1.68	110.41	99.8	14.19	6.74	6.25	5.73	1.31	92.5	1.15	17.7
	75V+PV	1.71	112.04	101.4	14.80	7.13	5.75	5.26	1.44	99.7	1.27	19.5
	100V+PV	1.75	116.72	102.1	15.56	7.19	6.24	5.59	1.42	103.7	1.28	18.7
	LSD (p=0.05)	*0.2876*	*27.25*	*27.27*	*1.79*	*1.19*	*1.31*	*2.19*	*0.16*	*21.0*	*0.14*	*1.31*

BS = Berry set; PS = Pea size; V = Véraison; PV = Post-véraison; Trm. av. = Treatment average; 75 = 75% field water capacity irrigation; 100 = 100% field water capacity irrigation; NI = No irrigation; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3; Before irrigation (I) = Means of measurements at berry set

to the diversity of its expression in different organs in response to water stress. The inhibition of phloem unloading, *e.g.* by down-regulation of ATP-ase, sucrose and hexose transporters, and K carriers have also been mentioned as mechanisms for decreased phloem flow during late ripening (Rogiers *et al.*, 2006, and references therein). Over and above the implication that ABA may be involved in triggering berry ripening (Coombe, 1992), it also has been related to the enhancement of sink (grape bunch) strength and the direction of photo-assimilates, impacting on total yield biomass and secondary metabolites, such as anthocyanins (Hunter *et al.*, 1991; Hiratsuka *et al.*, 2001; Quiroga *et al.*, 2009). From a general point of view, it seems logical that gibberellic acid also have a role to play in the ripening process, implicated by the higher seed number associated with a larger berry (Barbagallo *et al.*, 2011). Although addressing general plant metabolism, xylem cytokinins were implicated in nutritional signalling and phloem cytokinins in sink strength regulation (Kamínek *et al.*, 2006). This may have implications for grape berry development and ripening, particularly with regard to the fluctuation in importance of the berry as a sink during the season and late ripening, and the regulation of phloem/xylem mobility of minerals such as calcium and potassium, which can also have a large impact on organic acid salt formation and, from a practical point of view, on the pH of the juice. The latter could also lead to changes in anthocyanin intensity.

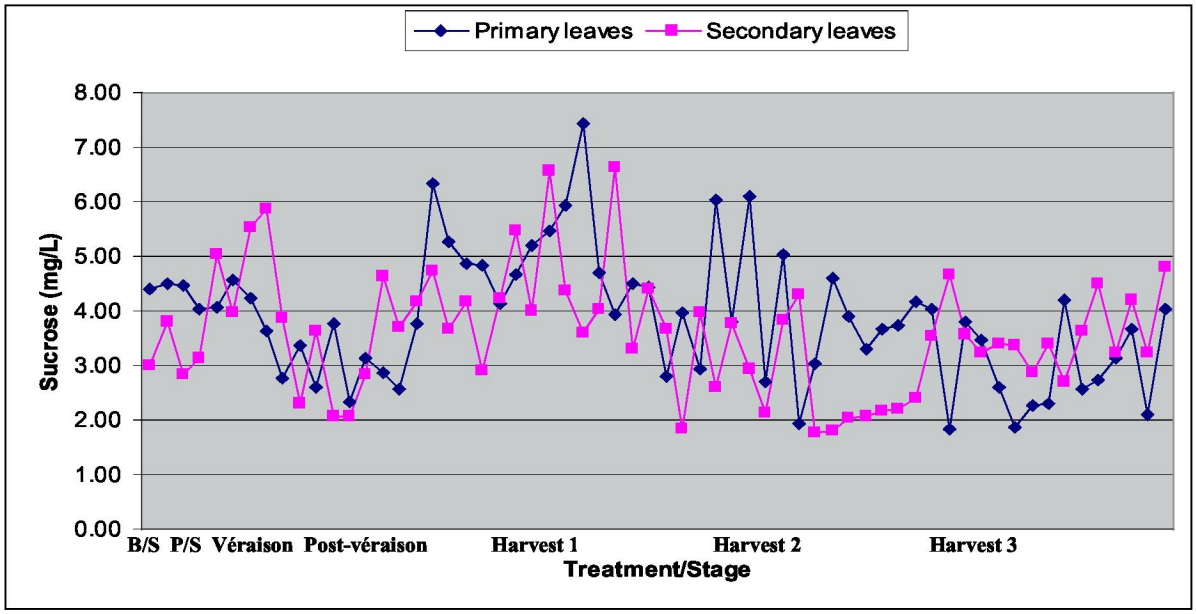

FIGURE 7
Trends in the evolution of sucrose contents of primary and secondary leaves of Shiraz/Richter 99 over stages.

