Grapevine (Shiraz/Richter 99) water relations during berry ripening

by

Warren Ellis

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Supervisor:

Prof. J.J. Hunter

Co-supervisors:

Prof. P.G. Goussard

Prof. A. Deloire

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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W. Ellis **Date**

SUMMARY

The effect of various irrigation strategies on grapevine water relations during the berry ripening period was investigated in a Shiraz/Richter 99 vineyard. Comparisons between different irrigation strategies (full/seasonal, véraison+post véraison, post véraison and no irrigation) were made.

During the day, the seasonally irrigated vines experienced less water stress than the deficit treatments. Non-irrigated vines seemed to maintain higher diurnal leaf water potentials. Lower leaf water potentials indicated lower water contents in the vegetative and reproductive tissue. Full irrigation seemed to stimulate primary shoot length. Longer water deficit induced earlier and more complete shoot maturation (reserve accumulation). Re-distribution of leaf area on the shoot may occur when vines are subjected to water deficit. Extended water deficit seemed to induce earlier and restricted water loss from vegetative tissue. The water relations were reflected in the berry size. Irrigation during ripening seemed to induce a continuation of berry water loss. Transpiration losses were apparently much higher in fully irrigated vines whereas stomatal control efficiently maintained water relations in non-irrigated vines.

Water deficit seemed to have enhanced the soluble solid accumulation. Irrigation treatments did not seem to affect the titratable acid and pH. The post véraison irrigation in particular seemed to favour a wide window for harvesting. Irrigation at post véraison and especially véraison+post veraison seemed to have a greater effect on the synthesis and extraction of phenolics, anthocyanins and tannins in the berry skins. Different irrigation strategies may affect grapes in such a way that different wine styles are obtained.

OPSOMMING

Die effek van verskillende besproeiingstrategieë op waterverhoudings in die wingerdstok tydens korrelrypwording is in ‘n Shiraz/Richter 99 wingerd ondersoek. Vergelykings is tussen verskillende besproeiingstrategieë (vol/seisoenaal, deurslaan+na-deurslaan, na-deurslaan en sonder besproeiing) gemaak.

Gedurende die dag het die seisoenaal-besproeide stokke minder watertekort simptome getoon as dié in behandelings wat tekort-besproeiing ontvang het. Die stokke wat nie besproei is nie, het oënskynlik hoër daaglikse blaarwaterpotensiaal behou. Laer blaarwater-potensiaal het op laer waterinhoud in die vegetatiewe en reproduktiewe weefsels gedui. Dit het voorgekom asof volbesproeiing hooflootlengte gestimuleer het. ‘n Langer watertekort het vroeër en meer volledige lootrypwording (akkumulasie van reserwes) geïnduseer. Herverspreiding van blaaroppervlak op die lote kan voorkom wanneer die stokke aan ‘n watertekort blootgestel word. ‘n Uitgebreide watertekort het klaarblyklik vroeër en beperkte waterverlies uit vegetatiewe weefsel geïnduseer. Die waterverhoudings is in die korrelgrootte weerspieël. Dit het geblyk dat besproeiing tydens rypwording ‘n voortsetting van waterverlies uit die korrel geïnduseer het. Transpirasieverliese was waarskynlik baie hoër in die volledig besproeide stokke, terwyl huidmondjie-regulering die waterverhoudings in nie-besproeide stokke doeltreffend behou het.

Watertekort het oënskynlik die akkumulasie van oplosbare vastestowwe verbeter. Besproeiingsbehandelings het skynbaar geen invloed op die titreerbare suur en pH gehad nie. Besproeiing ná deurslaan blyk veral gunstig te wees vir ‘n groot venstertydperk vir oes. Deurslaan en veral deurslaan+na-deurslaan besproeiing blyk ‘n groter effek uit te oefen op die sintese en ekstraksie van die fenole, antosianiene en tanniene in die doppe. Verskillende besproeiingstrategieë mag druiwe tot so ‘n mate beïnvloed dat verskillende wynstyle verkry word.

This thesis is dedicated to
the Lord my savior, my parents especially my farther for all his support,
and family being there for me every step of the way

BIOGRAPHICAL SKETCH

Warren Ellis grew up in Stellenbosch and attended Paul Roos Gymnasium where he matriculated in 1998. He then studied Viticulture and Oenology at the Stellenbosch University, South Africa, where he received his BScAgric degree in 2003. He is currently employed at Neil Ellis Wines where he practices wine making.

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PREFACE

This thesis is presented as a compilation of six chapters. Each chapter is introduced separately and is written according to the style of the *South African Journal of Enology and Viticulture*.

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Chapter I

INTRODUCTION AND PROJECT AIMS

INTRODUCTION AND PROJECT AIM

Water is one of the most important environmental factors impacting on growth, yield and grape composition of grapevines and is therefore critical for the quality of wine. Berry size at harvest for especially red grape varieties is considered a very important component of determining wine grape quality all over the world. It is envisaged that smaller berries may deliver wine with more complexity, aroma and colour because of the extractability of the skin. There is an increasing need to manipulate berry size in the vineyards and to obtain optimum levels of ripeness regarding soluble solids, pH, titratable acid and phenolic compounds to produce wine of high quality. The availability of water during certain periods of berry growth is known to cause changes in grape composition and berry size. These changes include an increase in berry size, and dilution of berry flavour compounds, sugars, and organic acids, and can cause a decrease in tannins and anthocyanins. Water deficit management of vineyards has therefore received much attention, the consequences of which have not been fully elucidated.

Evidence suggests that with regulated water deficit treatment during different periods of berry ripening, different levels of soluble solids, acidity, pH, and phenolics may be achieved. Crucial periods of water deficit are of the utmost importance. Determining critical periods during which berry growth is water sensitive, and understanding the contribution of plant water status in grape composition, may also contribute to obtaining different levels of ripeness for the production of different styles of wines.

The aim of the study was to determine the effect of varying vine water status on vegetative and reproductive growth as well as grape composition. The impact of vine water status in the duration of the ripening period and in grape composition, as related to the identification of different ripeness levels, was envisaged.

Chapter II

LITERATURE REVIEW

GENERAL EFFECT OF IRRIGATION ON VEGETATIVE AND REPRODUCTIVE GROWTH AND GRAPE COMPOSITION

1. VEGETATIVE GROWTH

Water stress has inhibitory effects on vegetative and reproductive growth and alters the phenology (Coombe & Dry, 1988). According to Van Zyl (1984), the number of young roots formed was reduced with moderate water deficit, with 50% plant available water in the soil, and with 25% plant available water. Reid & Wample (1985) found that the root system responds to drought by reducing growth of all organs, although a larger root system can increase their ability to collect water as a trade-off between shoot and root growth.

Van Zyl (1981) noted reduced shoot growth during early growth stages and argued that it might be an indication of water stress. According to Myburgh (1998), insufficient irrigation that induces severe water stress, may result in poor vegetative growth. Bravdo (2000) found that regulated water deficit at the early growth phase of vegetative growth could be used to reduce vigour and moderate water deficit after véraison could inhibit further vegetative growth. Active shoot growth may continue through the whole season in the presence of adequate water (Van Zyl, 1981). Inadequate water may reduce the length of the growth season, induce premature leaf fall, and decrease leaf size (Fanizza & Ricciardi, 1990), thus reducing active leaf area (Van Zyl, 1981). It may also lead to various other negative effects, such as a premature reduction in shoot growth (Van Zyl, 1981) and inadequate ripening of shoots and bunches (Bravdo *et al*., 1972; Van Zyl, 1981; Miller *et al*., 1996b).

Plant productivity, measured as the mass of dry matter produced, depends directly on leaf surface and photosynthetic activity (Bravdo *et al*., 1972; Miller *et al*., 1996b). In water stressed vines, photosynthetic activity is reduced because of stomatal closure (Dűring, 1990; Schultz, 1996). Water stressed plants with lower photo-synthesis, together with the reduced leaf area, result in lower productivity compared to vines not subjected to water deficit (Gomez-del-Campo *et al*., 2002). According to Mullins *et al*. (1992), grape bunches become the second strongest carbohydrate sink after véraison. Vigorous vines can often actively continue producing leaf area after véraison (Miller *et al*., 1996a). According to Hunter & Visser (1988), the apical, middle and basal leaves, translocate their photosynthetates mainly to the bunches from just after berry set up to véraison. After véraison, this pattern continues, whereas before and at harvest carbohydrates are again redistributed in the canopy.

2. REPRODUCTIVE GROWTH

According to Hardie & Considine (1976), Van Zyl (1984) and Sipiora & Gutiérrez-Granda (1998), the supply of water to the grapevine is an environmental factor affecting the berry size.

Ojeda *et al.* (2002) studied the effect of three different deficit treatments in different stages of berry ripening on the composition of Shiraz grapes. The treatments consisted of a strong deficit between anthesis and véraison, medium deficit between anthesis and véraison, and a late strong deficit between véraison and maturity. Their study showed that berry mass decreased substantially as a result of water deficit (Fig. 1). For water deficit treatments applied during the period between anthesis and véraison, the size reduction of the berries was greater than that of the late water deficit treatments applied between véraison and maturity. This indicates insensitivity of grape berries to water deficit during the ripening period. In all the treatments, water deficit reduced pulp mass, which paralleled whole berry mass. The skin mass was only affected when water deficit was applied between flowering and véraison. Intensive dehydration applied between véraison and maturity did not modify the skin mass (Fig. 2). The mass ratio of skin:pulp increased with the timing and intensity of water deficit, the strong deficit treatment applied in the period between anthesis and véraison leading to much higher values than the other treatments.

McCarthy (2000) concluded that berry mass was most sensitive to water stress during the post-flowering period. The absence of a consistent correlation between berry growth and soil water deficit indicated a reduced sensitivity of berries to water stress towards the post-flowering period. Post-flowering deficit reduced vegetative growth in some seasons and this may result in a greater proportion of older leaves with a reduced photosynthetic capacity during the ripening period (McCarthy, 2000).

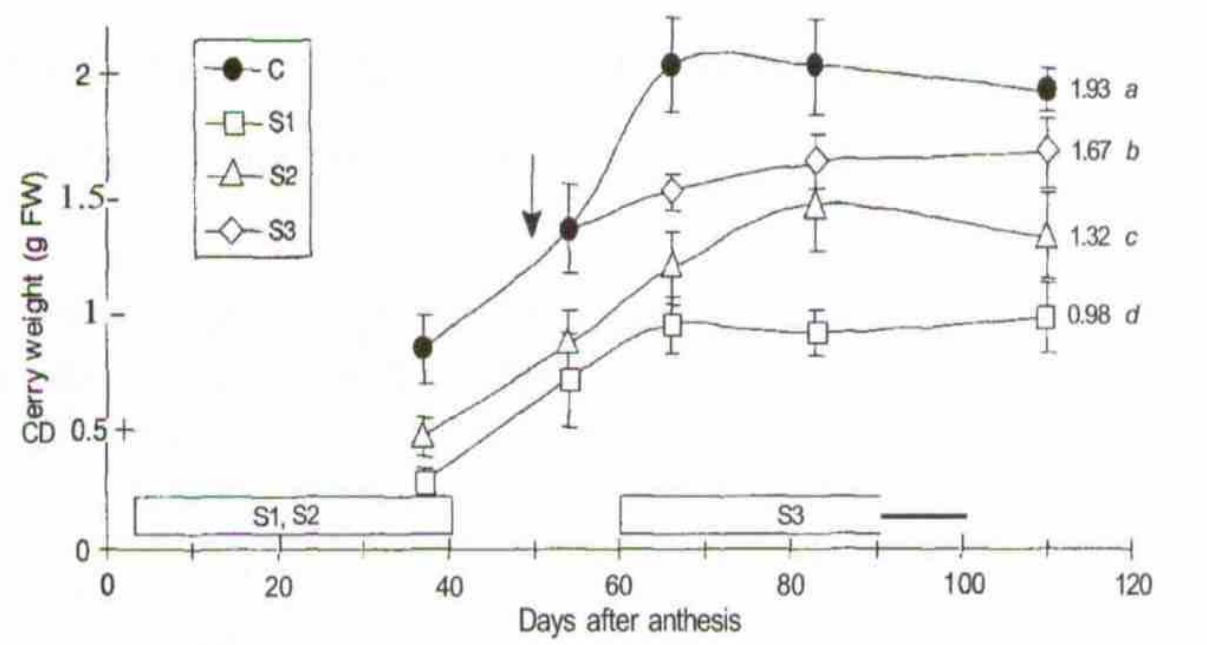

Fig. 1. Changes in fresh weight (FW) (g) of Shiraz berries subjected to water deficit treatments as a function of number of days after anthesis. C=control; S1=strong; S2=medium levels of early water deficit between anthesis and véraison; S3=strong late water deficit between véraison and harvest maturity. Arrow indicates onset of véraison. Vertical bars indicate standard deviation (n=6). Values followed by the same letter are not significantly different (p <0.05) (Ojeda *et al.,* 2002).

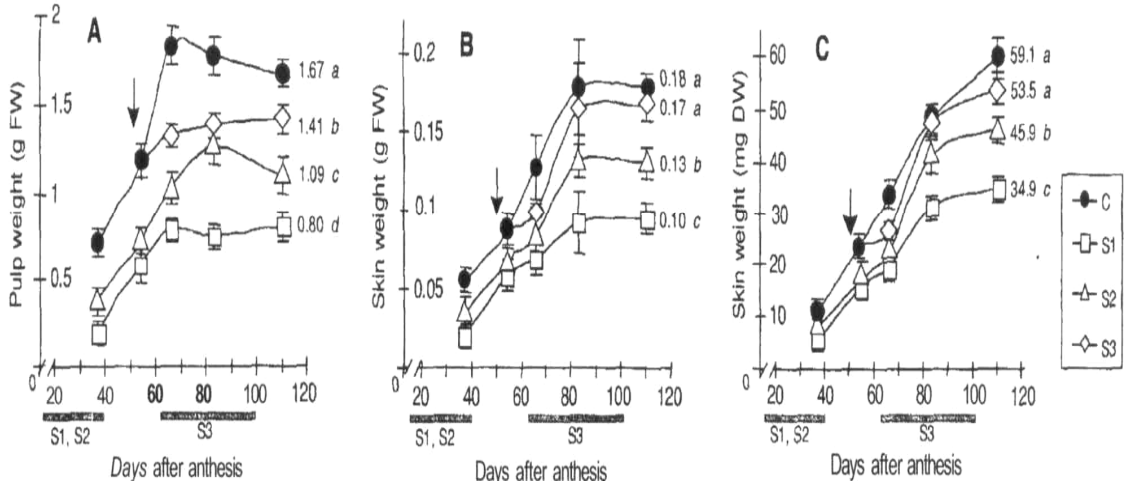

Fig. 2. Changes in seedless pulp fresh weight (FW) (A), skin fresh weight (B), and skin dry weight (DW) (C) of Shiraz berries subjected to water deficit treatments *as a* function of number of days after anthesis. C=control; S1=strong; S2=medium levels of early water deficit between anthesis and véraison; S3=strong late water deficit between véraison and harvest maturity. Arrow indicates onset of véraison. Vertical bars indicate standard deviation (n=10). Values followed by the same letter are not significantly different *(p* <0.05) (Ojeda *et al.,* 2002).

In contrast, Roby & Matthews (2004) found that low vine water status during the post véraison period, inhibited berry growth, but no differences in berry fresh mass occurred between high water status vines and the control. Skin mass was positively correlated with berry mass in all treatments. The berries of each treatment were classified in six categories from 0,5 g/berry to 1,5 g/berry. They observed that for the low water status vines, the three intermediate categories, where the majority of the berries occurred, had the largest berry skin mass. The skin mass of the low water status vines was up to 25% more, compared to that of the high water status vines and the control. The skin mass of the larger berries did not differ between the treatments. Most berry sizes for the low water status vines had slightly more skin mass (g/berry) and considerably more relative skin mass (% berry fresh weight) than the control and high water status berries. Although water deficits may be a possible reason for stimulated post véraison skin growth, it is more likely that expansive growth of the inner mesocarp was more inhibited by water deficits than the skin tissue itself.

Small berries are considered a key component of grape quality (Bravdo *et al*., 1985; McCarthy, 2000; Kennedy *et al*., 2002) for red cultivars such as *Vitis vinifera* L. cv. Shiraz (McCarthy, 2000). Vigour-reducing rootstocks, micro-irrigation, canopy manipulation by means of different trellis systems, as well as other management practices are not sufficient to increase grape quality.

3. BERRY COMPOSITION

Soil water status may lead to leaves and bunches developing in different conditions, varying from heavily shaded to exposed canopies. According to Hasselgrove *et al*. (2000), bunches developing in well-exposed canopies, as opposed to those developing in heavily shaded canopies, have smaller berries, higher must soluble solid concentration, lower pH, higher titratable acidity and less incidence of unripe flavours. By reducing berry size, bunches would be less compact. A more open framework would expose a greater surface area of such berries to sunlight. Higher sunlight levels within and around the bunch may improve the colour of grape berries (Smart, 1982).

The composition of phenolics depends on the cultivar, and is influenced by viticultural and environmental factors (Brossaud *et al*., 1999). Phenolic compounds are mainly

localised in the skin and seeds of the grape berry (Ojeda *et al*., 2002). In the case of red grape varieties, the skin is particularly rich in flavonols and anthocyanins. The phenolic concentration of the must is indirectly affected by the final size of the grape berry, in that this concentration depends on the skin surface-to-berry volume ratio (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004).

3.1. Soluble solids

The grape berry has a double sigmoid growth curve (Hunter, 1991; Coombe, 1992b). According to Coombe & Dry (1988), berry fruit development has two cycles: the first takes the berries to the hard, green, slow growing phase; berry ripening occurs during the second cycle, beginning at véraison. The ripening stage at véraison is associated with cell enlargement, a change in berry colour, berry softening, and sugar accumulation (Coombe & Dry, 1988; Hunter, 1991), with a decrease in acidity and astringency, loss in chlorophyll, and an increase in aroma (Hunter, 1991).

According to Coombe (1992), grapes begin to accumulate sugar from the moment of berry softening. Wang *et al.* (2003) found that the sugar concentration of the berries was not modified by water stress during the early stages of the second phase of berry growth. The size of the berries of the water-stressed and irrigated vines was different. During the later stage of the second phase of berry growth the sugar concentration of the two treatments was significantly different, being higher in the normally watered vines than in the water stressed vines.

According to Ojeda *et al.* (2002) and Castellarin *et al.* (2005), no significant differences were found in the final sugar concentration between irrigated and deficit treatments. The total soluble solids per berry were proportional to berry size as indicated by berry mass (Ojeda *et al.*, 2002). Roby *et al*. (2004) stated that the total soluble solids per berry increased linearly with berry size and the concentration of soluble solids in each berry was also dependent on size. According to Matthews & Anderson (1988), the amount of sugar was greater in continually irrigated vines than in the water stressed vines. Ginestar *et al*. (1998) also noted that the berry sugar in water stressed treatments was lower than in watered treatments. Hardie & Considine (1976) found a reduction in total sugar accumulation in the berries of stressed vines and grape ripening was delayed. On the other hand, Morris & Cawthon (1982) noted

that excess water normally reduces sugar, but with moderate irrigation, during dry years, it is increased.

According to Wang *et al.* (2003), non-stressed grapevines had a higher sugar-unloading rate than the water stressed grapevines during ripening. This was the reason why sugar concentration in the water stressed grape berries was lower than that of the control berries, in relation with the dynamics of photosynthesis, which depend on the vine water status. Thus when water stress is regularly or continually applied throughout the ripening period of the berries, the accumulation of sugar at maturity is also affected in a manner that is independent of berry volume, when the berry volume decreases during ripening due to water loss. Concentration has a more important effect than accumulation (through sugar unloading) on the final sugar level of the berries.

3.2. Titratable acidity and pH

The organic acid content of grape berries consists mainly of tartaric, malic and citric acids and can be measured by titration and expressed as total titratable acids (Ribéreau-Gayon *et al.*, 1998). Acid is a very important quality factor. Wine with too much acid is tart in taste, whereas wine with low acid levels may produce a bland taste. High pH levels increase the probability of microorganism activity; it also has a negative effect on the colour intensity of red wines and the aging ability of the wine (Ribéreau-Gayon *et al.,* 1998).

Increased water availability often causes an increase in the potassium and pH levels in the berry and wine (Freeman & Kliewer, 1983). The presence of potassium in the berries and wine appears to be linked to pH and acidity (Boulton, 1980; Freeman & Kliewer, 1983). Musts with a high potassium concentration tend to have high pH and malate. According to Hunter *et al*. (1991) and Hunter & Ruffner (2001), berries reach the highest malic and tartaric concentrations at pea size. From véraison to ripeness malic acid decreased (Iland & Coombe, 1988; Hunter, 1991; Hunter *et al*., 1991; Coombe, 1992) due to malic acid metabolism during ripening (Iland & Coombe, 1988). The tartaric acid content in the berries changed very little from véraison to ripening (Iland & Coombe, 1988; Hunter, 1991; Hunter *et al*., 1991; Coombe, 1992). Smart & Coombe (1983) noted that excessive irrigation slows ripening, increases yield partially by berry enlargement, and elevates must pH and acidity from shading

due to excessive shoot growth. An increase in shading within the canopy was especially associated with an increase in the must malic acid content (Coombe, 1987; Archer, 1988; Smart *et al*., 1988; Archer & Strauss, 1989) and a decrease in tartaric acid (Smart *et al*., 1985; Archer, 1988; Archer & Strauss, 1989). Water stress enhances early ripening but reduces yield, berry mass and malic acid due to excessive exposure (Smart & Coombe, 1983; Iland & Coombe, 1992). According to Ginestar *et al*. (1998), increased bunch exposure may lead to an increase in berry temperature causing an increase in respiration of malic acid, leading to higher pH values.

Excessive amounts of potassium in the berries are mostly because of excessive amounts of soil moisture and the availability of potassium in the soil. According to Gladstones (1992), the effects of irrigation or excessive soil moisture on must and wine pH are primarily because of impaired canopy light conditions and thus accumulation of potassium. The lower acidity in the irrigated vines occurred because of the increase of berry size that contributed to the reduction in total acid concentrations. Authors like Mullins *et al.* (1992) suggested that the decrease in tartaric acid concentration could be due to dilution resulting from the increase in berry size. Yuste *et al.* (2004) noted higher pH values in the water stressed vines. The high pH values in the water stressed vines should be related to potassium accumulation in the berries, since potassium concentration is one of the most critical factors linked to must pH (Boulton, 1980; Jackson & Lombard, 1993). According to Boulton (1980), the reduction in photosynthetic activity of the leaves is related to potassium transport from the leaves towards the berries. Thus water stress and the decrease in photosynthetic activity could have caused a higher potassium accumulation in the berries and also a higher pH (Yuste *et al*., 2004).

3.3. Phenolics

Phenolic compounds play an important role in the flavour of red wines (Ribéreau-Gayon *et al.*, 1998). Phenolic compounds are responsible for positive tasting characteristics, but are also responsible for unpleasant negative characteristics (Ribéreau-Gayon *et al.*, 1998). In red wines, body, backbone, structure, fullness and roundness are quality characteristics. Negative aspects such as bitterness, roughness, harshness, astringency and thinness should be avoided, as they are incompatible with quality. The overall organoleptic impression is based on a

harmonious balance between these two groups of sensations. These sensations are directly related to the type and concentration of the various molecules, such as phenolics and especially tannins (Ribéreau-Gayon *et al.*, 1998). Phenolic compounds are mainly localized in the skin and seeds of the grape berry (Ojeda *et al*., 2002). In the case of red grape varieties, the skin is particularly rich in flavonols and anthocyanins. Since phenolic concentration depends on the skin surface:berry volume ratio the final size of the berry affects the phenolic concentration (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004).

Previous work on water deficit treated vines done by Ojeda (1999), showed that the pericarp cellular volume, independently of period and intensity of water deficit, causes berry size and mass reduction. Cell multiplication and indirectly cell numbers per berry pericarp were not affected. The biosynthesis of phenolic compounds may be followed by their content expressed in terms of the skin mass per single berry (concentration of phenolics was expressed in mg/g of fresh skin mass). The results indicated that the phenol content of the berries was dependent on total skin mass, which was affected by water deficit, primarily when it was applied during the green growth stages of the berry, from anthesis to véraison.

3.4. Tannins

By definition, tannins are substances capable of producing stable combinations with proteins and other polymers, such as polysaccharides. The complex polymers in grapes and wines are condensed tannins (Ribéreau-Gayon *et al.,* 1998). Tannins are structurally diverse, resulting from the number of hydroxyl groups, their position on the aromatic nuclei, the stereochemistry of the asymmetrical carbons, as well as the number and type of bonds between the basic units. This diversity explains the existence of tannins with different properties in various types of grapes and wine (Ribéreau-Gayon *et al.,* 1998).

The type and concentration of tannins may produce a soft, balanced impression or, on the other hand, certain aggressiveness that is either perceived as bitterness at the end of the palate or as astringency on the aftertaste. The tannin balance of red wine results from the good harmonization of tannins from seed and skin origin. Tannins from the seeds give the wine structure and body, while tannins from the skin provide

fullness, roundness and colour. There is a high risk of excessive astringency if seed tannins dominate, while too much extract from the skins can cause bitterness and an herbaceous character, especially if the grapes are insufficiently ripe (Ribéreau-Gayon *et al*., 1998).

According to Ojeda *et al.* (2002), all the deficit treatments had higher flavonol concentration than that of irrigated vines. With medium levels of early water deficit between anthesis and véraison and strong deficits between véraison and harvest maturity, the biosynthesis of flavonols (expressed in mg per skin mass of individual berries) increased to a greater extent compared to the regularly irrigated vines and strong deficit between anthesis and véraison. They also found that for both the well irrigated and deficit treatments the concentration of the total tannins decreased 37 days after anthesis and stabilized later during the season (Fig. 3A). The total tannin content expressed per skin mass of a single berry was reduced by the two early season deficit treatments. This reduction in biosynthesis correlated with the severity of dehydration as indicated by berry mass loss (Fig. 3B).

Water deficit between anthesis and véraison resulted in an inhibition of the phenolic biosynthesis for the total tannins. Medium water deficit between anthesis and véraison and strong deficit between véraison and harvest maturity increased the biosynthesis of tannins. The results indicated the potential impact of both skin mass and berry size on fruit composition and the quality of wine.

According to Roby *et al.* (2004), the concentration of skin tannin was unchanged with berry size. Tannins in the skin of berries, with the same size, which had been exposed to different irrigation treatments, were measured. The skin tannins were higher in the low irrigation than in the high irrigation treatment for all, except the largest berries. Thus the concentration of the tannins increased by an effect of vine water status, which was independent of the role of water status on the size of the berry, because all the berries in comparison were of similar size. Kennedy *et al.* (2002) concluded that there were no significant differences in the final skin tannin in the presence of large differences in vine water status (Fig. 4). This suggests that the potential for water deficit to alter skin tannins is limited.

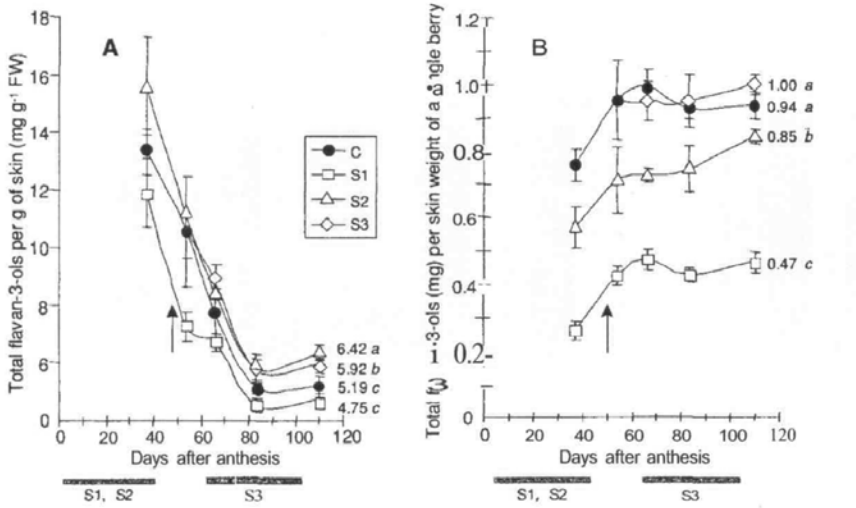

Fig. 3. Total flavan-3-ol content, expressed in mg catechin equivalent: (A) per g of fresh skin (FW); (B) mg per skin weight of a single berry, subjected to water deficit treatments as a function of the number of days after anthesis. C=control; S1=strong; S2=medium levels of early water deficit between anthesis and véraison; S3=strong late water deficit between véraison and harvest maturity. Continuous arrow indicates onset of véraison. Vertical bars indicate standard deviation (n=6). Values followed by the same letter are not significantly different *(p* <0.05) (Ojeda *et al.,* 2002).

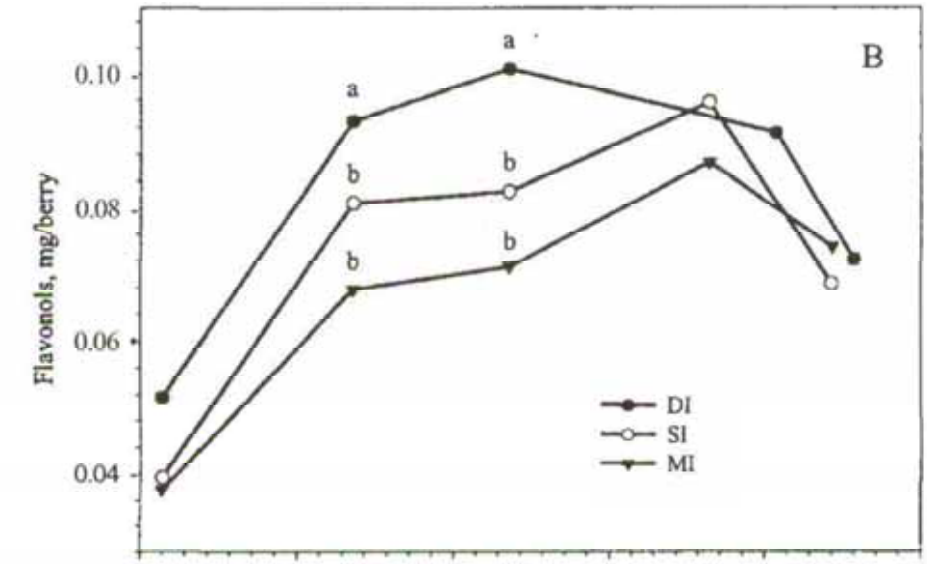

Fig. 4. Tannins expressed as flavonols and Flavan-3-ol monomers (B) amounts during fruit ripening for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at *p* = 0.05 (Kennedy *et al.*, 2002).

3.5. Anthocyanins

Anthocyanins are the red pigments located mainly in the skin of grapes and are located in the vacuoles of the skin cells (Ribéreau-Gayon *et al.,* 1998). The majority of these pigments combine and condense with tannins in wine to form another, more stable class of colour molecules. These combined complexes of anthocyanins are responsible for the colour in wine (Ribéreau-Gayon *et al.,* 1998). To obtain the optimum levels of anthocyanins is very important in the making of red wine. Irrigation, at different stages of irrigation on vine growth, is known to have an influence on the anthocyanins and knowing when these crucial times are, one can manipulate the water status of the vine in order to achieve the maximum amount of anthocyanins needed for the preparation of a specific style of wine.

Irrigation can have similar effects than rainfall on the ripening of berries, as well as the ripeness level of the grapes. Rainfall often delays ripening and affects the composition of the grapes. Kennedy *et al.* (2002) concluded that differences in vine water status were associated with differences in skin flavonoid composition in fruit. The anthocyanin concentration for the minimal irrigated vines was significantly higher than that of the other treatments on a concentration basis, but there were no differences on a per berry basis. A study conducted by Ginestar *et al.* (1998) also showed an increase in anthocyanins on a mass basis, but lower values on a per berry basis with water deficit. Water deficit treatments also showed an increased phenolic concentration of the juice and extracted phenols and anthocyanins from the skins (Matthews *et al*., 1986).

Irrigation at different stages of berry growth is known to have different effects on anthocyanin development. Ginestar *et al.* (1998) stated that water deficit during the period between anthesis and véraison resulted in the greatest reduction in berry mass for Shiraz compared to that of well irrigated vines. Matthews & Anderson (1989) and Van Zyl (1984) also stated that berry growth is more sensitive to water deficits before véraison. This resulted in an increase in the concentration of anthocyanins and total phenolics. Freeman & Kliewer (1983) noted that skin anthocyanin concentration in non-irrigated vines was higher (Freeman & Kliewer, 1983; Yuste *et al*., 2004) than that of irrigated vines when compared at the same soluble solid level or a given date.

In contrast, Ojeda *et al.* (2002) found that for all their treatments, the anthocyanin concentration increased a few days after véraison (Fig. 5A). The berries of treatments with water deficit between véraison and final harvest had increased biosynthesis of anthocyanins, whereas strong deficit treatment from anthesis to véraison significantly inhibited this biosynthesis (Fig. 5A). Higher anthocyanin biosynthesis and concentration for water deficit treatment between véraison and maturity occurred.

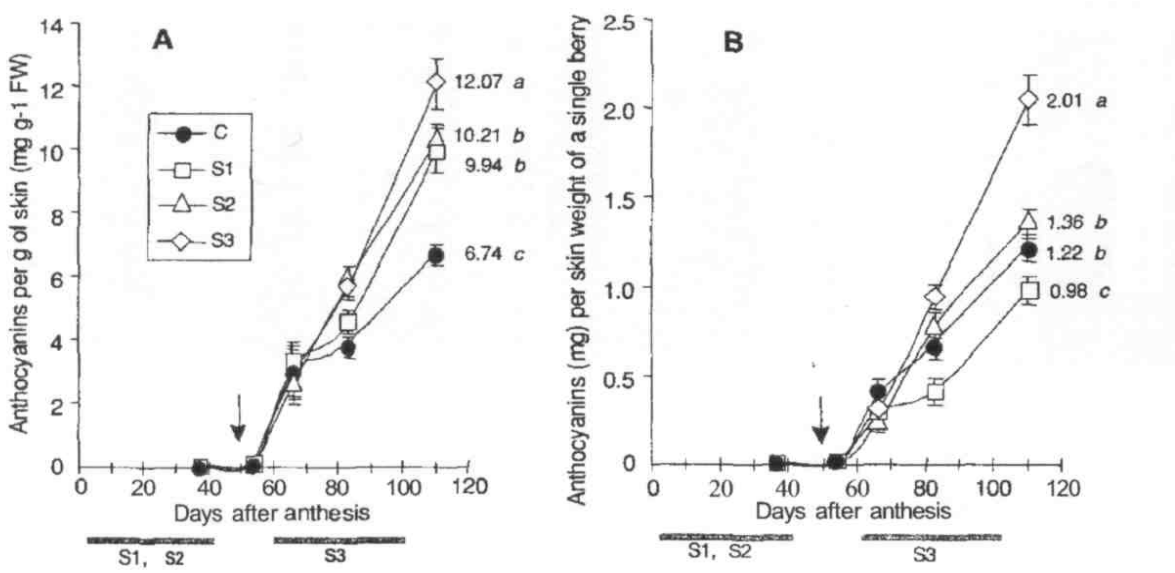

Fig. 5. Anthocyanin content, expressed in mg malvidin equivalent: (A) per g of fresh skin (FW); (B) mg per skin weight of single berry, of Shiraz berries subjected to water deficit treatments as a function of number of days after anthesis. C=control; S1 =strong; S2=medium levels of early water deficit between anthesis and véraison; S3= strong late water deficit between véraison and harvest maturity. Arrow indicates onset of véraison. Vertical bars indicate standard deviation (n=6) (Ojeda *et al.,* 2002).

According to Freeman & Kliewer (1983) and Hardie & Considine (1976), wine colour was reduced due to irrigation, by reducing the proportion of pigments in the coloured form. This was due to an increase in pH, which was associated with larger berries as a result of irrigation. However, Hardie & Considine (1976) noted that a decrease in colour had also been observed where yields have been increased by irrigation. These differences were related to greater skin area to volume ratio of small, non-irrigated berries.

4. CONCLUSIONS

There appears to be strong evidence that water availability can affect vegetative and reproductive growth as well as grape and must composition and thus wine quality. Soils saturated with water causes, with unrestricted, vigorous growth, increased berry size and reduced wine quality (Coggan, 2002). With excessive vegetative growth, canopy management practices are important to create and maintain an optimum canopy. Unrestricted growth reduces grape quality due to shade, which causes secondary effects such as low light intensity reaching the berries.

It is important to realise that water stress may have the same negative effects than over-irrigation. From existing literature it is still difficult to deduct when to apply deficit irrigation during different stages of berry growth to produce grapes and wine with the required quality. Different times of irrigation may increase or decrease grape composition. However, existing evidence suggests that deficit irrigation between anthesis and véraison may reduce berry size and may favour the obtainment of a lower pH, and higher sugar, tannins, anthocyanins, and degree of polymerization of tannins. Excess irrigation between véraison and maturity must be avoided.

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Chapter III

RESEARCH RESULTS

WATER STATUS OF VEGETATIVE AND REPRODUCTIVE TISSUE OF SHIRAZ/RICHTER 99 GRAPEVINES DURING BERRY RIPENING

ABSTRACT

In this study, grapevine water relations during the berry-ripening period, under the influence of various irrigation strategies, were investigated in an attempt to quantify the water status of vegetative and reproductive tissue, in a Shiraz/Richter 99 vineyard. Comparisons based on water status of vegetative and reproductive tissue during ripening were made between different irrigation strategies (no irrigation; and irrigation at all phenological stages; at véraison and post véraison; and at post véraison). During the day, vines of the full irrigation treatment experienced less water deficit than the other treatments, with the non-irrigated and post véraison irrigated vines generally experiencing higher water deficit. The water potential of irrigated vines seemed to remain more constant than that of the water deficit vines. Non-irrigated vines seemed to maintain higher diurnal leaf water potentials, compared to the véraison+post véraison and post véraison irrigated vines. Lower leaf water potentials indicated lower water contents in the vegetative and reproductive tissue. Higher water contents were observed in the basal parts of the primary shoots, in the primary leaves and secondary shoots in this region, with the apical parts having the lowest water contents. The water relations were reflected in the berry size. Transpiration losses were probably much higher in fully irrigated vines, whereas stomatal control efficiently maintained water relations in non-irrigated vines.

INTRODUCTION

According to Smart (1974), water stress effects on the grapevine involve reactions at intercellular, cellular and tissue level. A decrease in stomatal opening is one of the most significant responses, which enable the plant to alleviate unfavourable conditions of water status and environmental stress, but reduces the uptake of CO_2 and hence photosynthesis.

Grapevine water status depends on the degree of imbalance between water uptake and loss through transpiration (Smart, 1974; Smart & Barrs, 1974). Soil water availability and root distribution determine the rate of water uptake. Transpiration depends on availability of energy to vaporize water and the resistance to vapour and liquid in the soil-plant-atmosphere system. There is thus a close relationship between the plant water status, evaporative demand from the environment and soil water availability.

According to Naor & Wample (1994), water stress decreases the stomatal conductance and photosynthetic rate of grapevine leaves, despite an apparent osmotic adjustment of the stressed leaves. Lopes *et al*. (2005) noted that with time, the soil water was exhausted in the non-irrigated grapevines, as indicated by their lower pre-dawn leaf water potential. Small differences were found in diurnal water potential values between the non-irrigated grapevines and irrigated grapevines. The similar diurnal plant water status between non-irrigated grapevines and irrigated grapevines is due to the efficient control of water loss by reduced stomatal conductance. As water stress intensifies, stomata close early in the morning, preventing an excessive drop in leaf water potential (Naor & Wample, 1994; Correira *et al*., 1995; Lopes *et al*., 2005).

This experiment was conducted to determine the water relations of vegetative and reproductive tissue during the ripening period under the influence of various irrigation strategies.

MATERIALS AND METHODS

Experimental vineyard

A seven-year-old *Vitis vinifera* L. cv. Shiraz (clone SH1A), grafted onto Richter 99 (*Vitis Berlandieri x Vitis rupestris*) (clone RY2A) was used for this study. The experimental vineyard is situated on the Experiment farm of ARC Infruitec-Nietvoorbij in the Stellenbosch Region, Western Cape. The area is characterised by a Mediterranean climate. The vines are spaced 2.75 m x 1.5 m on a Glenrosa soil with a western aspect (26º slope) and orientated in a North-South direction. The vines are trained onto a 7-wire lengthened Perold trellising system (VSP) of which three sets of wires are movable. Vines were pruned to two-bud spurs with a spur spacing of approximately 15 cm. Canopies were suckered, shoot positioned and tipped/topped during the pre-véraison period. Irrigation was applied through a micro-sprinkler system.

Treatments and layout

Four treatments, comprising irrigation combinations to field water capacity at different stages, were applied (field water capacity of the soil was determined before the start of the experiment). The treatments were completely randomised in two blocks,

representing two replications. Thirty vines were used per replication. The treatments were: (i) seasonal irrigation from berry set with further irrigation at pea size, (ii) véraison and one month post véraison, (iii) irrigation at véraison with further irrigation at post véraison, (iv) irrigation at post véraison and (v) no irrigation. The sampling of the treatments was split into five stages: véraison, one month after véraison, and three times during the ripening period.

Measurements

Vegetative parameters: Five randomly selected shoots from the thirty vines per replicate were used for each treatment and replicate at each ripening stage. The primary shoots were divided into three categories: basal, middle and apical. The measurements of the primary and secondary leaves, shoots, and petioles of the primary leaves, were taken in these three parts. The roots were sampled randomly from a 0.027 m^3 (30X30X30 cm) soil profile, 20 cm from the grapevine trunk. Mass (g) of the roots, primary and secondary leaves, shoots, and petioles of primary leaves was measured. Water content [as mass (g) and percentage] of the roots, shoots, leaves and petioles was determined by drying the tissue for 72h in an oven at 70°C and using the formula: (fresh mass)-(dry mass)/(fresh mass) X 100. The percentage water distribution throughout the season was also determined [(total water in tissue)/(sum of the total amount of water in all the tissues)] X 100.

Reproductive parameters: Bunches were sampled from the five randomly selected shoots. The mass (g) of 50 randomly selected berries was measured for each treatment and replicate. The skin, pulp, and seeds were separated and the mass (g) determined. Water content (%) of the skin, pulp, and seeds was determined by the same formula described above, after drying the tissue in an oven at 70°C. The percentage water distribution throughout the season was also determined [(total water in tissue)/(sum of the total amount of water in all the tissues)] X 100.

Water potential measurements: Leaf water potential was measured throughout the season at each sampling date. Measurements were done by means of a pressure chamber (Scholander *et al*., 1965) at predawn, 10:00, 14:00 and 16:00. The leaf water potential was determined by measuring the water potential of the mature primary shoot leaves (exposed to the sun during the day).

Soil water: Soil water was determined gravimetrically by means of a neutron moisture probe.

Statistical procedures

A random split-plot experiment was performed with main plot treatments as four irrigations (full irrigation; véraison and at post véraison irrigation; post véraison irrigation; and no irrigation), replicated randomly within each of the two blocks. The sub-plot treatments were five different stages of ripening (véraison, one month after véraison, and three further stages approximately two weeks apart). The whole experiment was repeated over two seasons on the same experimental plots. The repeated measurements for the two seasons were considered as sub-sub-plot treatments (Little & Hills, 1972). The appropriate analyses of variance were performed on all the variables measured (SAS Institute, Inc., 1999).

Shapiro-Wilk test was performed to test for non-normality of the residuals (Shapiro & Wilk, 1965). Deviation from normality was mainly due to kurtosis and not skewness; the data were therefore considered as reliable (Glass *et al*., 1972). Students' t-LSD (least significant difference) was calculated at a 5% significance level to compare means of significant effects (Snedecor & Cochran, 1967).

RESULTS

Soil water

Irrigated and non-irrigated treatments differed in soil water content in spite of rain during the ripening period, with non-irrigated vines being subjected to lower soil water contents from véraison (Fig. 1)

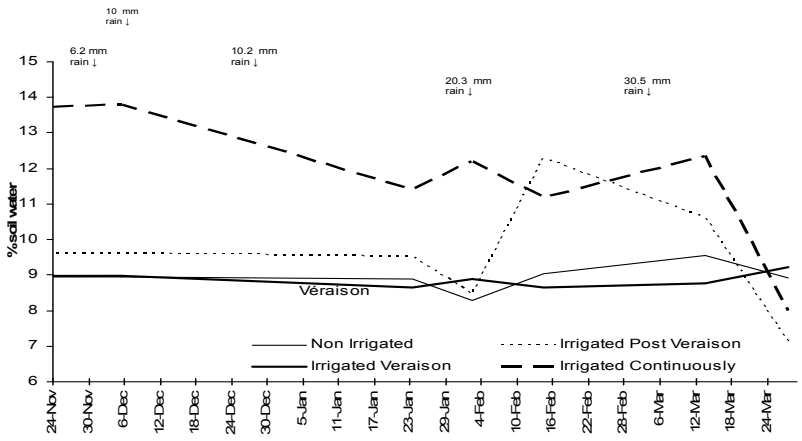

Fig. 1. Soil water content of irrigation treatments at different development stages (values above the graph indicate precipitation).