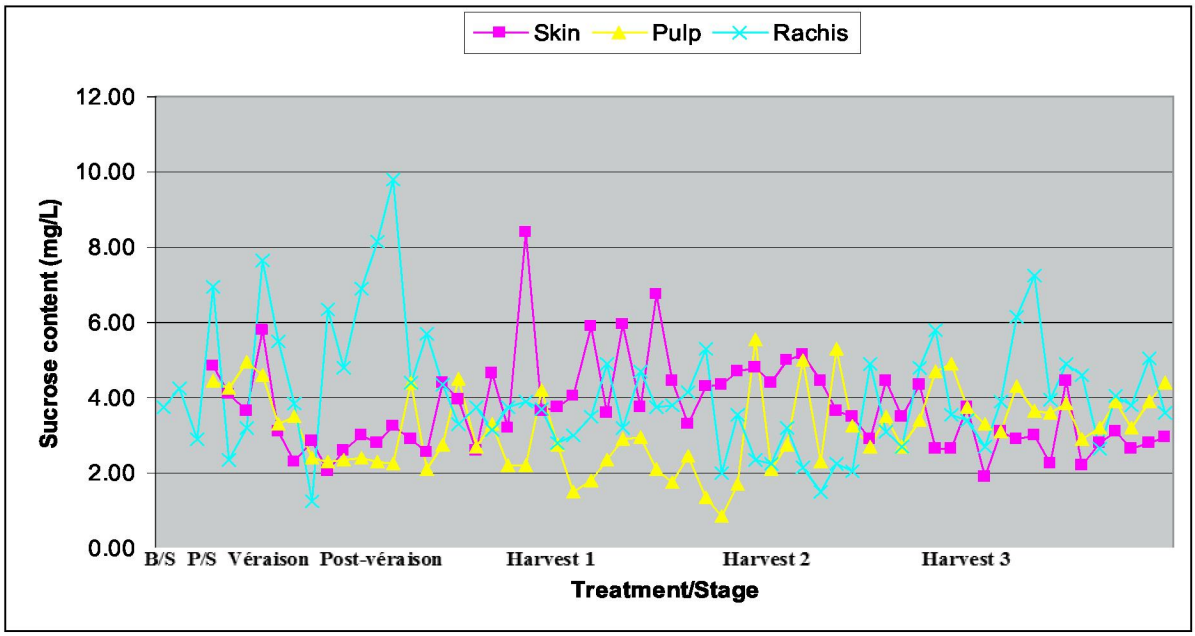

FIGURE 8
Trends in the evolution of sucrose contents of bunch parameters of Shiraz/Richter 99 over stages.

TABLE 8
Effect of level and stage of irrigation on grape must and berry skin contents of Shiraz/Richter 99.