Leaf water potential

Comparing the two seasons of measurement (2004-2005 & 2005-2006), similar leaf water potentials were found at predawn (Fig. 2). The full irrigation treatment displayed the highest water potential, followed by no irrigation, véraison+post véraison irrigation and post véraison irrigation. At 10:00, 14:00 and 16:00, more pronounced differences between the two seasons occurred (Figs. 3, 4 & 5), the 2005-2006 season generally displaying higher leaf water potential. The patterns between the treatments, however, largely stayed the same.

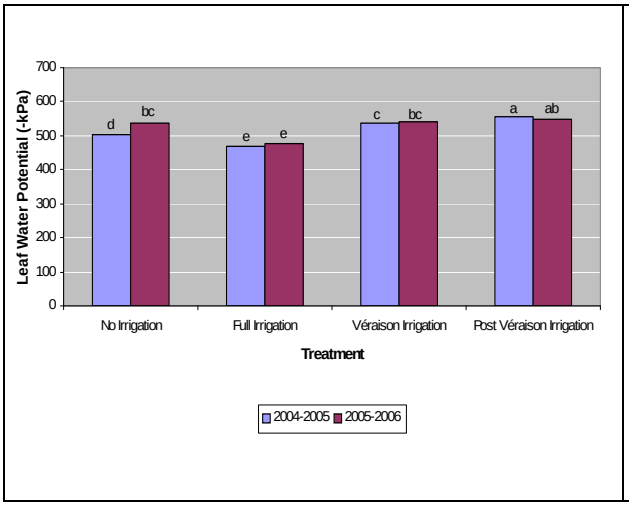

Fig. 2. Average predawn water potential for seasons 2004-2005 and 2005-2006 for different treatments.

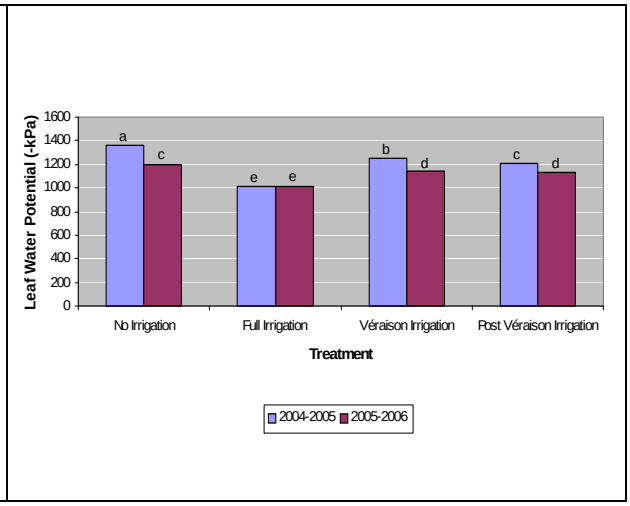

Fig. 3. Average water potential at 10:00 for seasons 2004-2005 and 2005-2006 for different treatments.

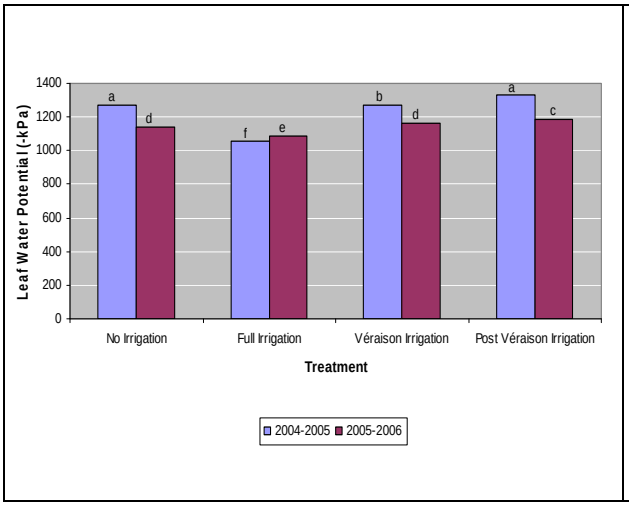

Fig. 4. Average water potential at 14:00 for seasons 2004-2005 and 2005-2006 for different treatments.

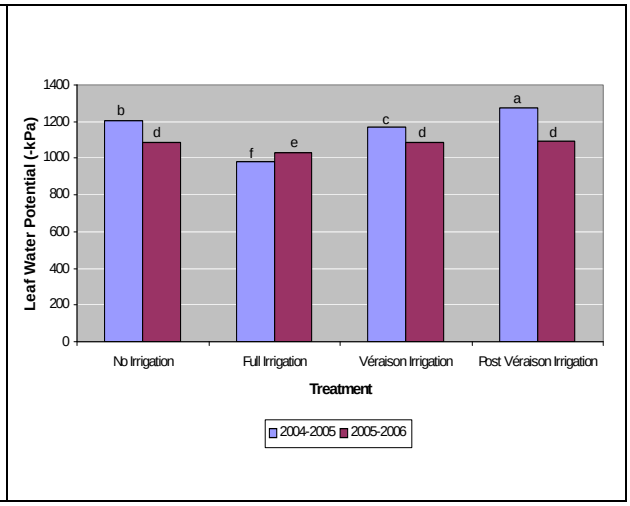

Fig. 5. Average water potential at 16:00 for seasons 2004-2005 and 2005-2006 for different treatments.

Over seasons, the average predawn leaf water potential of the full irrigation treatment was significantly higher than that of the other treatments (Fig. 6). The leaf water potential of the no irrigation treatment was also higher than that of the véraison+post véraison and post véraison irrigation treatments. At 10:00, a significantly higher water potential occurred for full-irrigated vines compared to the other treatments (Fig. 7).

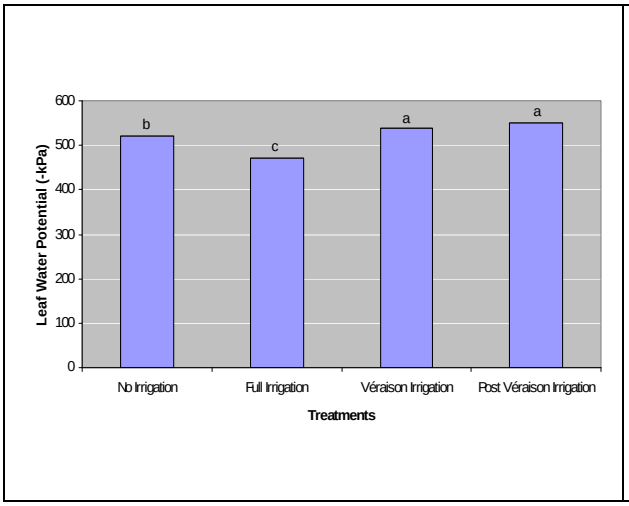

Fig. 6. Average predawn water potential over two seasons for different treatments.

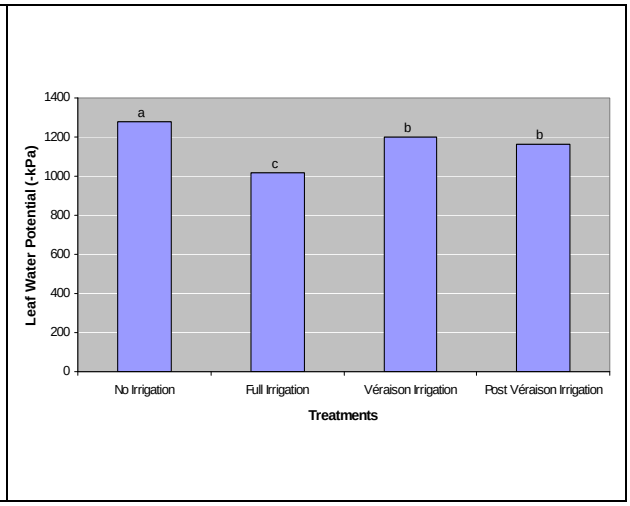

Fig. 7. Average water potential at 10:00 over two seasons for different treatments.

The no irrigation treatment had the lowest water potential. At 14:00 and 16:00, similar general patterns were found, i.e. highest water potential for fully irrigated vines, followed by no irrigation, véraison+post véraison irrigation and post véraison irrigation (Figs. 8 & 9).

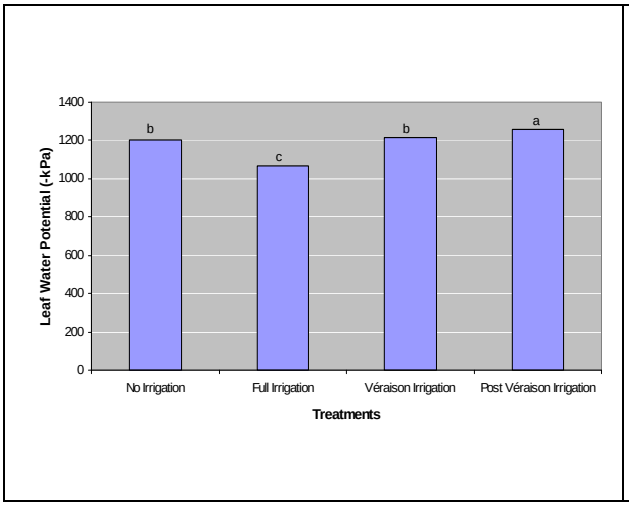

Fig. 8. Average water potential at 14:00 over two seasons for different treatments.

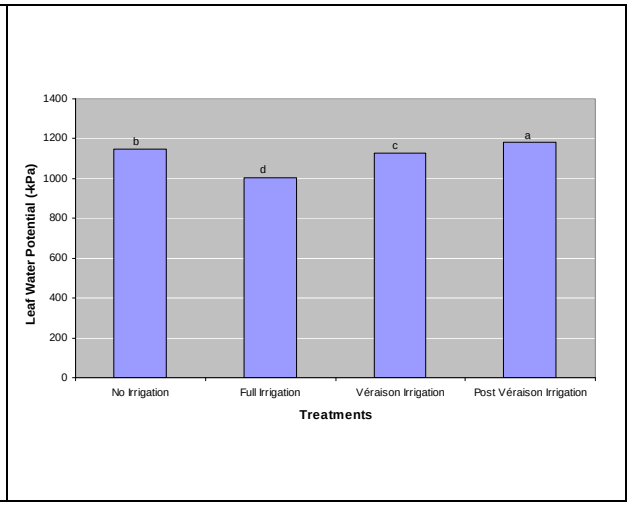

Fig. 9. Average water potential at 16:00 over two seasons for different treatments.

Considering all the treatments at the different stages and times of measurement during the day, it was clear that the vines recuperated well during the night at véraison and at one month after véraison (Figs. 10, 11, 12 & 13). However, at the different ripening stages after that, differences were more pronounced and mostly maintained during the night. The full irrigation treatment always displayed highest leaf water potential, generally followed by the no irrigation treatment, and the véraison+post véraison and post véraison treatments.

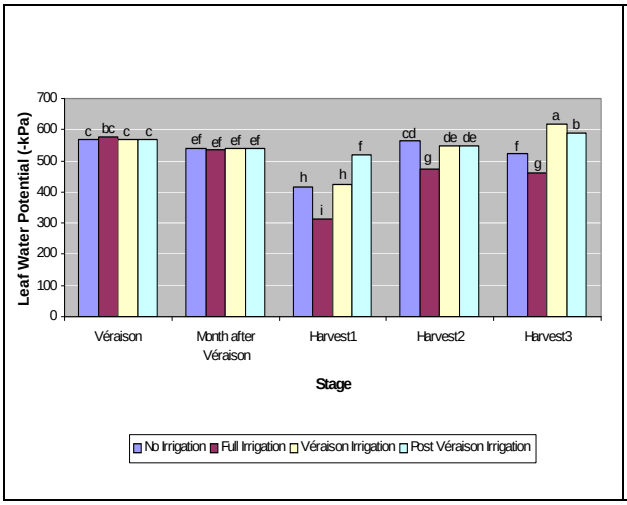

Fig. 10. Average predawn water potential over two seasons harvested at different stages of ripening and for different treatments.

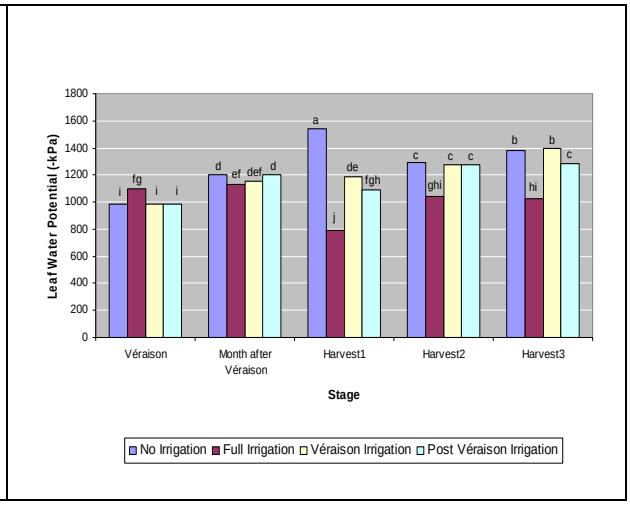

Fig. 11. Average water potential at 10:00 over two seasons harvested at different stages of ripening and for different treatments.

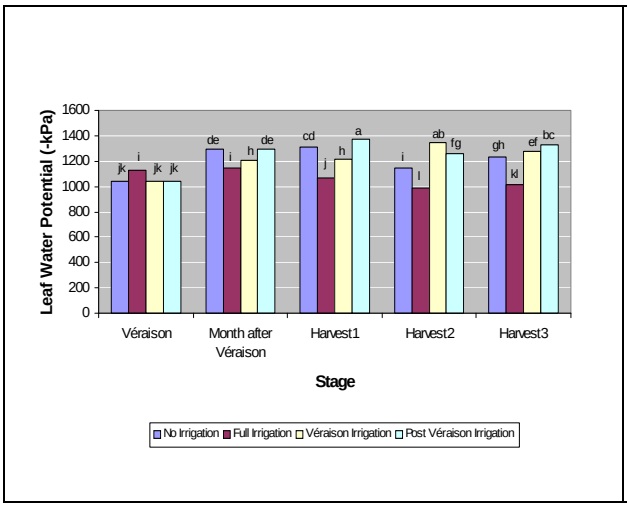

Fig. 12. Average water potential at 14:00 over two seasons harvested at different stages of ripening and for different treatments.

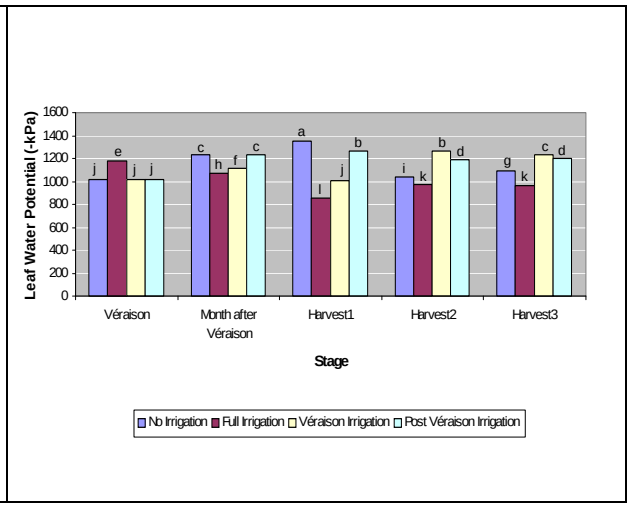

Fig. 13. Average water potential at 16:00 over two seasons harvested at different stages of ripening and for different treatments.

Water status of the vegetative organs

Root water content: The water content in the roots generally followed similar trends to the water potential of the leaves, both in terms of the season as well as the differences between the treatments (Figs. 14 & 15). The full irrigation treatment had significantly higher root water content (Fig. 15). In correspondence with the leaf water potential, the full irrigation treatment also tended to have higher root water content when considering the different stages of measurement (Fig. 16).

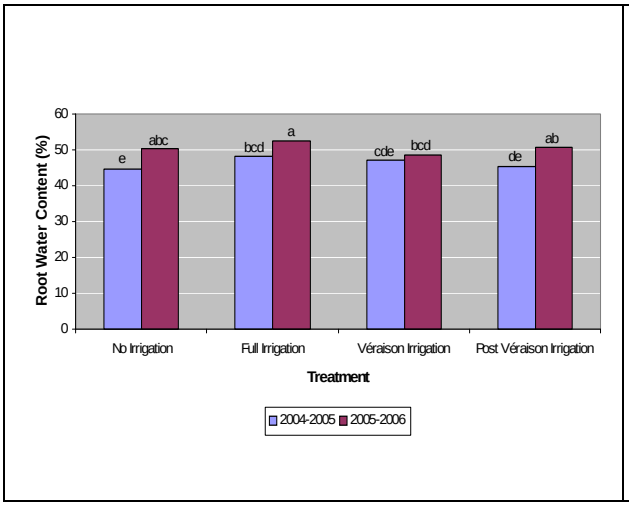

Fig. 14. Percentage water in roots for seasons 2004-2005 and 2005-2006 for different treatments.

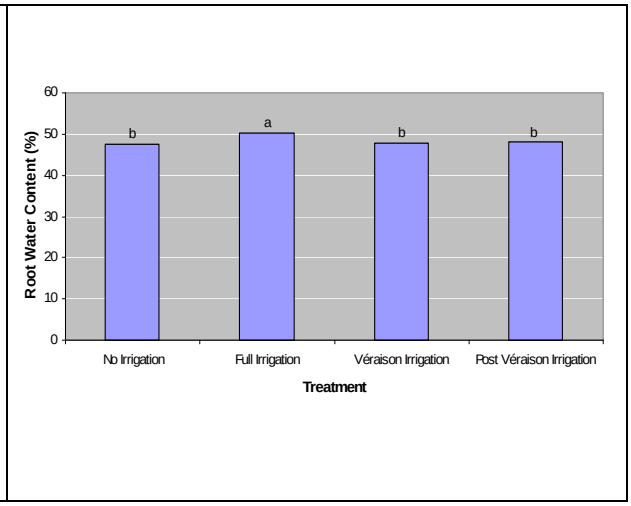

Fig. 15. Percentage water in roots over two seasons for different treatments.

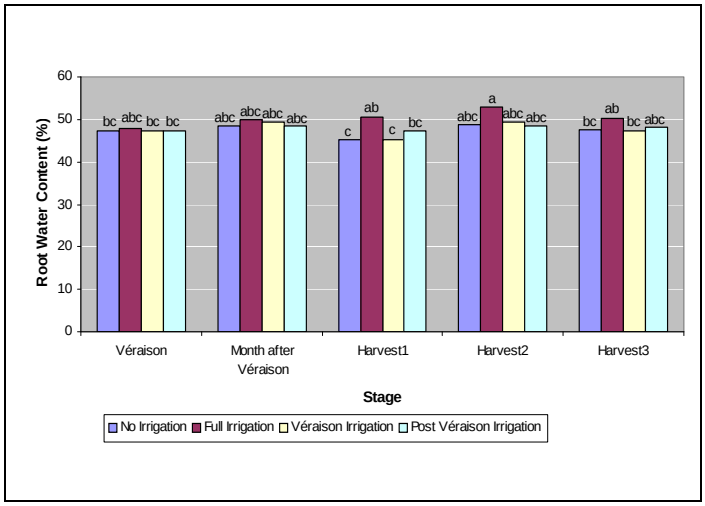

Fig. 16. Percentage water in roots over two seasons harvested at different stages of ripening and for different treatments.

Primary shoot water content: The seasonal differences in primary shoot water content between the treatments showed similar trends to those found for the roots

(Figs. 17, 18 & 19). Seasonal differences were more pronounced in the apical parts of the shoots (Fig. 19).

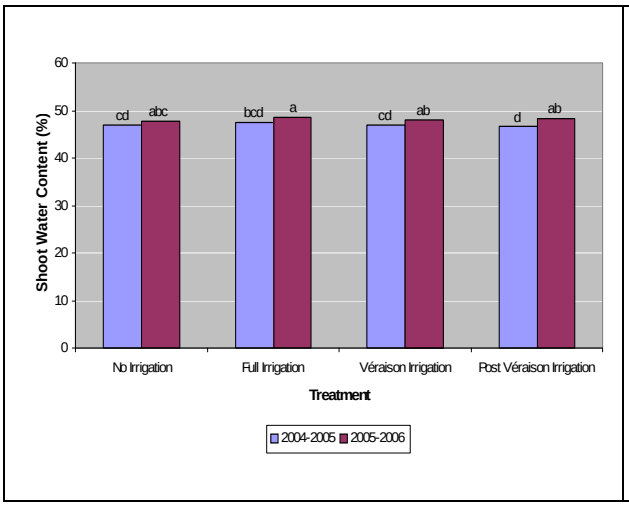

Fig. 17. Percentage water in basal parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

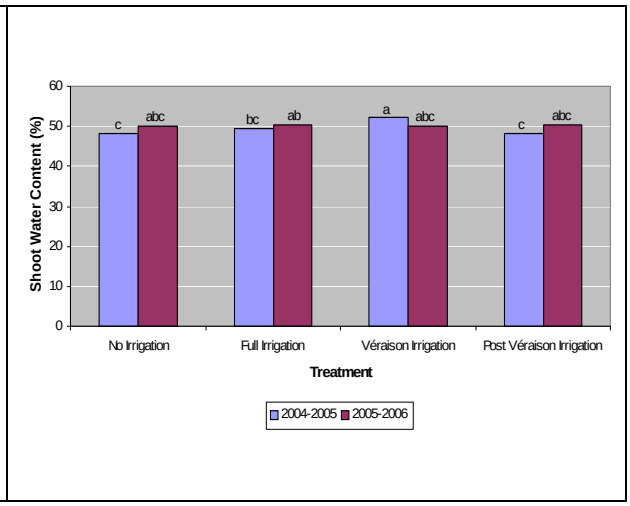

Fig. 18. Percentage water in middle parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

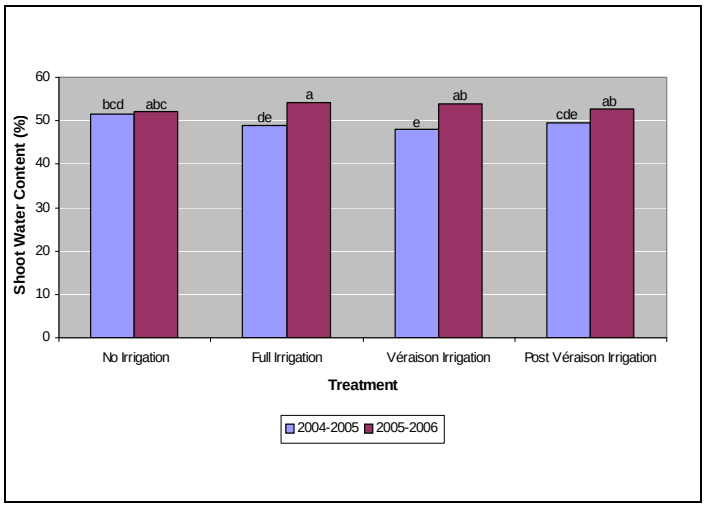

Fig. 19. Percentage water in apical parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

No major differences between treatments occurred. The water content of the primary shoot progressively decreased from basal to apical (Fig. 20).

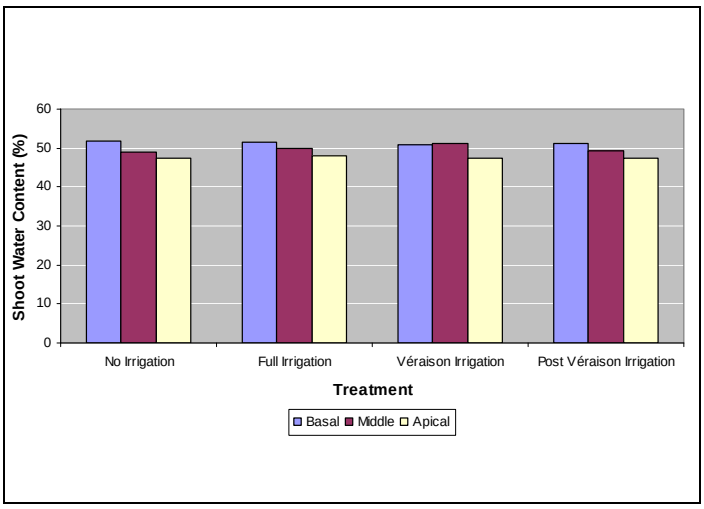

Fig. 20. Percentage water for basal, middle and apical parts of primary shoots over two seasons for different treatments.

Considering the treatments per stage of measurement, a slow reduction from véraison to the second ripeness level seemed to occur for particularly the middle and apical parts of primary shoots (Figs. 21, 22 & 23). The water content of the véraison+post véraison treatment in particular further decreased after that, whereas the rest either stabilised or even slightly increased.

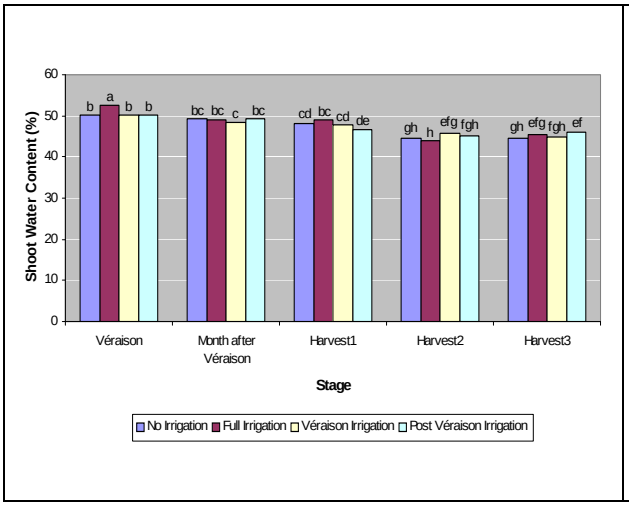

Fig. 21. Percentage water in basal parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

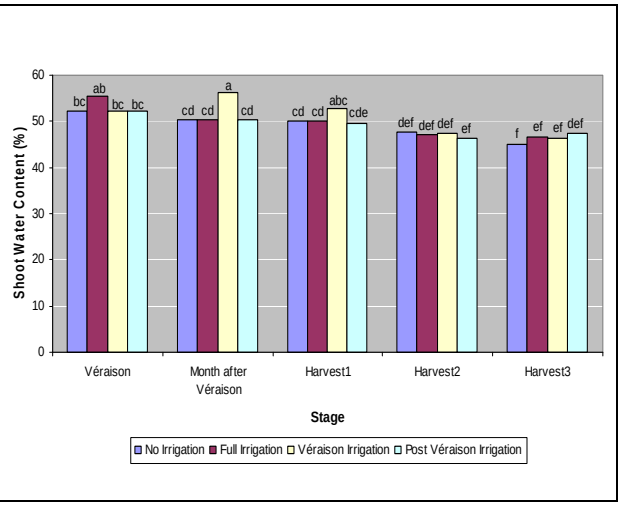

Fig. 22. Percentage water in middle parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

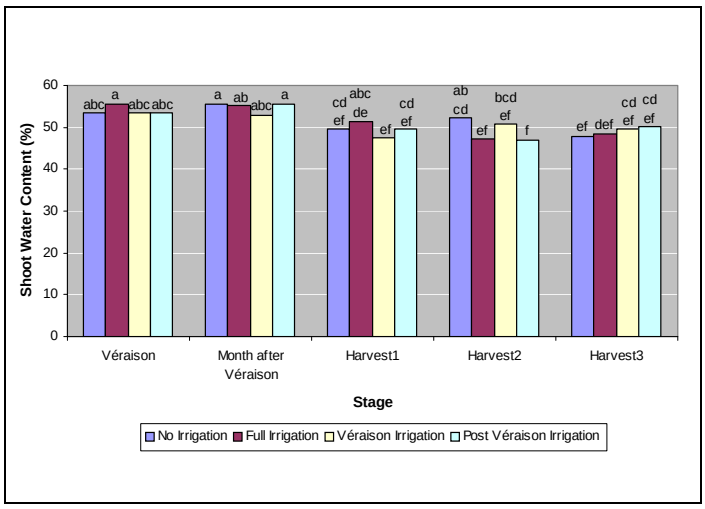

Fig. 23. Percentage water in apical parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

Secondary shoot water content: No specific trends or major differences were found for secondary shoots in any of the years of study (Figs. 24, 25 & 26).

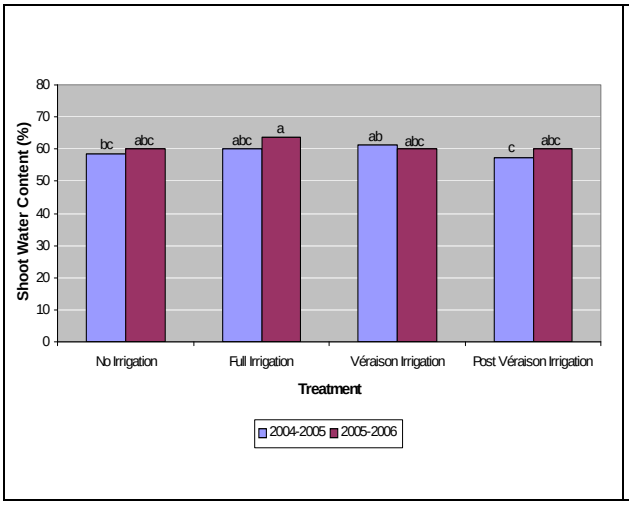

Fig. 24. Percentage water in secondary shoots on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

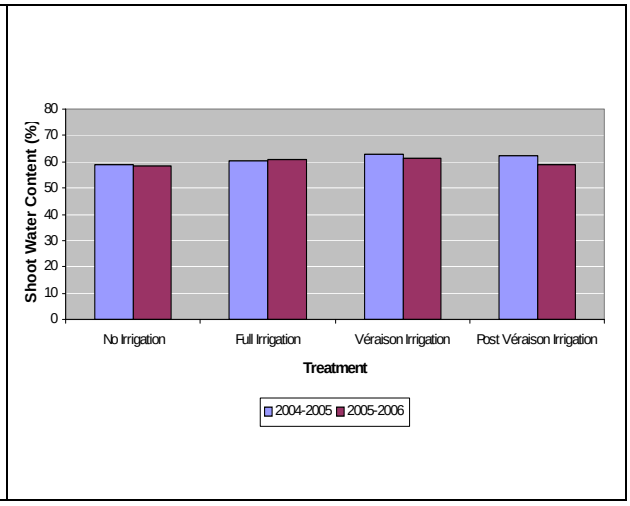

Fig. 25. Percentage water in secondary shoots on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

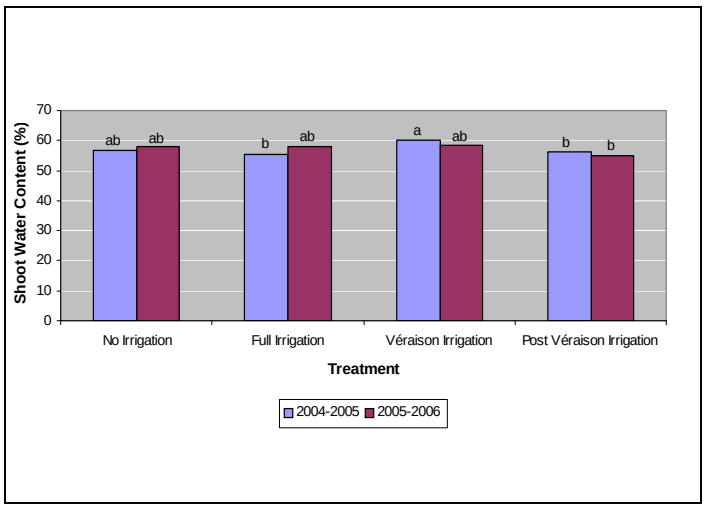

Fig. 26. Percentage water in secondary shoots on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

As for primary shoots the secondary shoots located in the different primary shoot zones, apparently also decreased in water content from basal to apical (Fig. 27).

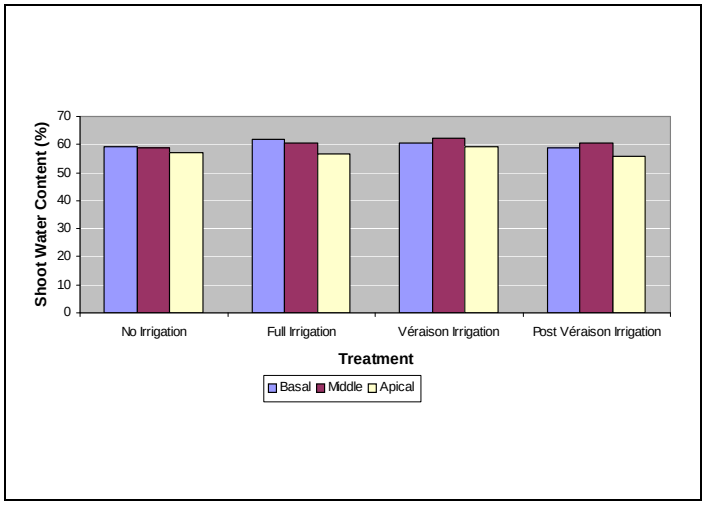

Fig. 27. Percentage water for secondary shoots on basal, middle and apical parts of shoots over two seasons for different treatments.

As in the case of the primary shoot water content, a decreasing trend occurred for all treatments until the second ripeness level (Figs. 28, 29 & 30), after which the water contents seemed to increase again. The vines apparently recuperated their water status at this time and were less affected by environmental demands.

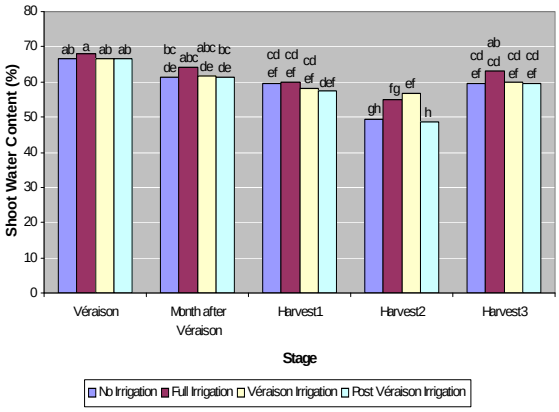

Fig. 28. Percentage water in secondary shoots on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

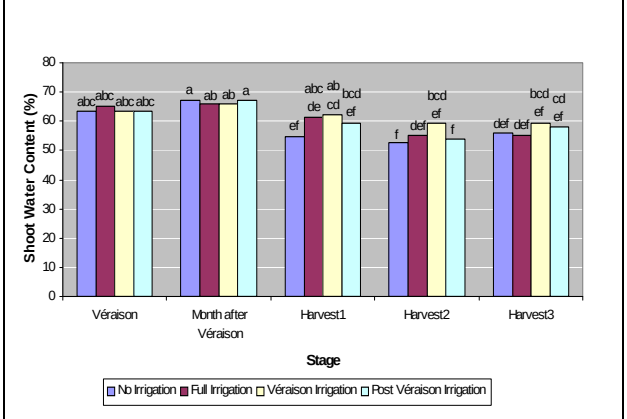

Fig. 29. Percentage water in secondary shoots on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

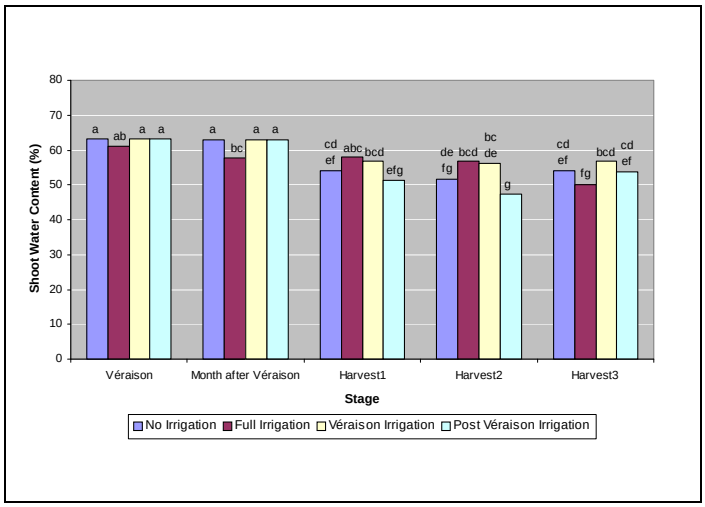

Fig. 30. Percentage water in secondary shoots on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Petiole water content: In petioles, seasonal water content differences were opposite to those found in the shoot (Figs. 31, 32 & 33). The trends of differences between treatments were, however, similar. As found for the shoots, the petiole water content decreased from basal to apical for all treatments (Fig. 34).

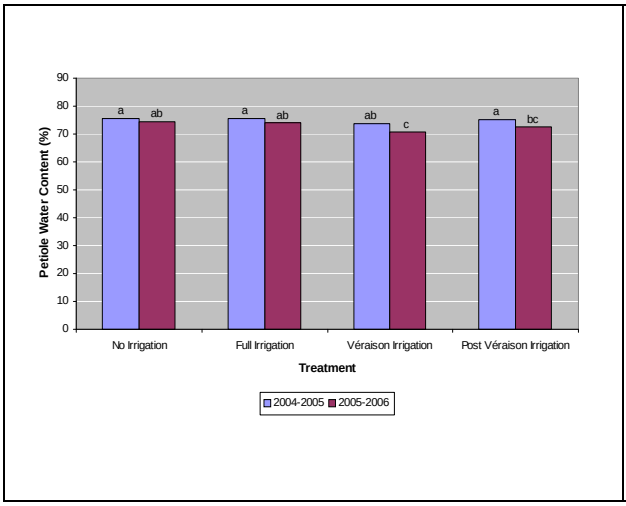

Fig. 31. Percentage water in petioles on basal parts of shoots parts for seasons 2004-2005 and 2005-2006 for different treatments.

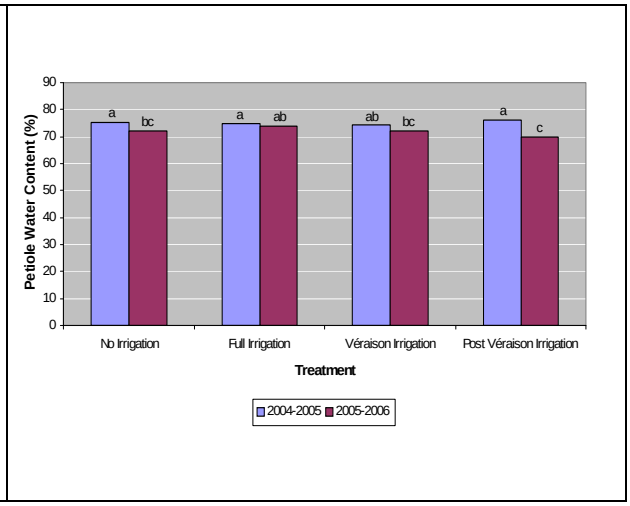

Fig. 32. Percentage water in petioles on middle parts of shoots parts for seasons 2004-2005 and 2005-2006 for different treatments.

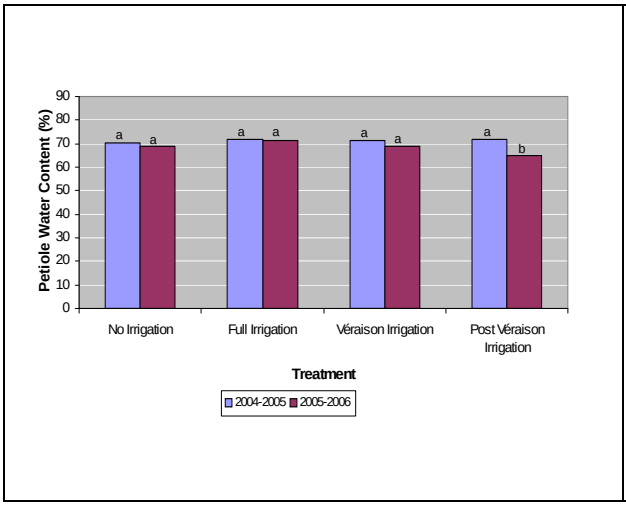

Fig. 33. Percentage water in petioles on apical parts of shoots parts for seasons 2004-2005 and 2005-2006 for different treatments.

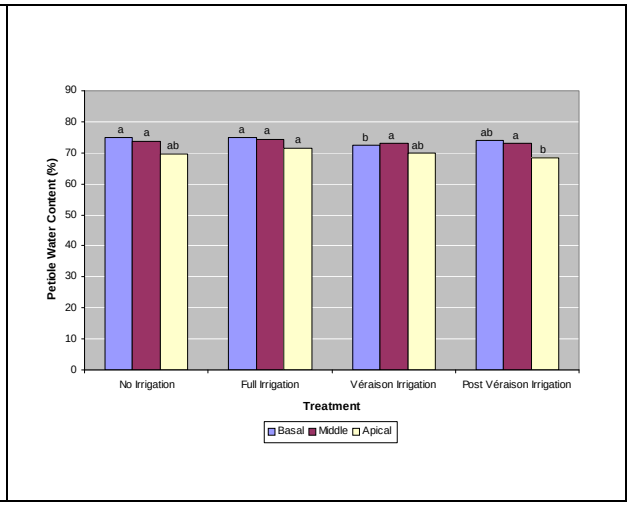

Fig. 34. Percentage water in petioles on basal, middle and apical parts of shoots over two seasons for different treatments.

Similar results to those found for the shoots occurred between the different stages (Figs. 35, 36 & 37).

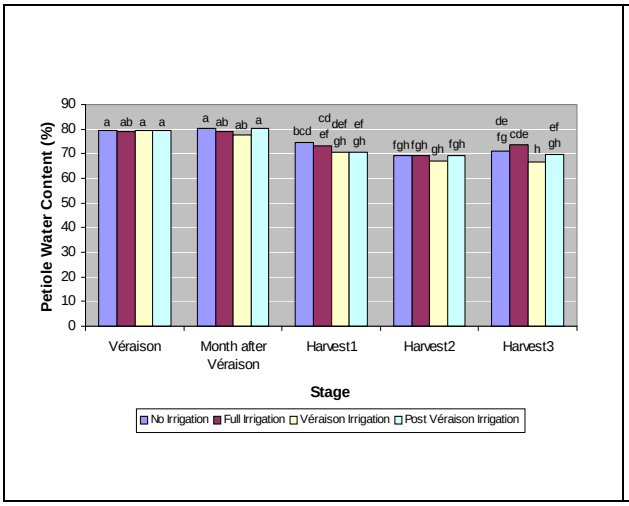

Fig. 35. Percentage water in petioles on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

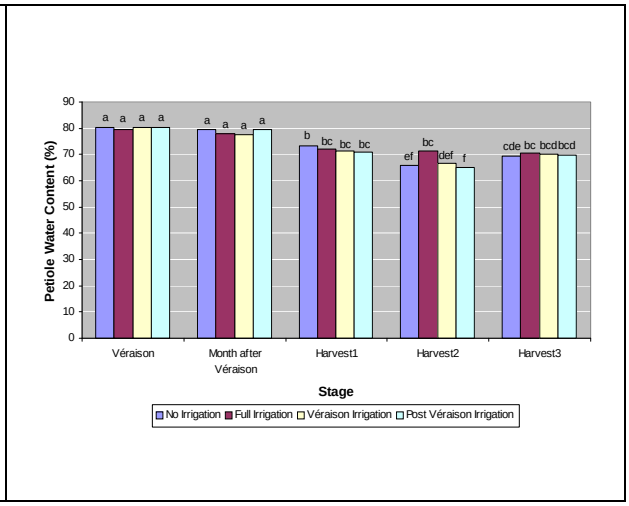

Fig. 36. Percentage water in petioles on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

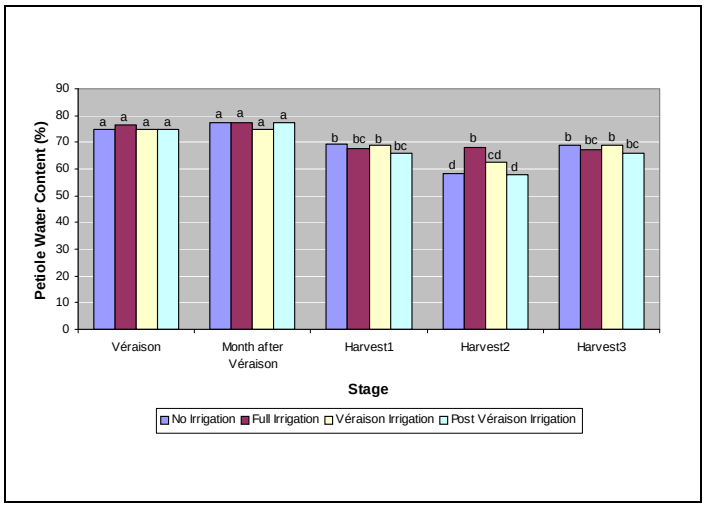

Fig. 37. Percentage water in petioles on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Primary leaf water content: Seasonal differences in primary leaf water content corresponded with those of the shoots (Figs. 38, 39 & 40) and also followed a decreasing pattern from basal to apical on the shoot for all the treatments (Fig. 41).