Stage	Irrigation Treatment (stage+level)	Total soluble solids (^{0}B)	Soluble solid content/ berry (g)	Skin water (%)
V	**NI**	12.3	0.22	76.2
	75All stages	10.6	0.18	75.9
	100All stages	11.2	0.18	74.8
	75PS	11.2	0.19	75.2
	100PS	10.6	0.18	76.6
PV	**NI**	19.6	0.35	71.6
	75All stages	18.7	0.35	73.9
	100All stages	18.0	0.33	73.7
	75PS	20.9	0.38	71.0
	100PS	18.3	0.34	72.4
	75V	20.1	0.35	71.8
	100V	19.0	0.35	72.6
	75PS+V	20.0	0.36	70.9
	100PS+V	18.3	0.34	72.1
H1	**NI**	21.6	0.39	70.3
	75All stages	21.3	0.35	70.5
	100All stages	21.0	0.38	71.0
	75PS	23.3	0.40	67.8
	100PS	21.8	0.38	70.1
	75V	22.4	0.38	69.3
	100V	21.7	0.38	70.0
	75PV	23.0	0.38	69.6
	100PV	22.5	0.36	68.2
	75PS+V	22.0	0.37	68.3
	100PS+V	20.2	0.37	71.4
	75PS+PV	22.5	0.35	68.9
	100PS+PV	21.9	0.37	69.8
	75V+PV	22.5	0.38	68.8
	100V+PV	20.5	0.32	70.9
H2	**NI**	25.1	0.40	65.9
	75All stages	24.6	0.35	67.9
	100All stages	24.1	0.36	66.5
	75PS	25.8	0.37	64.9
	100PS	25.5	0.39	66.0
	75V	25.3	0.38	66.0
	100V	24.5	0.38	67.6
	75PV	26.3	0.38	65.2
	100PV	26.6	0.40	66.2
	75PS+V	26.5	0.38	66.0
	100PS+V	22.6	0.35	68.4
	75PS+PV	26.0	0.38	66.2
	100PS+PV	25.6	0.36	66.1
	75V+PV	25.5	0.38	67.2
	100V+PV	24.3	0.34	67.1

TABLE 8 (CONTINUED)

Stage	Irrigation Treatment (stage+level)	Total soluble solids (°B)	Soluble solid content/ berry (g)	Skin water (%)
H3	**NI**	25.7	0.36	62.6
	75All stages	26.6	0.39	64.8
	100All stages	25.7	0.38	65.0
	75PS	28.6	0.38	61.4
	100PS	26.9	0.36	63.3
	75V	28.2	0.40	62.1
	100V	26.5	0.39	63.7
	75PV	28.0	0.40	62.9
	100PV	29.1	0.41	63.1
	75PS+V	26.9	0.34	63.4
	100PS+V	25.2	0.37	64.5
	75PS+PV	27.7	0.36	62.3
	100PS+PV	28.3	0.37	62.5
	75V+PV	28.4	0.41	62.2
	100V+PV	26.2	0.37	64.3
	LSD (p=0.05)	***1.60***	***0.04***	***1.86***

PS = Pea size; V = Véraison; PV = Post-véraison; 75 = 75% field water capacity irrigation; 100 = 100% field water capacity irrigation; NI = No irrigation; Trm. av. = Treatment average; H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3

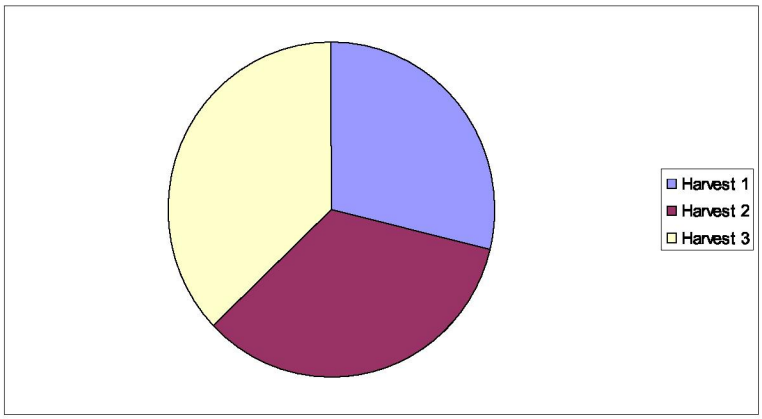

FIGURE 9
General effect (over level / stage of irrigation) on rachis:pulp+skin sucrose ratio of Shiraz/Richter 99 at different harvest stages.

It is well known that metabolic reaction is rather the result of hormonal cross-talk and interaction, *e.g.* between auxin and cytokinin, than individual hormonal action (Kamínek *et al.*, 2006).