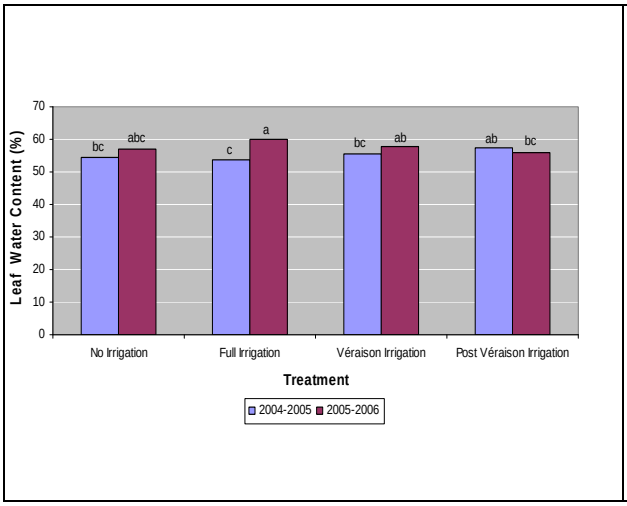

Fig. 38. Percentage water in primary leaves on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

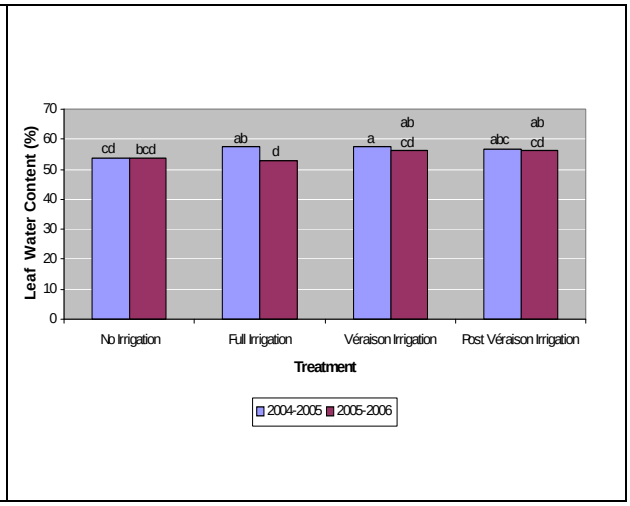

Fig. 39. Percentage water in primary leaves on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

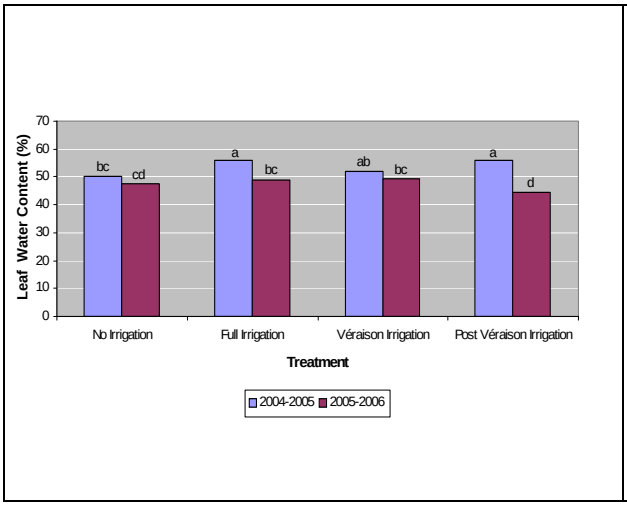

Fig. 40. Percentage water in primary leaves on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

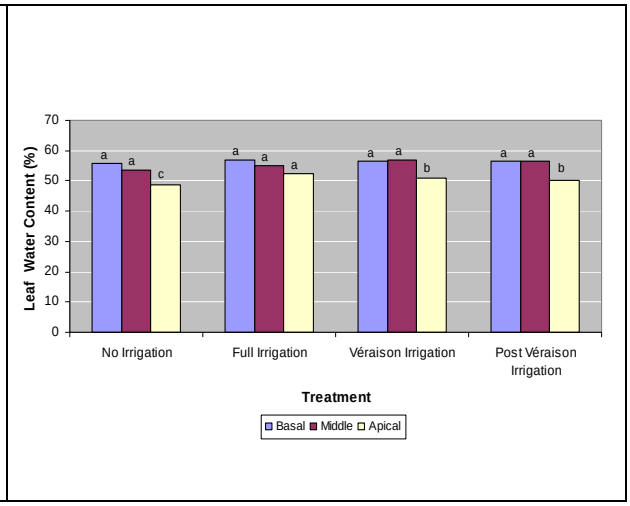

Fig. 41. Percentage water in primary leaves on basal, middle and apical parts of shoots over two seasons for different treatments.

The recuperation of the water content at the last stage of harvest seemed more pronounced for the leaves (Figs. 42, 43 & 44).

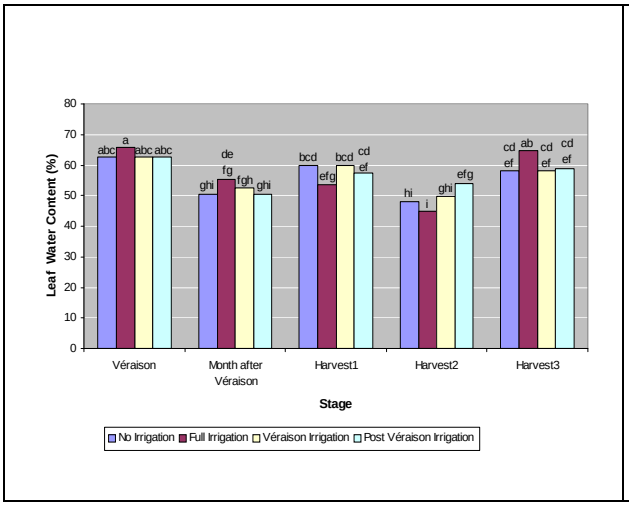

Fig. 42. Percentage water in primary leaves on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

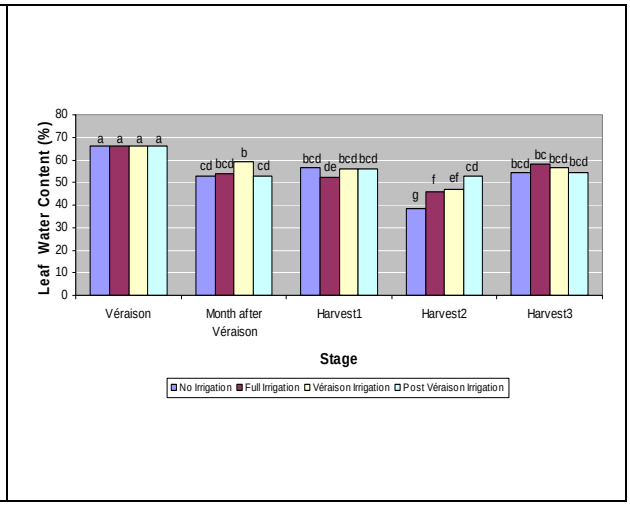

Fig. 43. Percentage water in primary leaves on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

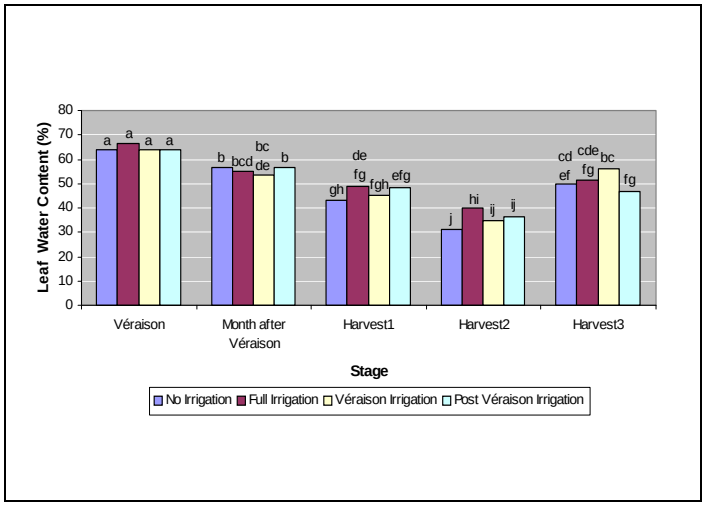

Fig. 44. Percentage water in primary leaves on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Secondary leaf water content: Similar seasonal trends than found for primary leaves occurred for secondary leaf water content (Figs. 45, 46 & 47). The distribution of water over the shoot also corresponded with that found for the other canopy vegetative parameters (Fig. 48).

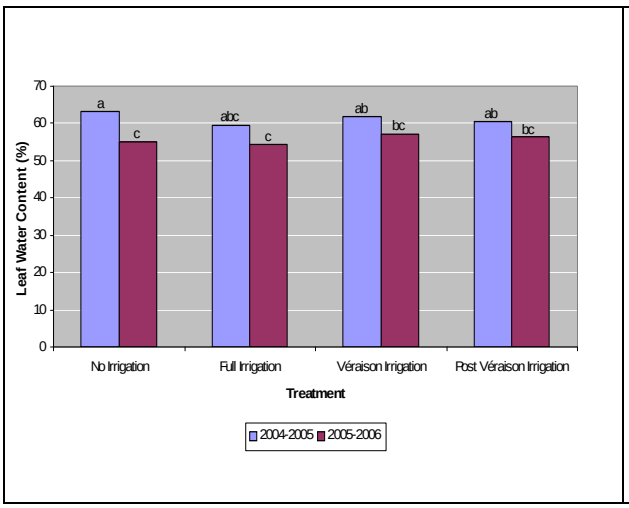

Fig. 45. Percentage water in secondary leaves on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

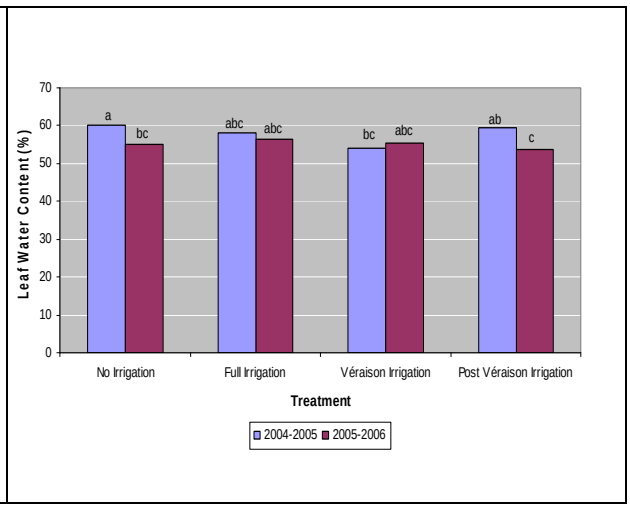

Fig. 46. Percentage water in secondary leaves on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

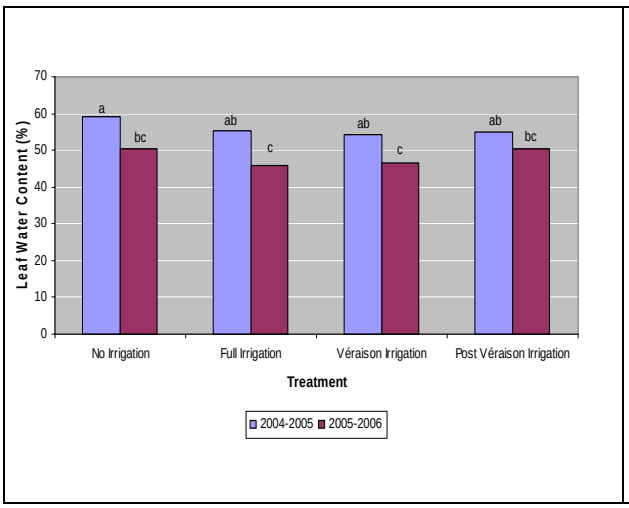

Fig. 47. Percentage water in secondary leaves on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

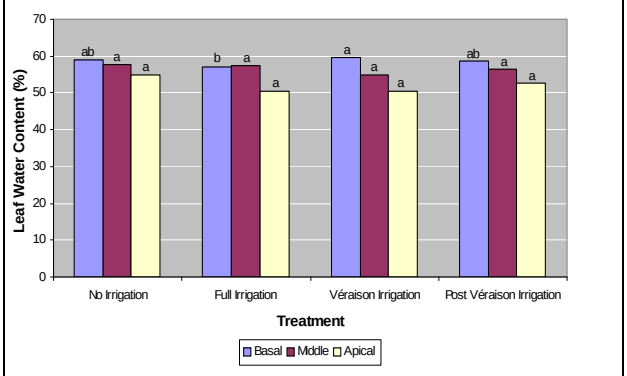

Fig. 48. Percentage water in secondary leaves on basal, middle and apical parts of shoots over two seasons for different treatments.

The reduction in water content until the penultimate ripening stage was even more pronounced than for the primary leaves (Figs. 49, 50 & 51). The drier treatments apparently retained more water than the fully irrigated vines.

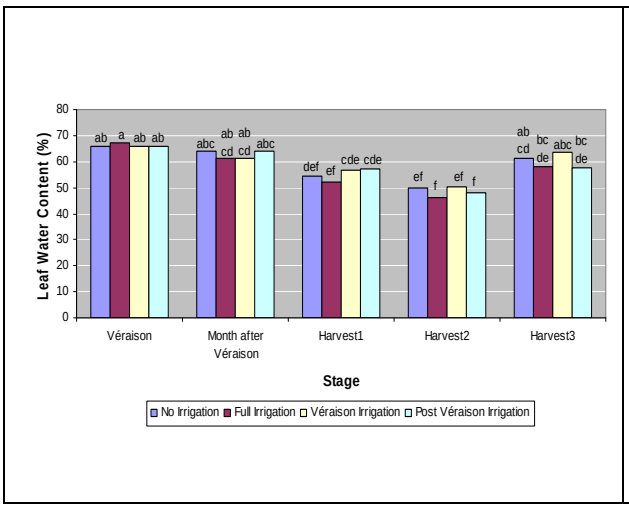

Fig. 49. Percentage water in secondary leaves on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

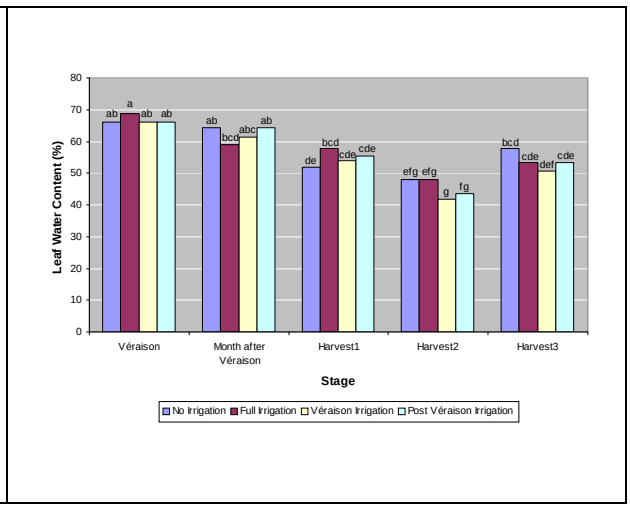

Fig. 50. Percentage water in secondary leaves on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

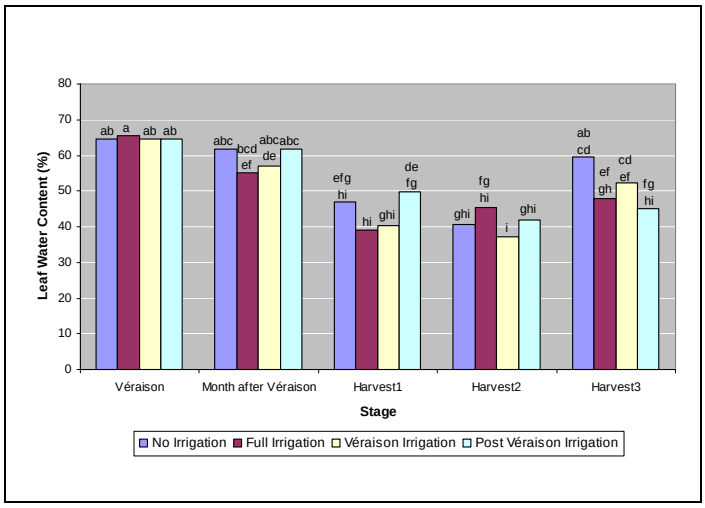

Fig. 51. Percentage water in secondary leaves on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Rachis water content: The water content patterns in the rachis were similar to those found for the shoots and leaves (Figs. 52, 53 & 54).

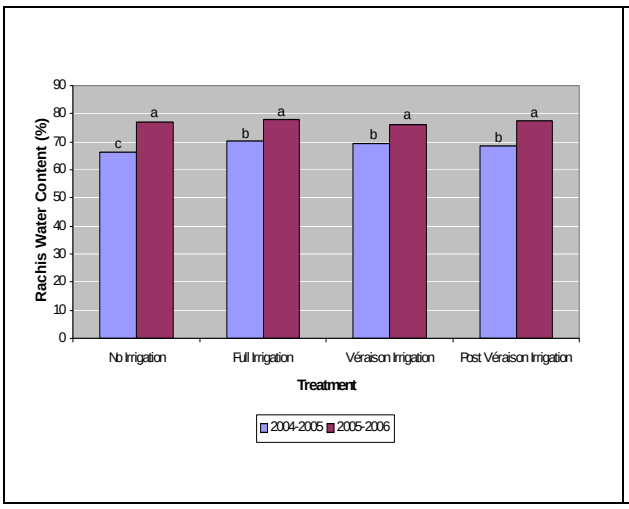

Fig. 52. Percentage water in bunch rachis for seasons 2004-2005 and 2005-2006 for different treatments.

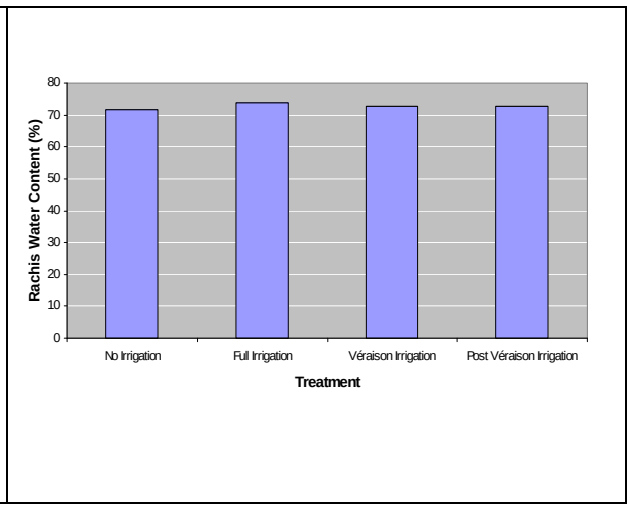

Fig. 53. Percentage water in bunch rachis over two seasons for different treatments.

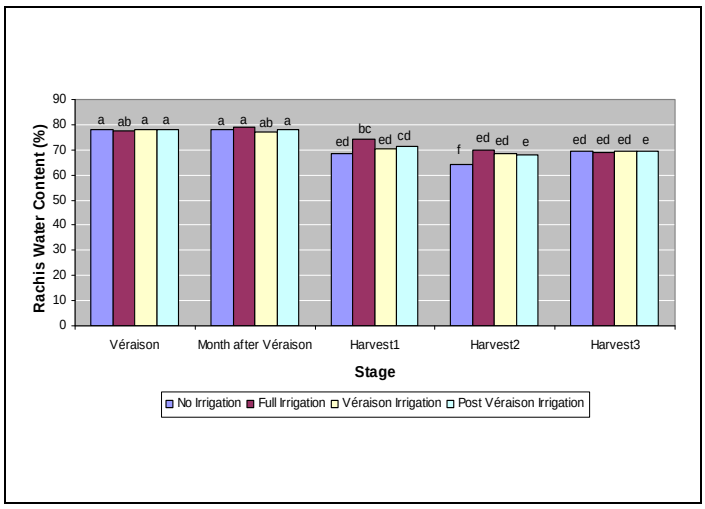

Fig. 54. Percentage water in bunch rachis over two seasons harvested at different stages of ripening and for different treatments.

Water status of the reproductive organs

Berry water content: Although the berry water content trends were largely similar to those of the vegetative parameters (Figs. 55, 56 & 57), the berry apparently did not show the hydraulic recovery during the last harvest stage (Fig. 57). The driest (no irrigation) treatment apparently maintained the water content better from the second to the third ripeness level. The rest of the treatments continued to lose water.

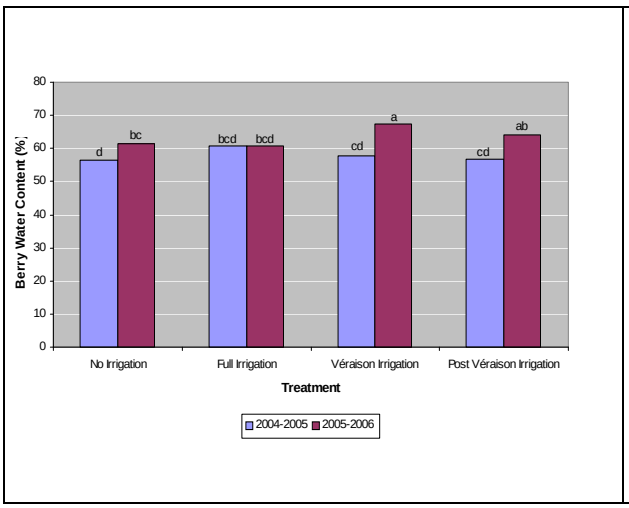

Fig. 55. Percentage water in berry for seasons 2004-2005 and 2005-2006 for different treatments.

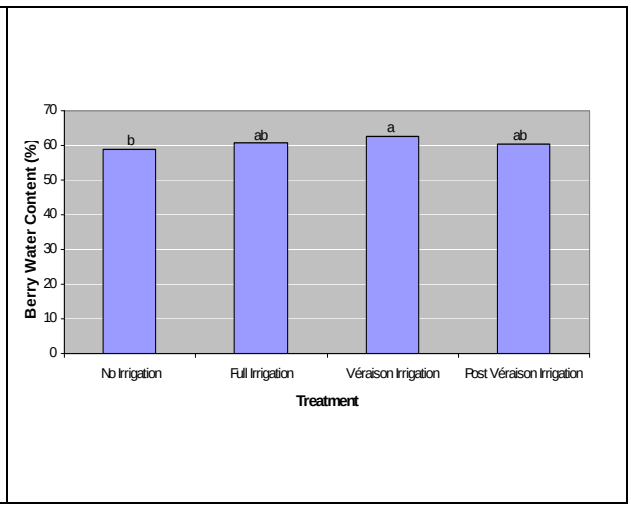

Fig. 56. Percentage water in berry over two seasons for different treatments.

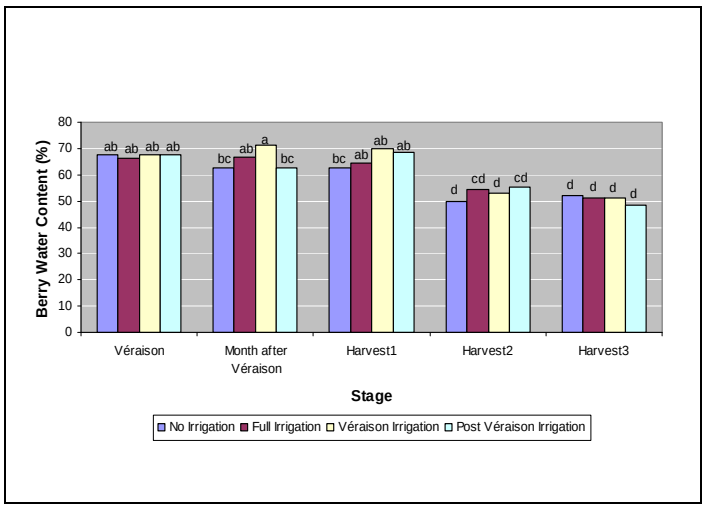

Fig. 57. Percentage water in berry over two seasons harvested at different stages of ripening and for different treatments.

Pulp, seed and skin water content: The seasonal pulp, seed and skin water content patterns were similar, except in the case of the full irrigation seed water content which reacted opposite to the other treatments, but in line with the patterns found for the pulp and skins (Figs. 58, 59 & 60).

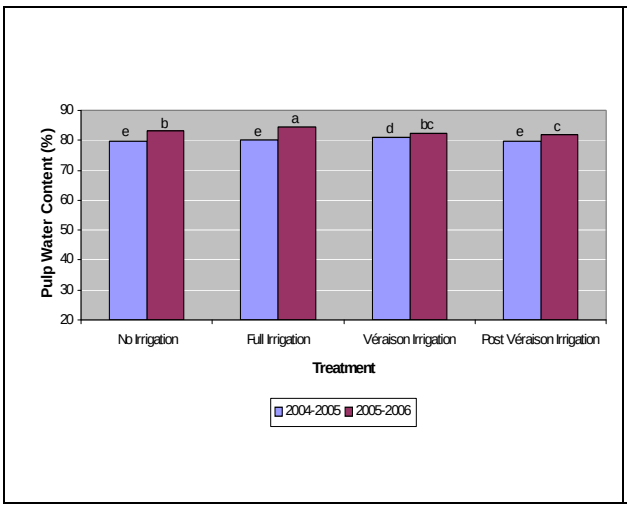

Fig. 58. Percentage water in pulp for seasons 2004-2005 and 2005-2006 for different treatments.

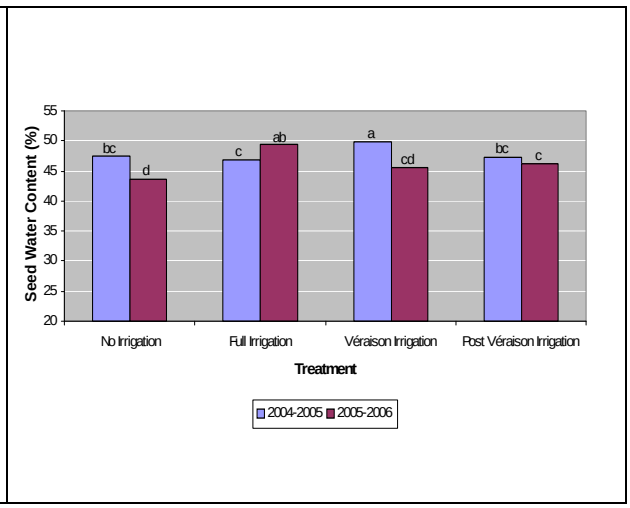

Fig. 59. Percentage water in seeds for seasons 2004-2005 and 2005-2006 for different treatments.

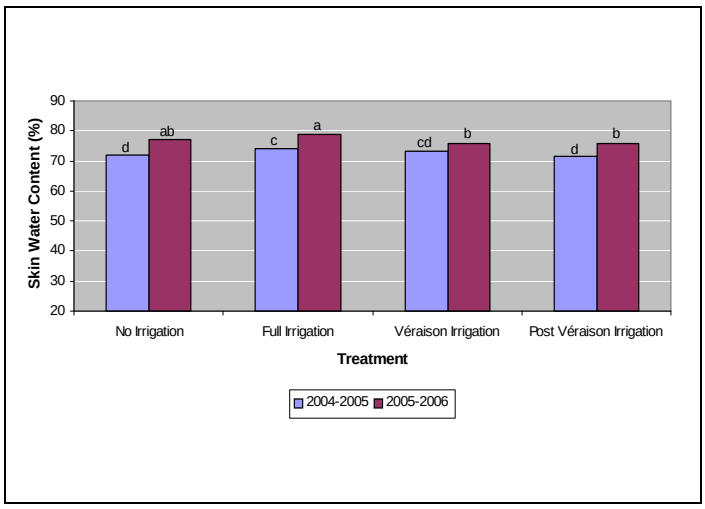

Fig. 60. Percentage water in skins for seasons 2004-2005 and 2005-2006 for different treatments.

The pulp generally contained approximately 80% water, the skins approximately 75% and the seeds approximately 45% (Fig. 61).

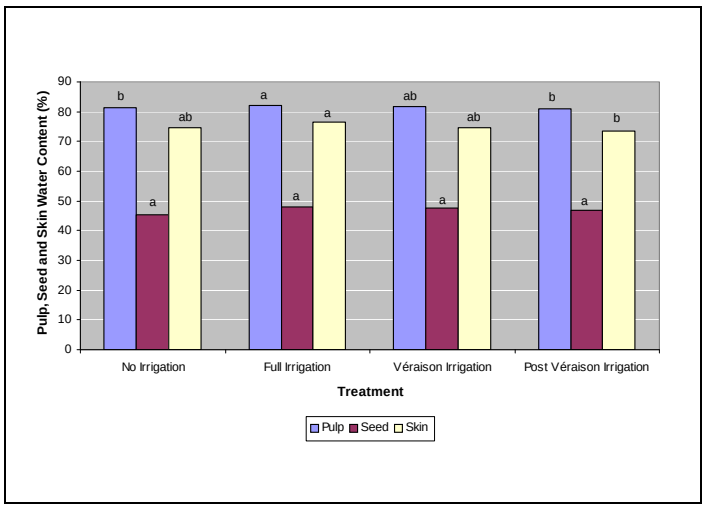

Fig. 61. Percentage water for pulp, seeds and skins over two seasons for different treatments.

Although a loss of water from the different parts of the berry was already evident at a month after véraison, the reduction in water content of the full irrigation treatment was delayed during the ripening period (Figs. 62, 63 & 64). The water loss was particularly noticeable for the seeds and skins.

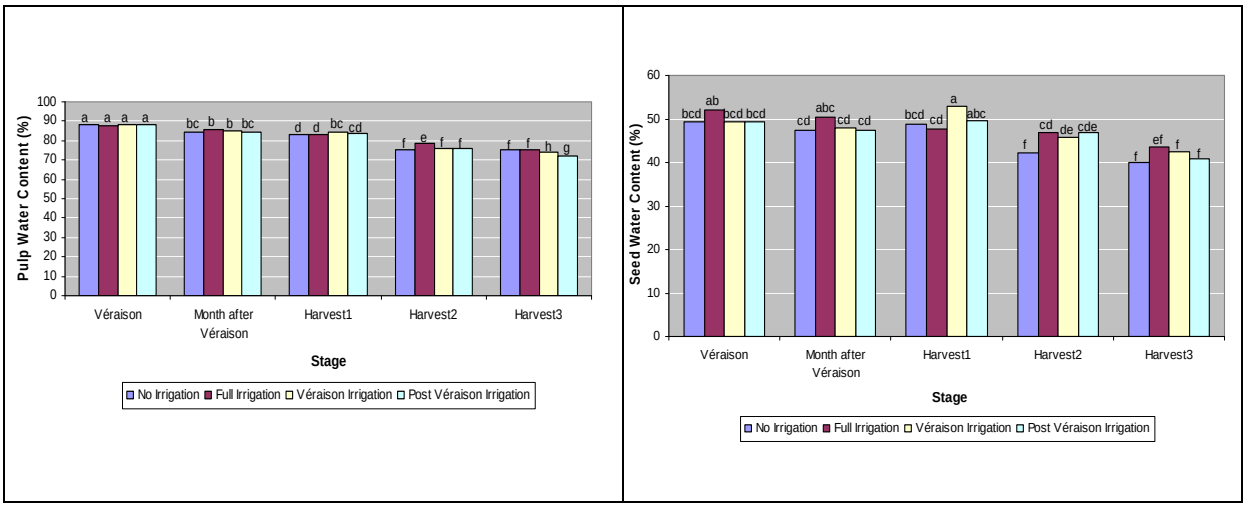

Fig. 62. Percentage water in pulp over two seasons harvested at different stages of ripening and for different treatments.

Fig. 63. Percentage water in seeds over two seasons harvested at different stages of ripening and for different treatments.

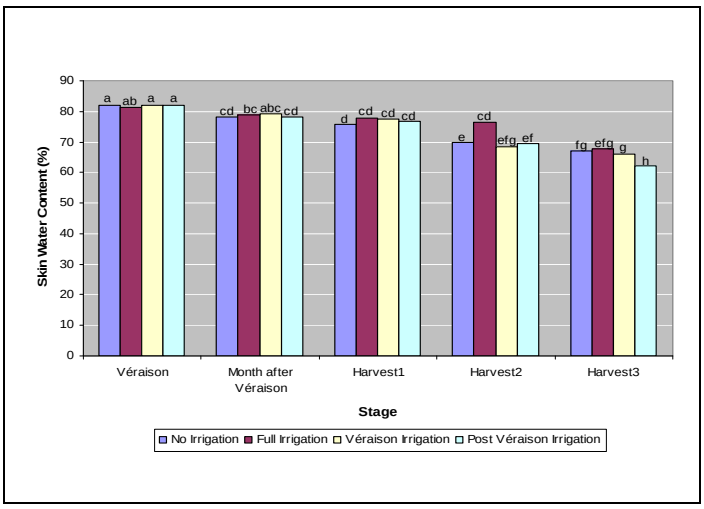

Fig. 64. Percentage water in skins over two seasons harvested at different stages of ripening and for different treatments.

The berry water loss of the no irrigation treatment apparently largely stopped at the second harvest stage, whereas berries of the full irrigation, véraison+post véraison irrigation and post véraison irrigation continued to lose water. In the seeds in particular, drier conditions seemed to induce earlier water loss.

Water distribution

As expected, the largest amount of water accumulated in the berries, especially in the pulp (Figs. 65, 66, 67 & 68). The tissues on all the basal parts had the highest accumulation of water for all the treatments. The full irrigation treatment distributed the water in the secondary shoots and leaves equally between the different parts (basal, middle and apical) on the primary shoots, with the apical parts having a slightly higher water content (Fig. 66).

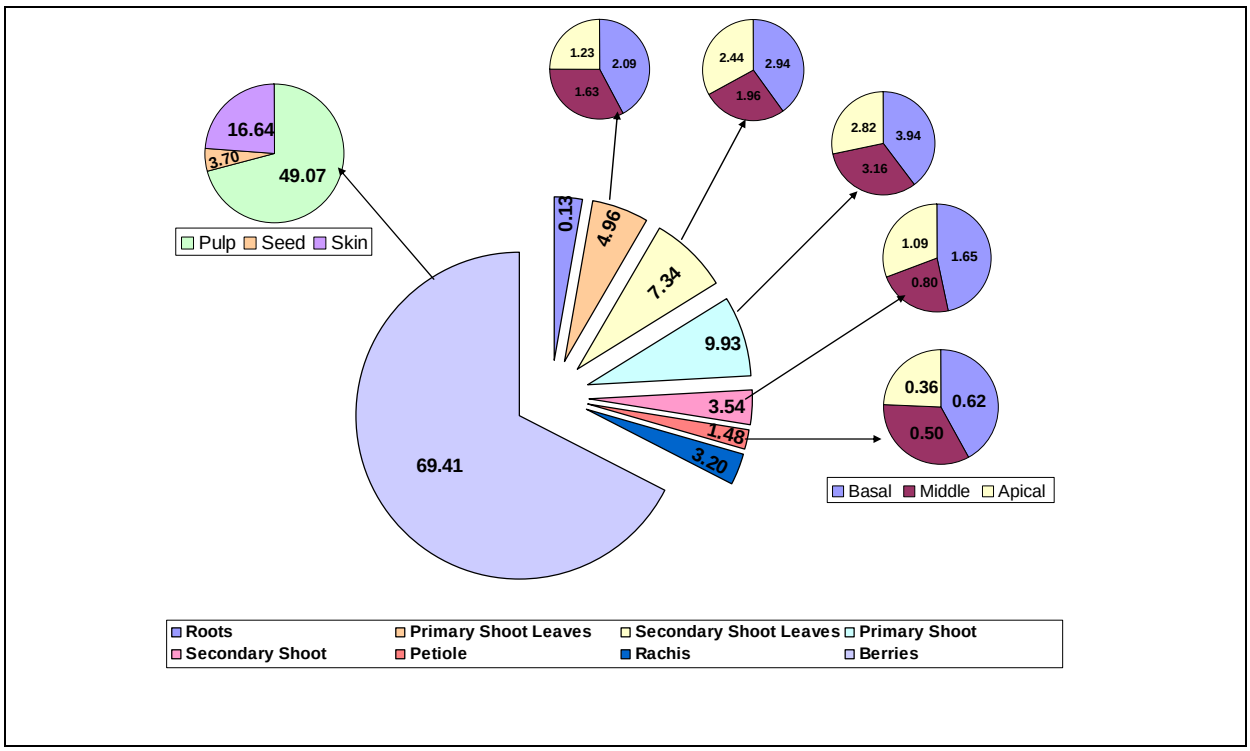

Fig. 65. Percentage water distribution in non-irrigated vines (data represents average of the three harvest dates).

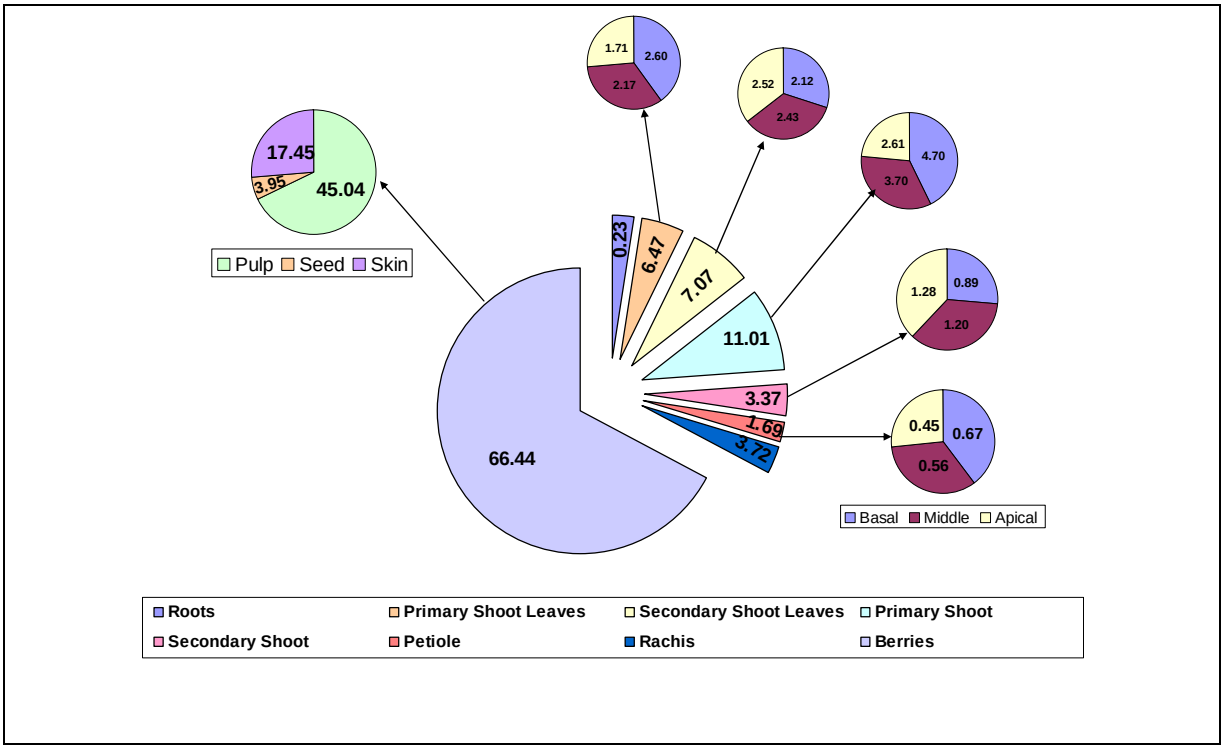

Fig. 66. Percentage water distribution in fully irrigated vines (data represents average of the three harvest dates).

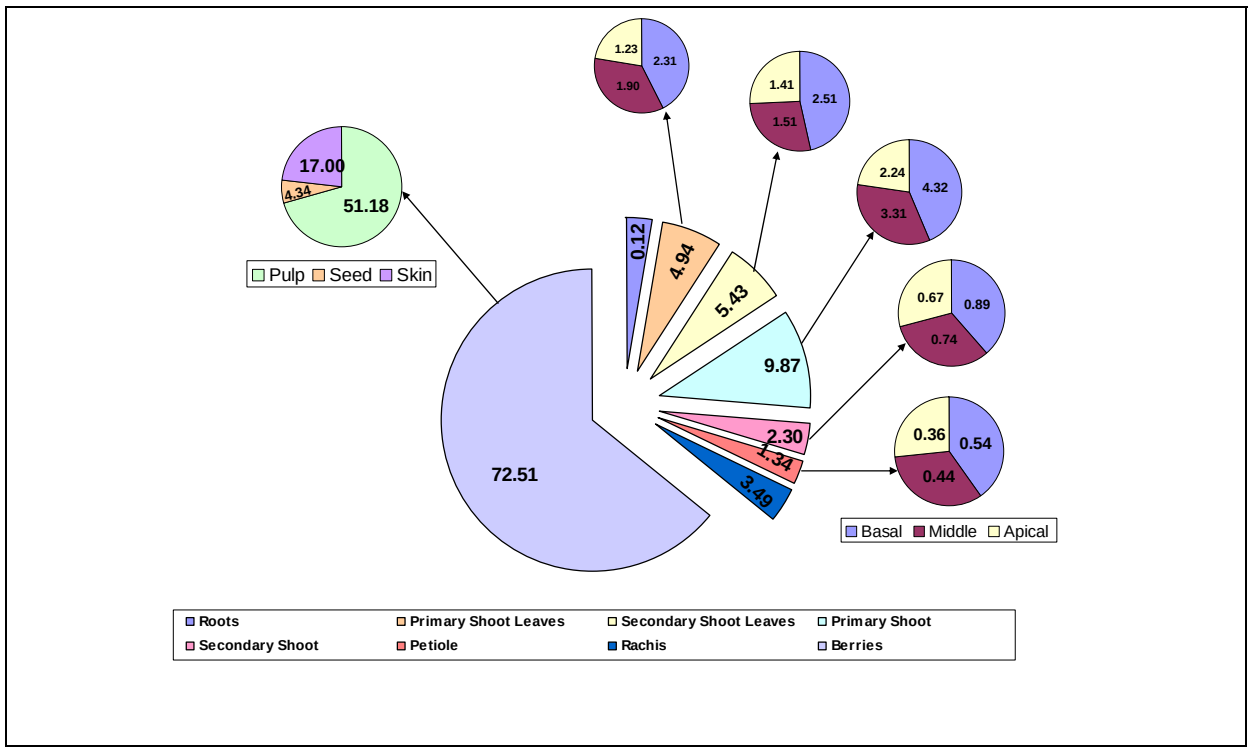

Fig. 67. Percentage water distribution in véraison irrigated vines (data represents average of the three harvest dates).

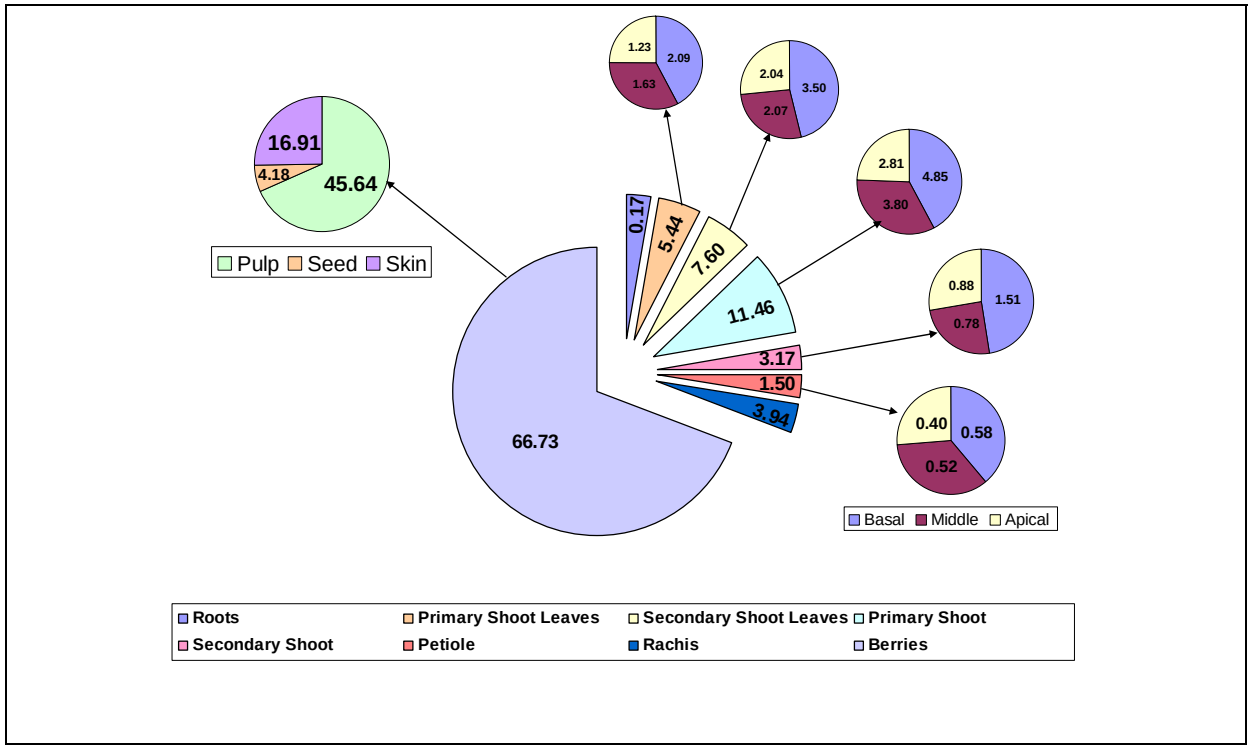

Fig. 68. Percentage water distribution in post véraison irrigated vines (data represents average of the three harvest dates).