No less important in berry water relations is the role of semi-permeable membranes in the mesocarp cells (Tilbrook & Tyerman, 2009; Fontes *et al.*, 2011). Neither transpiration (suggested to be supported by night fluxes; being in accordance with diurnal VPD fluctuations; and a low stomatal density supporting a cuticular pathway of water loss – Greer & Rogiers, 2009), phloem flow or xylem flow (whether partly or fully functional) (Lang & Düring, 1991; Greenspan *et al.*, 1994; Rebucci *et al.*, 1997; Chatelet *et al.*, 2008a, 2008b) seemed to be able to sustain influx during late ripening and to maintain berry turgor. Xylem backflow to the parent vine (Tyerman *et al.*, 2004; Tilbrook & Tyerman, 2009) may have been an accompanying possibility, at least through the central xylem bundles (Düring *et al.*, 1987; Findlay *et al.*, 1987; Lang & Thorpe, 1989). The latter may even have contributed to a better maintenance of the rachis, as found in this study. If the hydraulic status of the parent plant is (largely) recovered and sucrose is building up in the leaves (albeit at a slow rate) during late ripening because of a decrease in active sink demand (Hunter *et al.*, 1994), osmotic gradients would be expected to diminish in the phloem, and it may be argued that control for a cease in flow to the berry may be exerted by such mechanism, despite evidence that the vine is osmotically adjusting by increasing proline

concentrations during this time (Matthews & Anderson, 1988; Esteban *et al.*, 2002; Hilbert *et al.*, 2003).

For Shiraz, a physiological endpoint regarding sucrose demand by the berry seemed to occur during late ripening (Hunter *et al.*, 2004). This was accompanied by a build-up of sucrose in the leaves, and preceded by a phloem-supported sucrose drain from the leaves and a physiological endpoint regarding active leaf function. Demand for sucrose by the rest of the plant, including the berry, seemed to continue during the senescing phase of the canopy, but a point was also clearly reached when active demand by the berry was terminated and reserve build-up was favoured. The build-up of sucrose in the leaves was judged to be the result of a low, but continuing, largely maintenance-orientated photosynthetic rate and a largely inactive sucrose-hydrolysing enzyme pool in the senescing tissue, *i.e.* both leaves and berries (Ruffner *et al.*, 1990; Hunter *et al.*, 1994; Hunter & Ruffner, 2001), which led to a coordinated and regulated metabolic platform that was not supportive of phloem flux of water and sucrose.

Environmental conditions are changing late during ripening, with the senescing canopy having a lower evaporative demand during this time, photosynthetic output and concomitant loss of water by transpiration diminishing, sink demand on the canopy decreasing, sucrose building up in leaves, and the vine generally seeming at least to maintain water relations (Hunter *et al.*, 1994; Hunter & Ruffner, 2001; Hunter *et al.*, 2004). It seems reasonable to assume that these events would lead to a reduction or balancing in the Ψ gradient between the canopy/conduits of the parent plant and that of the berry, and that water flow and concomitant transport of sucrose to the berry would diminish, even under visually normal, intact bunch stem, rachis and pedicel occurrence. Sucrose loading and unloading of phloem-translocated substances are bound to be affected. An osmotic gradient-driven mass transport to the berry (based on the hypothesis of passive phloem transport – Münch, 1930), created by osmotic differences between the vascular tissue and the berry mesocarp (see also Hunter & Ruffner, 2001), may well be diminishing, particularly during late ripening. The Münch hypothesis relates to an influx and efflux of water, with a bulk flow of solution from source to sink that tends to balance solute concentrations through the passive mediation of water. Phloem sap is hydro-dynamically moved longitudinally under the force of pressure from the region of the source to that of the sink, changing its status from locally hyper-osmotic to locally hypo-osmotic, accommodating tube friction and losses on the way; major, active sources and sinks are most likely required for significant velocity (see also Pickard & Abraham-Shrauner, 2009). It is argued that an apoplasmic route of sugar unloading is realised in ripening berries (Patrick, 1997), which may be preceded by a symplasmic route during the green berry phase (Zhang *et al.*, 2006). However, under circumstances of saturated membrane transport (high sucrose concentrations in the free space), both apoplasmic and symplasmic routes may be operative during ripening, simultaneously or sequentially, depending on the conditions (Ho, 1988). The turnover rate of sucrolysis, particularly by the various forms of invertase (neutral, cytosolic, acid), may be critical to sustain the transfer of sucrose from sieve element/companion cell complexes in the berry brush (Hawker, 1985; Ruffner *et al.*, 1990; Zhang *et al.*, 2006). To prevent the dampening of phloem pressure and hence import into the berry, the depletion of apoplasmic sucrose must be offset by the maintenance of apoplasmic osmolarity (Lang & Düring, 1991; Patrick, 1997). The unloading of sucrose into the berry apoplast would raise the osmotic pressure, causing water efflux from the phloem. This, in turn, would release the pressure in the phloem at the unloading site and phloem influx would result. The water released from the phloem would need to be transpired at a rate that would sustain gradients. It can be argued that the lack of strong gradients during late ripening would most likely reduce sucrose loading in sources (in the leaves into the phloem) and downloading at sinks (berries), despite the ostensibly still ample availability of sucrose in the leaves (see also Hunter *et al.*, 2004). Transpiration seemed to outweigh the influx of sucrose during this time, hence the continued shrinking of the berry.

The relationship during early ripening between water influx (primarily *via* the phloem) and water efflux (*via* transpiration) apparently became weaker during late ripening (see also Etchebarne *et al.*, 2007). During the latter period, water flux would seem rather to be physical/non-metabolic (Dreier *et al.*, 2000), with environmental factors playing a larger role in establishing final soluble solid concentration. This does not refrain from the complex regulatory processes involved in berry development, be they physically, fluxomically or metabolomically related. The osmotic potential of the berry is also not determined solely by sugar, but an array of compounds, including amino acids, organic acids, inorganic cations and anions, and other taste and flavour compounds, all contributing to the soluble solid/osmotic status of the berry. Although partial degeneration seems more evident (Krasnow *et al.*, 2008; Fontes *et al.*, 2011), the viability of the internal structure of the berry (mesocarp cell integrity) changes during this time, be it because of compartmentation/membrane breakdown and/or cell death (Dreier *et al.*, 1998; Tilbrook & Tyerman, 2008), hence the decrease in firmness and observed shrivelling (Krasnow *et al.*, 2008). According to Thomas *et al.* (2006), cell membranes (assumed plasmalemma and tonoplast) remain intact post-véraison [the difficulty is that few studies dealing with phloem transport, unloading, berry/cell integrity, etc. pinpoint the berry ripeness level, and it is mostly impossible to determine what exactly véraison/post-véraison/ripening/early ripening/late ripening, etc. mean]. The apparent inability of the berry to maintain hydraulic vitality in distal parts *versus* proximal (brush) parts is evidence of osmotic imbalances and improper cell function (Tyerman *et al.*, 2004). It can be assumed that these changes would be mainly turgor, cell wall matrix and membrane related (Dreier *et al.*, 1998; Goulao & Oliveira, 2008; Tilbrook & Tyerman, 2009; Fontes *et al.*, 2011). According to Nunan *et al.* (1998), no major changes in cell wall polysaccharide composition occurred during the softening of berries, but specific components were modified. Protein composition was largely affected, and the possibility of reinforcement of the cell walls with hydroxyproline-rich glycoproteins to maintain the integrity of mesocarp cells during softening was also mentioned. The pectin polymers in the cell walls consist of

linear polygalacturonan chains (so-called smooth regions) interspersed with branched rhamnogalacturonan chains (so-called hairy regions), some of which are modified by methyl and/or acetyl esterification (Shevchik & Hugouvieux-cotte-pattat, 2003). Although many factors are involved, the softening process is bound to be accompanied by enzyme activity, particularly endo- or exo-hydrolases capable of depolymerising the (1→4)-β-galactan constituents of the pectic polysaccharides and increasing their solubility through degradation (Nunan *et al.*, 1998). Pectin methylesterases catalyse the hydrolysis of methyl-ester groups and control the accessibility of polygalacturonans to polygalacturonases and/or pectate lyases (Barnavon *et al.*, 2000; Glissant *et al.*, 2008). The rate of unloading therefore would be affected by the physiological and structural properties of the unloading pathway, in concert with sink metabolism/compartmentation. It seems evident that a scenario as described above may lead to a mixing of cytoplasm and vacuole contents, which would further affect taste, as well as the structure and flavour compounds of the berry at harvest *via* bonding, co-pigmentation, polymerisation and oxidation reactions. Although specifics are lacking at this stage, this may not necessarily be detrimental to wine quality.