DISCUSSION

The predawn water potential was significantly lower in the water deficit vines than in the vines that had continual irrigation. The lower average predawn water potentials in the véraison+post véraison irrigation treatment and post véraison irrigation treatment, compared to the no irrigation treatment, were unexpected. The non-irrigated vines might have been positively affected by rainfall in spite of the treatment. The measurements of the leaf water potential taken at 10:00 indicated higher levels of stress in the non-irrigated vines as well as the véraison+post véraison irrigated vines and post véraison irrigated vines, compared to the fully irrigated vines. The water potential of the deficit irrigated vines was below -1200 kPa. At 14:00 similar patterns occurred and then the water potential increased to above -1200 kPa, also for the deficit vines at 16:00. This indicated that the vines probably started to recuperate in terms of water status, already during late afternoon. Recuperation of non-irrigated vines seemed more efficient. The similar diurnal plant water status between the water deficit irrigated grapevines may be due to the efficient control of water loss by reduced stomatal conductance. As water stress intensifies, stomata close early in the morning, preventing an excessive drop in leaf water potential (Naor & Wample, 1994; Correira *et al*., 1995; Lopes *et al*., 2005).

The predawn leaf water potentials measured at different times of ripening for the irrigation treatments were relatively stable for all the treatments up to a month after véraison, where after it increased suddenly up to the first harvest stage, probably due to rain in the week before the measurements were taken. After this, the water potential steadily decreased up to the last harvest stage and only the fully irrigated vines managed to stay under -500 kPa at predawn. The decline in vine water status in the different treatments at the different stages of ripening may be due to the continued transpiration and the demand for water exceeding the capacity of the roots to supply water to the transpiring leaves, despite the soil water content (Matthews *et al*., 1987). Aged induced inefficient opening and closing of stomata may also have affected water loss. This may affect the absorption of water by the roots as well as the regulation of photosynthetic activity.

Water status of the vegetative organs

Root water content: The water content measured in the roots over two seasons indicated that there is a correlation between the leaf water potential and water

content in the roots. The full irrigation treatment, having the highest water potential also had the highest root water content. Although there were no real differences between the water deficit treatments, the no irrigation treatment still had the lowest root water content. The root water content measured over two seasons harvested at different times of ripening showed no differences of any significance between treatments. This may be due to the availability of soil moisture in deeper soil layers providing buffer capacity to the vine, as the grapevine is known for its extensive root system. Water deficit seems to impact to a larger extent on desiccation, sap flow and transpiration than on water absorption and storage in the root system.

Shoot water content: No significant differences were found in the average water content between the primary shoot parts of the different treatments. The basal parts of the shoots showed the highest water content of all parts. According to Bravdo *et al*. (1985), the physical closeness of the leaves to the bunches and the direct translocation of water to the bunches may place a high demand for water on the basal part of the shoot. The fact that the basal leaves were already at an advanced age during the ripening time may also have contributed to the maintenance of a higher water content (reduced transpiration) in this part of the shoot. In contrast, the apical leaves were photosynthetically very active during this time and higher stomatal activity, transpiration and photosynthetic activity occurred for these leaves (Hunter & Visser, 1989; Hunter *et al*., 1994). The water content in the secondary shoots showed similar patterns than those found for the primary shoots. It seemed as if the water content in the secondary shoots was generally slightly higher than in the primary shoots. The variation per position may be largely due to age differences from the apical to basal position of the secondary shoots.

Petiole water content: The water content in the petioles of the primary leaves followed a similar pattern to that of the primary and secondary shoots, with higher water content in the petioles located basally and lowest water content in the petioles located apically. The véraison+post véraison irrigation treatment showed a higher water content in the middle petioles than in the basal petioles. The water content in the petioles of the different parts for all the treatments decreased towards the second stage of harvest and then increased at the end. This may be due to a drop in temperature towards the end of the season, resulting in a lower water demand in the canopy.

Leaf water content: The average primary leaf water content in the basal and apical shoot parts showed a relationship between the water content and the irrigation treatment. The full irrigation had the highest primary leaf water content and the no irrigation had the lowest water content. For the véraison+post véraison irrigation and post véraison irrigation treatments, the middle leaves had higher water contents than the basal leaves.

The average secondary leaf water content in the basal parts of primary shoots of the véraison+post véraison irrigated, post véraison irrigated and non-irrigated shoots was the highest. This could be due to the secondary leaves on the basal parts being more active during berry ripening than the primary leaves in the same position. They were probably largely used to support the grapes in the position where the primary leaves were senescing. The secondary leaf water content also decreased up to the second stage of harvest and then increased.

The distribution of water in the shoot seems concerted between the different shoot organs. The leaves (particularly the secondary leaves) showed more pronounced water loss towards the penultimate ripeness stage. A recuperation of water content seemed evident at the last harvest date.

Rachis and berry water content: Similar patterns than for the other vegetative parameters were found for rachis water content. This is evidence of the rachis reacting like a vegetative organ, although it is commonly considered as part of the bunch. The average water content in the no irrigation treatment berries was lowest. The véraison+post véraison irrigation treatment had the highest water content, probably due to the sudden increase in water after irrigation was applied. The berry water content of the post véraison irrigation treatment also increased suddenly after irrigation was applied. During the different stages of harvesting, the water content decreased due to berry water loss. Berry water loss from fully irrigated vines was delayed during the three harvesting stages.

The water content of the different berry tissue parts (pulp, seed and skin) was noticeably affected by the different treatments. Water loss was particularly noticeable for seeds and skins. Drier conditions seemed to induce earlier water loss

in the seeds in particular. The average water content of the pulp was highest for the fully irrigated vines and lowest for the non-irrigated and post véraison irrigated vines.

Water distribution

Concerning vegetative tissue, the basal parts of the primary shoots and leaves showed the highest water content as a percentage of total water content. According to Bravdo *et al*. (1985), the physical closeness of the leaves to the bunches and the direct translocation of water to the bunches may place a high demand for water on the basal part of the shoot and may contributed to the maintenance of a higher water content in this part of the shoot.

The water content of secondary leaves and shoots located on the basal parts of primary shoots of the véraison+post véraison irrigated, post véraison irrigated and non-irrigated vines was the highest. This could be due to the secondary leaves on the basal parts being more active during berry ripening and responsible for translocation of water and photosynthetates to the bunches. A probable reason for the equal distribution of water in the fully irrigated vines could be that adequate water was available for the bunches and further vegetative growth. The berries contained highest water, with the pulp clearly dominating, followed by the skins and seeds.

CONCLUSIONS

Predawn leaf water potential clearly showed the differences between the irrigation treatments. The leaf water potentials decreased from predawn to 10:00. Water deficit vines experienced basically similar diurnal plant water status due to efficient control of water loss by the stomata. As water stress intensified during the morning, the stomata apparently closed, preventing an excessive drop in the leaf water potential during the day. During the day, vines of the full irrigation treatment experienced less water stress than the other treatments, with the non-irrigated and post véraison irrigated vines generally experiencing higher water stress. The water potential of irrigated vines seemed to remain more constant than that of the water deficit vines. As the season progressed, a decrease in water potential for all of the treatments from late morning throughout the day occurred. Non-irrigated vines seemed to maintain higher diurnal leaf water potentials, compared to the véraison+post véraison and post véraison irrigated vines.

Lower leaf water potentials indicated lower water contents in the vegetative and reproductive tissue. The root water content was higher in the full irrigation treatment, but no significant differences were observed in the water deficit treatments. Higher water contents were observed in the basal parts of the primary shoots, in the secondary shoots in this region, and in leaves in this region, with the apical parts having the lowest water contents. Secondary leaves in the basal position in particular, clearly had a significant role in water and photosynthetate translocation to the berries.

Irrigation at véraison+post véraison and post véraison caused a sudden increase in water accumulation in the berries of the vines. It seems as if the water deficit conditions caused the berries to accumulate water rapidly after irrigation. The berries seemed more sensitive to irrigation after the water deficit period. The fully irrigated and non-irrigated vines seemed to manage their water accumulation; berry water content was kept constant through the season before water loss and berry shrinkage occurred. The water relations were reflected in the berry size. Transpiration losses were probably much higher in fully irrigated vines, whereas stomatal control efficiently maintained water relations in non-irrigated vines.

The water content in the seeds, skin and pulp was reduced towards the end of the season. The vegetative organs also experienced water loss through the season, but the water content increased at the end of the season, probably due to a lower demand for water from the largely senescing canopy and changing environmental conditions (e.g. lower temperatures). The vine therefore recuperated in terms of water relations, seemingly irrespective of soil water availability.

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Chapter IV

RESEARCH RESULTS

VEGETATIVE AND REPRODUCTIVE GROWTH OF SHIRAZ/RICHTER 99 GRAPEVINES AS AFFECTED BY WATER STATUS DURING BERRY RIPENING

ABSTRACT

In this study, grapevine water relations during the berry ripening period, under the influence of various irrigation strategies were investigated, in an attempt to quantify the effect of water status on vegetative and reproductive growth in a Shiraz/Richter 99 vineyard. Comparisons based on certain vegetative and reproductive growth parameters during ripening were made between different irrigation strategies (no irrigation; and irrigation at all phenological stages; at véraison and post véraison; and at post véraison). Full irrigation seemed to stimulate primary shoot length. With longer water deficit, earlier and more complete shoot maturation (reserve accumulation) was induced. The rate of development and position of occurrence of secondary shoots were affected by irrigation. Water deficit (seasonal and post véraison irrigated) seemed to induce fewer, but longer, secondary shoots in basal parts of primary shoots. Re-distribution of leaf area on the shoot seemed to occur when vines were subjected to water deficit conditions. Irrigation during ripening seemed to induce a continuation of berry water loss, whereas extended water deficit seemed to induce earlier and restricted water loss. Full irrigation treatment during the season induced larger berry skin surface. The highest skin:pulp ratio occurred for the post véraison irrigation treatment.

INTRODUCTION

Water is an important factor influencing vegetative and reproductive growth. Most farmers use non-deficit irrigation programs, meaning that the soil is simply saturated with water, resulting in unrestricted plant growth (Coggan, 2002). Unrestricted growth results in an increase in berry size and reduced wine quality. In such cases, excess vegetation has to be removed by topping and leaf removal in order to improve canopy microclimate. Water deficit may reduce the size of grapevine canopies, decreasing labour costs and facilitating the obtainment of an optimal canopy microclimate.

All over the world, wine industries aim to produce grape and wine quality suited to meet the increasing national and international requirements. The need to manipulate berry size has increased (McCarthy, 2000), particularly in so-called warm wine producing countries, facilitating the buffering of pH increases and resulting negative effects on wine quality. Small berries are considered a key component of grape

quality (Bravdo *et al*., 1985; McCarthy, 2000; Kennedy *et al*., 2002) for cultivars varieties such as *Vitis vinifera* L. cv. Shiraz (McCarthy, 2000).

Manipulation of vegetative and/or reproductive growth to maintain or to enhance wine grape quality without adversely affecting yield would be a practical benefit to many viticulturists (McCarthy, 1997). A possible method of manipulating berry size is by controlling the soil water availability during berry development. Water deficit has inhibitory effects on vegetative and reproductive growth and alters the phenology (Coombe, 1992). Inadequate water will reduce the length of the vegetative growing season, and induce premature leaf fall and smaller leaves (Fanizza & Ricciardi, 1990), thus reducing leaf area formation, which may lead to reduced yields (Bravdo *et al*., 1972; Miller *et al*., 1996b). Plant productivity, measured as the amount of dry matter produced, depends directly on leaf surface and photosynthetic activity (Bravdo *et al*., 1972; Miller *et al*., 1996b). In water stressed vines, photosynthetic activity is reduced because of stomatal closure (Dűring, 1990; Schultz, 1996).

Water stressed plants with lower photosynthesis, together with the reduced leaf area, result in lower production compared to vines not subjected to water deficit (Gomez-del-Campo *et al*., 2002). According to Mullins *et al*. (1992), the grape bunches become the second strongest carbohydrate sink after véraison. Vigorous vines can often continue producing leaf area after véraison (Miller *et al*., 1996a). According to Hunter & Visser (1988), the apical, middle and basal leaves translocate their photosynthetates mainly to the bunches from berry set up to véraison. After véraison, the bunches were still highly nourished by the basal leaves.

Varying soil water status leads to leaves and bunches developing in different conditions, changing from heavily shaded to exposed canopies. According to Hasselgrove *et al*. (2000), berries developing in well-exposed canopies, as opposed to those developing in heavily shaded canopies, have higher must soluble solid concentration, lower pH, higher titratable acidity and less incidence of unripe flavours. By reducing berry size, bunches would be less compact. A more open bunch framework would expose a greater surface area of such berries to sunlight. Higher sunlight levels within and around the bunch may improve the colour of grape berries (Smart, 1982).

Phenolic compounds are mainly localised in the skin and seeds of the grape berry (Ojeda *et al*., 2002). In the case of red grape varieties, the skin is particularly rich in flavonols and anthocyanins. The composition of phenolics depends on the cultivar, and is influenced by viticultural and environmental factors (Brossaud *et al*., 1999). The phenolic concentration of the must is indirectly affected by the final size of the grape berry, in that this concentration depends on the skin surface:berry volume ratio (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004). According to Hardie & Considine (1976), Van Zyl (1984) and Sipiora & Gutiérrez-Granda (1998), the supply of water to the grapevine is an environmental factor affecting berry size.

Although the effect of water deficit on vegetative and reproductive growth is largely known, the timing of inducing water deficit for obtaining the optimum result, in combination with ripeness level, has not been systematically investigated. In view of this, an experiment was conducted over two seasons with different irrigation treatments and sampling dates, to determine an optimal irrigation strategy and harvest date for favourable vegetative and reproductive growth characteristics.

MATERIALS AND METHODS

Experimental vineyard

A seven-year-old *Vitis vinifera* L. cv. Shiraz (clone SH1A), grafted onto Richter 99 (*Vitis Berlandieri x Vitis rupestris*) (clone RY2A) was used for this study. The experimental vineyard is situated on the Experiment farm of ARC Infruitec-Nietvoorbij in the Stellenbosch Region, Western Cape. The area is characterised by a Mediterranean climate. The vines are spaced 2.75 m x 1.5 m on a Glenrosa soil with a western aspect (26º slope) and orientated in a North-South direction. The vines are trained onto a 7-wire lengthened Perold trellising system (VSP) of which three sets of wires are movable. Vines were pruned to two-bud spurs with a spur spacing of approximately 15 cm. Canopies were suckered, shoot positioned and tipped/topped during the pre-véraison period. Irrigation was applied through a micro-sprinkler system.

Treatments and layout

Four treatments, comprising irrigation combinations to field water capacity at different stages, were applied (field water capacity of the soil was determined before the start of the experiment). The treatments were completely randomised in two blocks,

representing two replications. Thirty vines were used per replication. The treatments were: (i) seasonal irrigation from berry set with further irrigation at pea size, (ii) véraison and one month post véraison, (iii) irrigation at véraison with further irrigation at post véraison, (iv) irrigation at post véraison and (v) no irrigation. The sampling of the treatments was split into five stages: véraison, one month after véraison, and three times during the ripening period.

Measurements

Vegetative parameters: Five randomly selected shoots from the thirty vines per replicate were used for each treatment and replicate at each ripening stage. The primary shoots were divided into three categories: basal, middle and apical. The measurements of the primary and secondary leaves and shoots, were taken in these three parts. Primary and secondary shoot length (cm) and mass (g), and number of secondary shoots, were measured. The number of primary and secondary leaves per shoot, primary and secondary leaf area (cm^2), and primary and secondary leaf mass (g), were determined. Primary and secondary leaf area (cm^2) was determined by means of a Li-cor LI-3100 leaf area meter.

Reproductive parameters: All bunches on the sampled shoots were used. Bunch size (length and shoulder width), mass (g), volume (cm^3), and number of bunches, were determined. The berries were separated after which the number, mass (g), and volume (cm^3) of the berries and rachis were determined. Surface area of the skin (cm^2) and mass (g) of the skin, pulp and seeds were also determined.

Berry mass (g) were measured by determining the average of 200 randomly selected berries. Skin, pulp and seed mass (g) were determined by separating the skin, pulp and seeds of 50 randomly selected berries. The skin:pulp ratio was obtained by dividing the fresh mass of the skins (average of the 50 berries) by the separated mass of the pulp. These skins were also used to determine the surface area (cm^2). This was done by spreading the skins open and laying them flat on a transparency paper. The area (cm^2) was then determined by means of the Li-cor leaf area meter.

Statistical procedures

A random split-plot experiment was performed with main plot treatments as four irrigations (full irrigation; véraison and at post véraison irrigation; post véraison irrigation; and no irrigation), replicated randomly within each of the two blocks. The

sub-plot treatments were five different stages of ripening (véraison, one month after véraison, and three further stages approximately two weeks apart). The whole experiment was repeated over two seasons on the same experimental plots. The repeated measurements for the two seasons were considered as sub-sub-plot treatments (Little & Hills, 1972). The appropriate analyses of variance were performed on all the variables measured (SAS Institute, Inc., 1999).

Shapiro-Wilk test was performed to test for non-normality of the residuals (Shapiro & Wilk, 1965). Deviation from normality was mainly due to kurtosis and not skewness; the data were therefore considered as reliable (Glass *et al*., 1972). Students' t-LSD (least significant difference) was calculated at a 5% significance level to compare means of significant effects (Snedecor & Cochran, 1967).

RESULTS

Primary shoot growth

Shoot length: The primary shoot length of the treatments differed between seasons 2004-2005 and 2005-2006 (Fig. 1). The primary shoot length in season 2004-2005 was longer than in season 2005-2006 for all treatments. Shoot lengths of vines under full irrigation during season 2004-2005 were statistically longer than those of the vines of the no irrigation and post véraison irrigation treatments. During season 2005-2006 no significant differences were found. The primary shoots of the fully irrigated vines were significantly longer than those of the non-irrigated vines (Fig. 2). The average primary shoot length measured over two seasons harvested at different times of ripening indicated irregularities in shoot length (Fig. 3). At véraison and post véraison there were no significant differences between the shoot lengths, whereas irregular trends occurred there after.

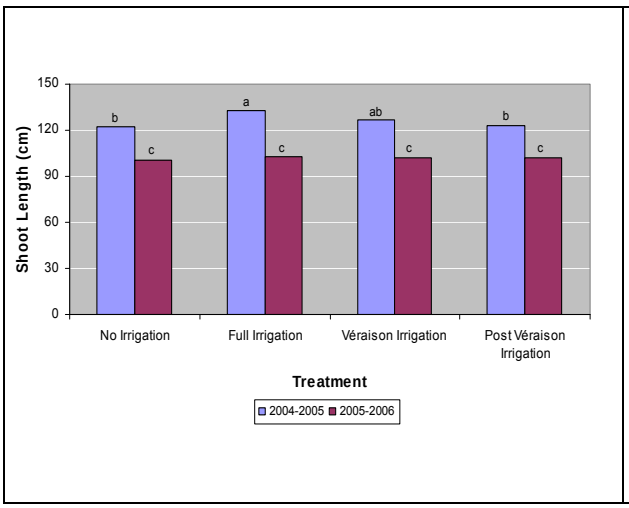

Fig. 1. Average length of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

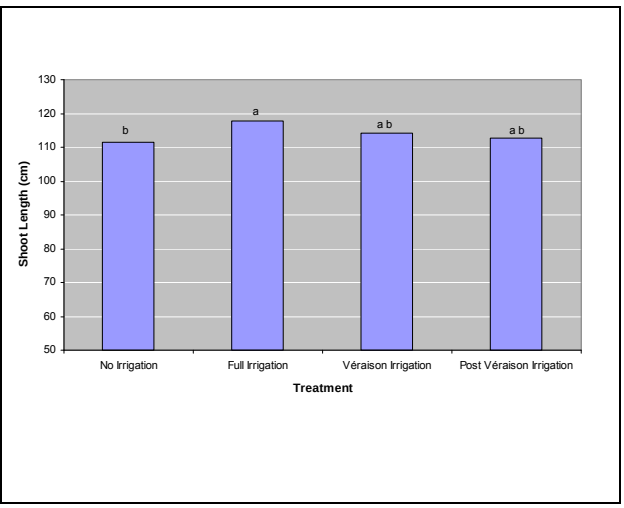

Fig. 2. Average primary shoot length over two seasons for different treatments.

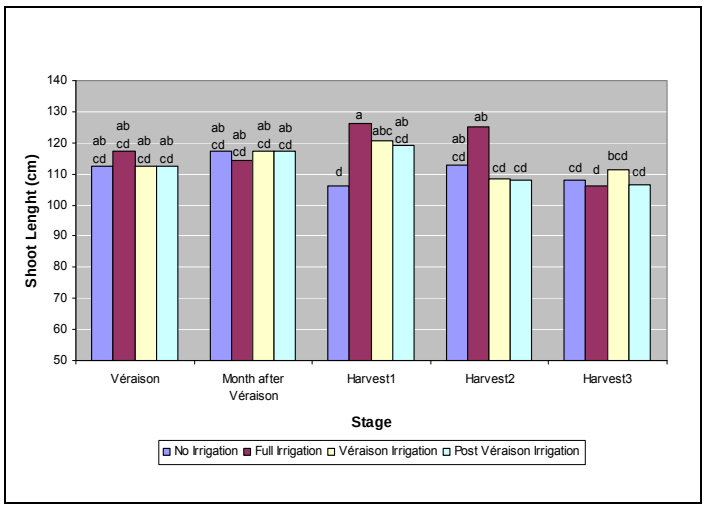

Fig. 3. Average length of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

Shoot mass: The mass of the basal, middle and apical primary shoot parts of the treatments differed between seasons 2004-2005 and 2005-2006, with a lower mass occurring in the latter season (Fig. 4, 5 & 6). No statistical significant differences in the average shoot mass for the basal, middle and apical parts of the primary shoots were found between treatments; basal parts were, however, clearly higher in mass (Fig. 7).