It seems reasonable to assume that the vines in this study were not highly stressed and that ample water was available to sustain demands. The berries seemed to go through phases during the ripening period that resemble a change from a high to a low "sucrose plus water transport":"berry transpiration" ratio (Hunter *et al.*, 2004). Active transport seemed to be followed by passive transport, which finally ceased under the ultimate influence of a diminishing driving force combining lower sucrose supply, lower phloem sucrose concentration, lower sucrose demand, decreasing sucrose metabolism/compartmentation, low water potential gradients, reducing canopy and berry transpiration and changing (decreasing) atmospheric VPD. A continued loss of water from the berry finally led to a reduction in both mass and volume, which may be envisaged to lead to physicochemical changes. Late during ripening, berry size reduction was not sufficient to compensate for the diminishing inflow of sugar to the berry, leading to declining rates of accumulation of soluble solids. It seems evident that the berry water relations are largely independent of those of the parent plant, at least during late ripening (see also Chatelet *et al.*, 2008; Greer & Rogiers, 2009). This is also deductible from the observed patch of dead tissue in the brush region found by Fuentes *et al.* (2010). The water relations of the parent plant seem to be focused on maintenance and recovery requirements, as well as transport to and accumulation in reserve-building areas during this time. This is further accentuated by a passive and even non-existing demand by the berries.

Giving the lack of a clear soluble solid and berry volume response to late-season irrigation, the distribution of recently available sucrose during late ripening may have been re-directed to areas of reserve accumulation. This may have been a physical, largely intra-vine water gradient-facilitated balancing flow of water (and concomitant sucrose flow into the phloem, despite the much reduced photosynthetic activity) that bypassed the berry, excluding the berry mesocarp in re-establishing a largely closed system focused on maintenance metabolism, displaying typical perennial behaviour. Although the use of reserve carbohydrate is not likely under conditions of ample canopy sucrose availability (Candolfi-Vasconcelos & Koblet, 1990; Vasconcelos & Castagnoli, 2000), a contribution to the phloem-located carbon pool for distribution to the berry, especially during late ripening (high ripeness level), should not be ignored and may also interfere with the debated canopy-berry relationships. In any event, the grape berry is (primarily) a sink organ that competes for solute partitioning priority right through the growth season by its (physical and metabolic) ability to attract water and solutes (Van Bel, 1993; Ho, 1988; Minchin *et al.*, 1993; Patrick, 1997; Van Bel *et al.*, 2013), under the influence of (often very demanding and unfavourable) environmental and cultivation conditions.

CONCLUSIONS

The water-holding capacity of the soil and changes in summer rainfall patterns from year to year within the high winter rainfall Mediterranean climate affected the reaction of the vines to treatments, complicating the data set and deductions and, in many cases, exerting an equalising effect. Yet, under the conditions of the terroir (with steep slope and expected deficit-inducing aspect) in which the grapevines were grown, additional water was still required and had a steering effect on physiological, vegetative and reproductive behaviour. Non-uniformity occurred in the duration of organ response and physiological response. Basic trends were in accordance with those found in other studies, whereas new information was obtained on the inter-relationships between the behaviour of the root system, canopy and grapes and changing terroir conditions (as affected by volume and timing of irrigation) during the ripening period.

The physical and compositional changes in the berry during late ripening under field conditions were clarified further. The study provided a further dimension to grapevine water relation effects on physiological behaviour and vegetative and reproductive growth, during grape ripening in particular. The effects of soil water on these multiple, interactive effects largely diminished as ripening proceeded. It is clear that soil water does not exert a direct causal effect, but rather an indirect effect, on grapevine physiological behaviour, steering a concert of processes within a whole plant system, under a strong influence of other environmental factors as the growth season progresses. This also confirms the complicating role that climate change and the expected exacerbated marginal abiotic conditions for grapevine cultivation may play in future interpretations of grapevine behaviour and grape and wine production.

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