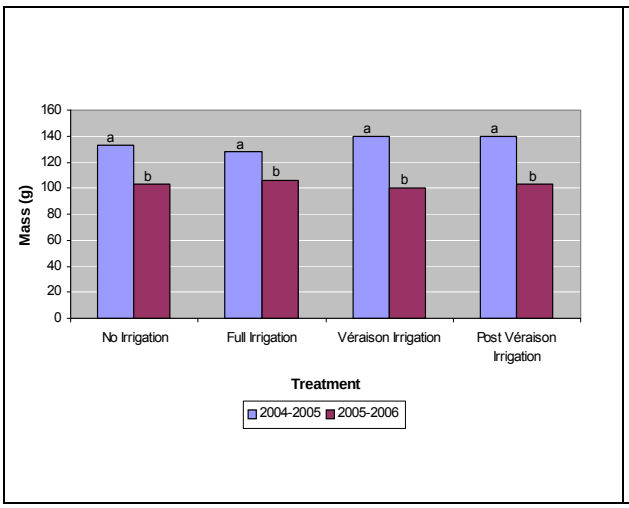

Fig. 4. Average mass for basal parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

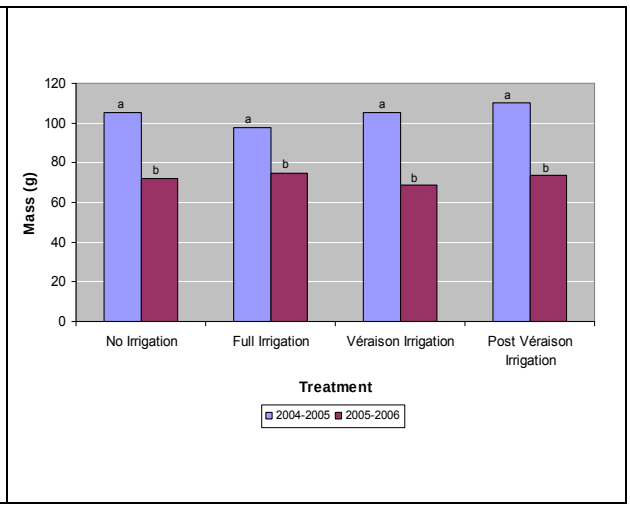

Fig. 5. Average mass for middle parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

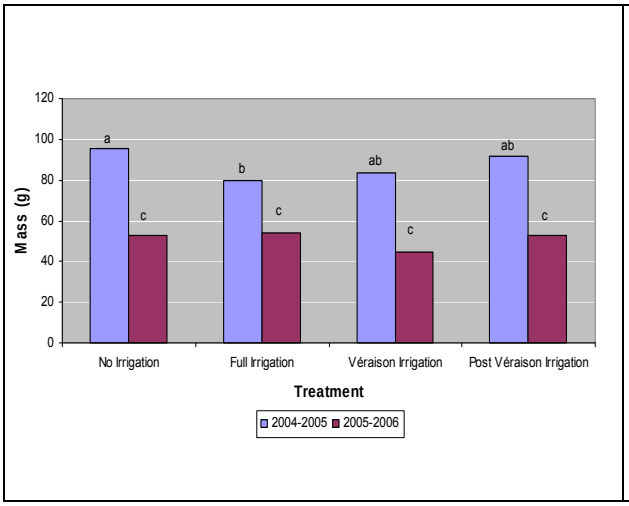

Fig. 6. Average mass for apical parts of primary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

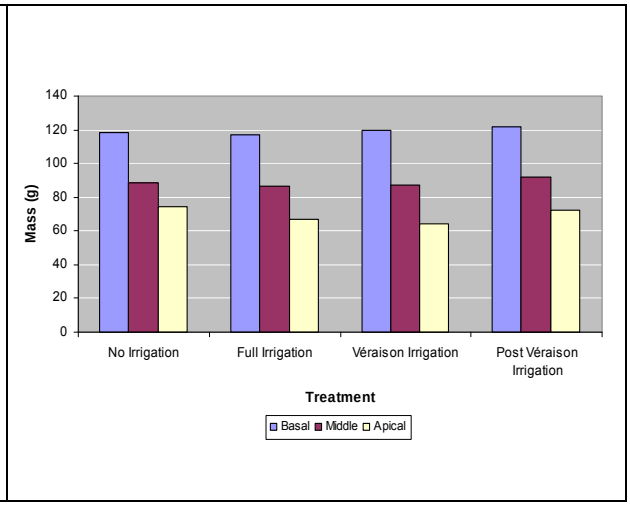

Fig. 7. Average mass for basal, middle and apical parts of primary shoots over two seasons for different treatments.

The average primary shoot mass measured between two seasons and harvested at different times of ripening showed irregular trends, but with a reduction in mass as the season progressed. Faster growth of vines receiving full irrigation probably led to longer primary shoots (Fig. 3), but with lower mass (Figs. 8, 9 & 10) at véraison. This difference was largely nullified as the season progressed. Re-growth seemed to occur again at the last harvest stage for the vines that received the full irrigation.

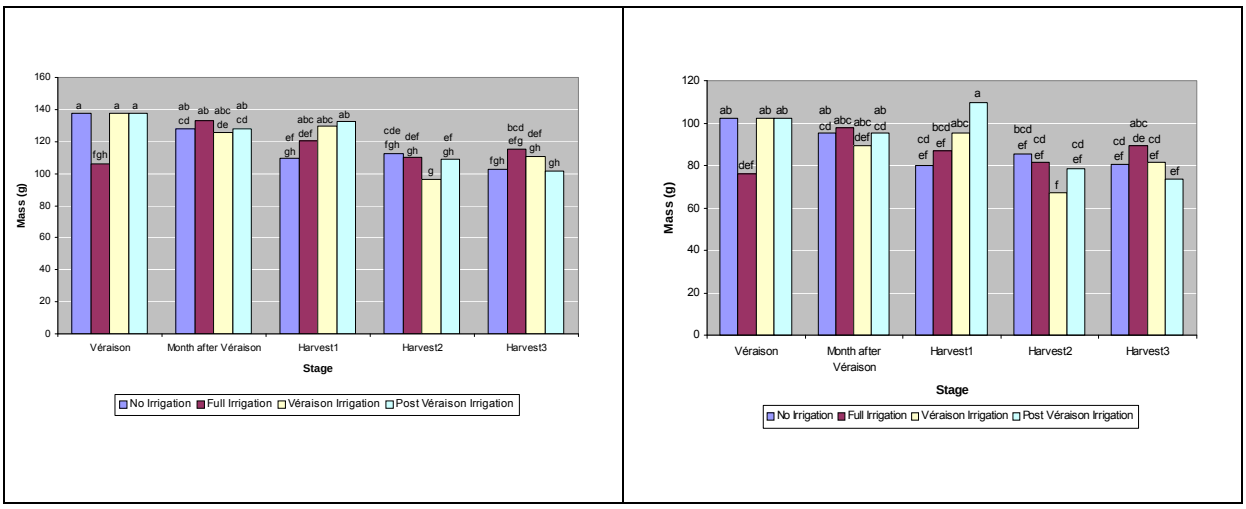

Fig. 8. Average mass for basal parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

Fig. 9. Average mass for middle parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

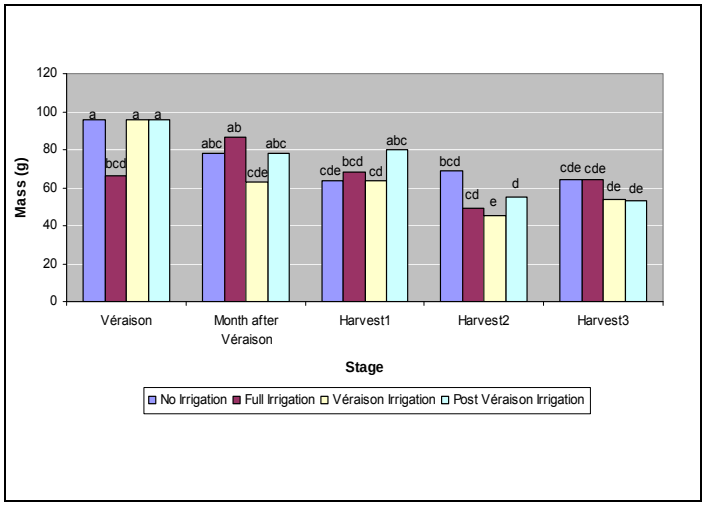

Fig. 10. Average mass for apical parts of primary shoots over two seasons harvested at different stages of ripening and for different treatments.

Secondary shoot growth

Number of shoots: The number of secondary shoots of the treatments seemed to be higher in 2004-2005 than in 2005-2006 (Figs. 11, 12, 13 & 14). The average number of secondary shoots tended to be higher with full irrigation (Fig. 15).

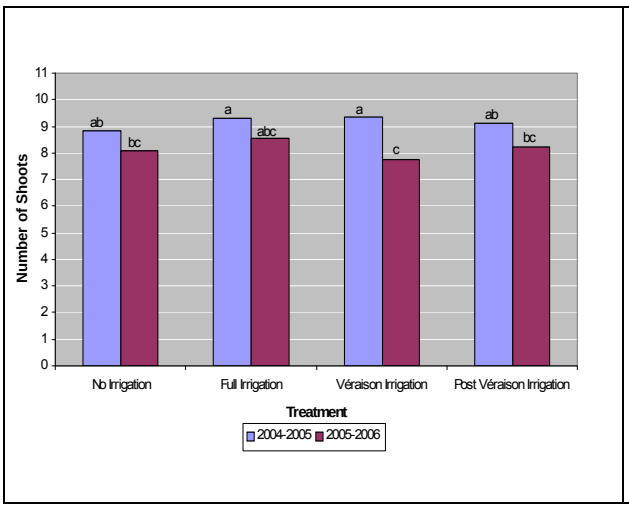

Fig. 11. Average number of secondary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

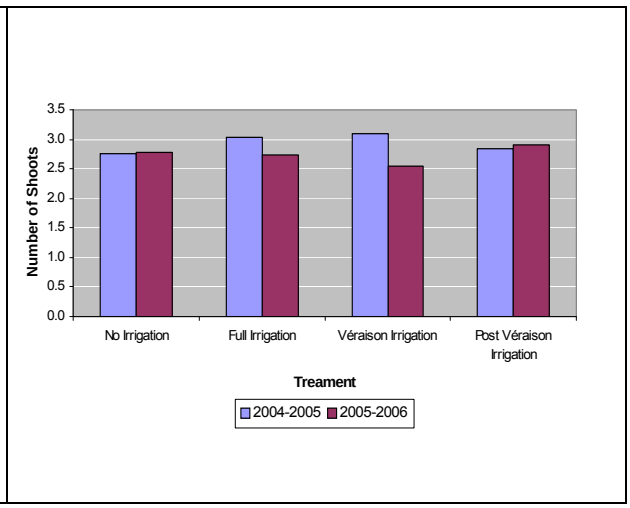

Fig. 12. Average number of secondary shoots on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

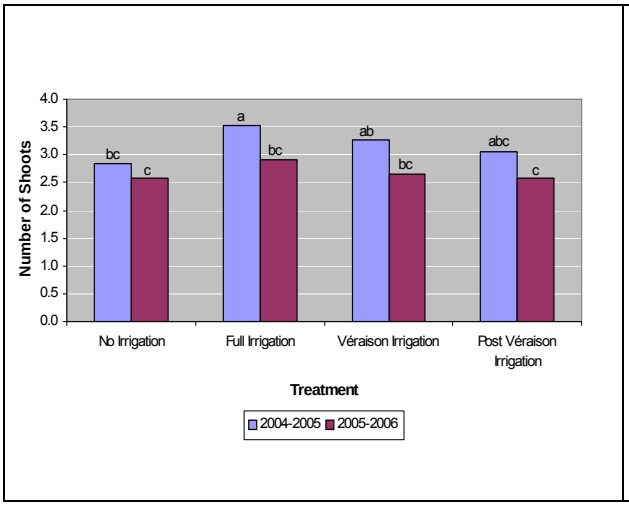

Fig. 13. Average number of secondary shoots on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

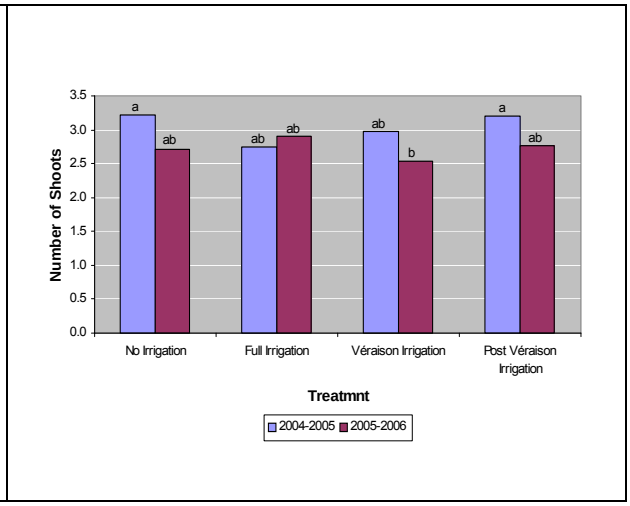

Fig. 14. Average number of secondary shoots on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

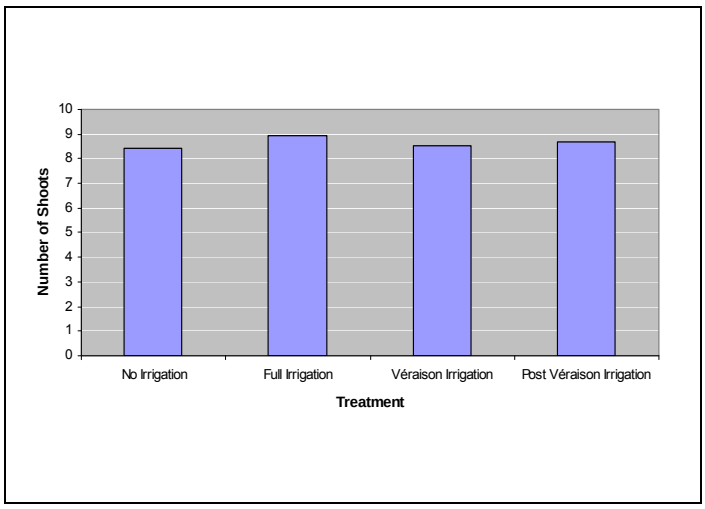

Fig. 15. Total number of secondary shoots over two seasons for different treatments.

The average number of secondary shoots on the basal, middle and apical parts of primary shoots indicated higher values for particularly the middle parts of the full irrigation treatment (Figs. 16, 17, 18, 19 & 20). The latter treatment also had higher values during the early ripening period (Fig. 17). During the later ripening period, the deficit irrigation treatments apparently had higher values, probably because of slower development.

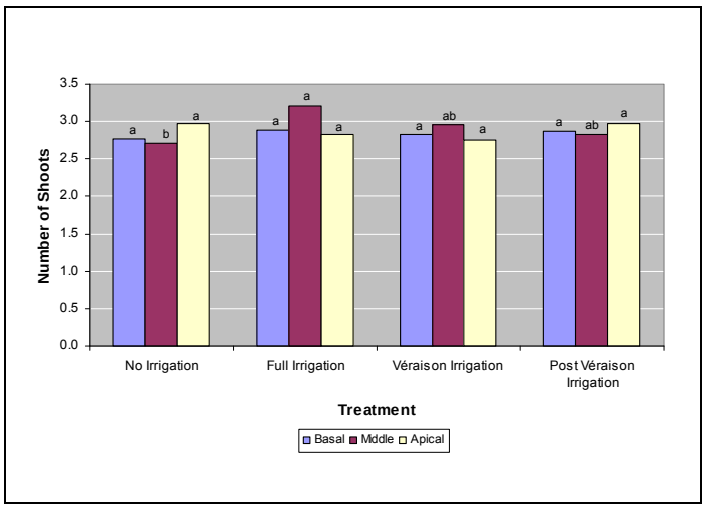

Fig. 16. Average number of secondary shoots on basal, middle and apical parts of shoots over two seasons for different treatments.

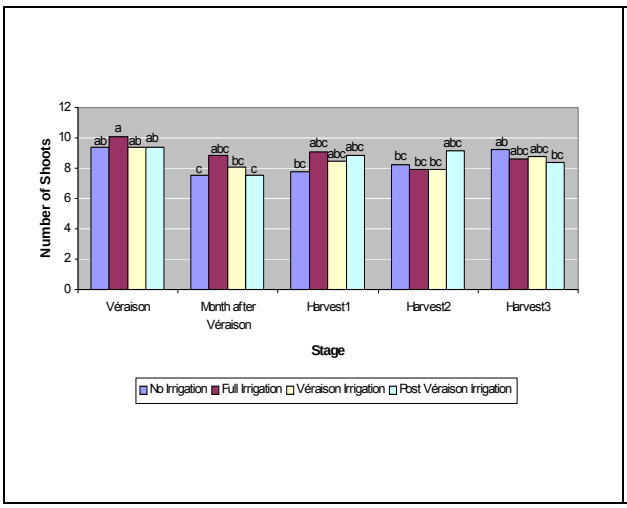

Fig. 17. Average number of secondary shoots over two seasons harvested at different stages of ripening and for different treatments.

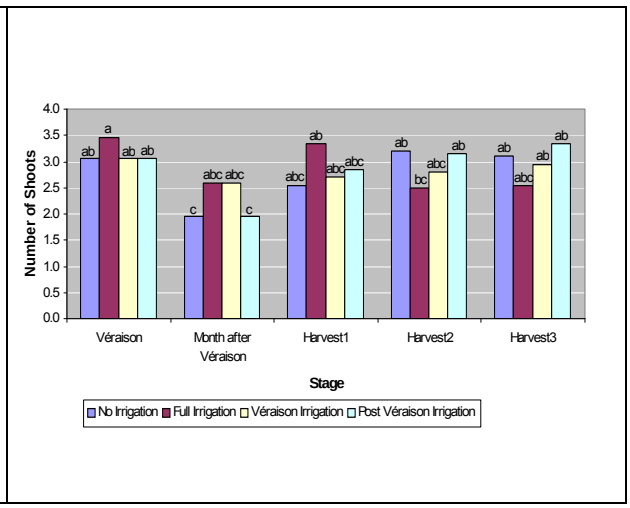

Fig. 18. Average number of secondary shoots on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

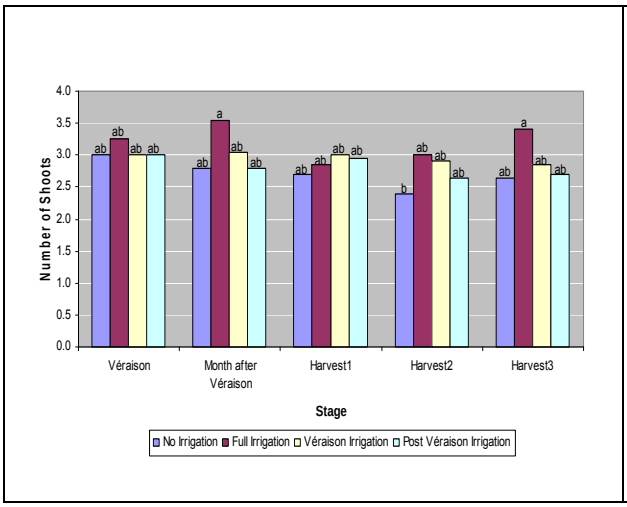

Fig. 19. Average number of secondary shoots on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

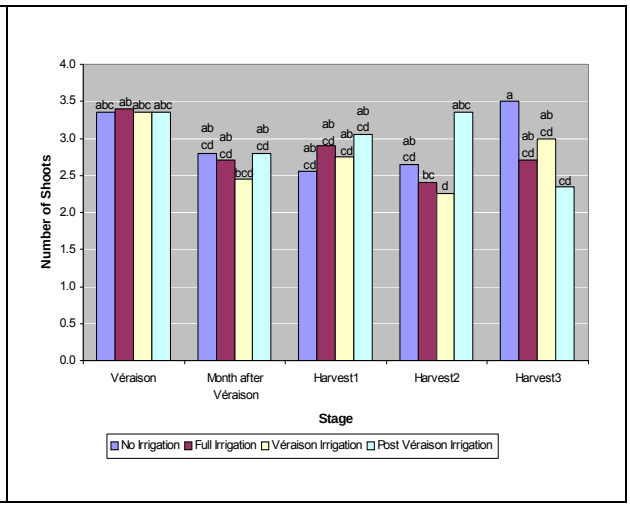

Fig. 20. Average number of secondary shoots on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Shoot length: The secondary shoots were significantly longer in season 2004-2005 (Fig. 21). The difference mainly occurred for basal and middle parts of the primary shoots, displaying higher values of secondary shoot length during the 2004-2005 season (Figs. 22, 23 & 24).

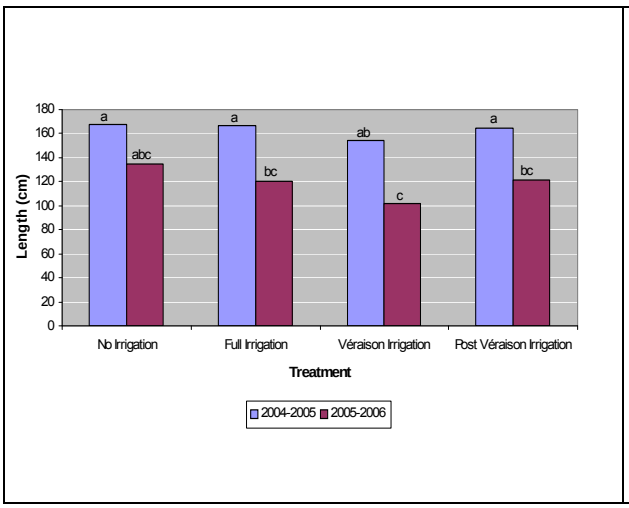

Fig. 21. Average length of secondary shoots for seasons 2004-2005 and 2005-2006 for different treatments.

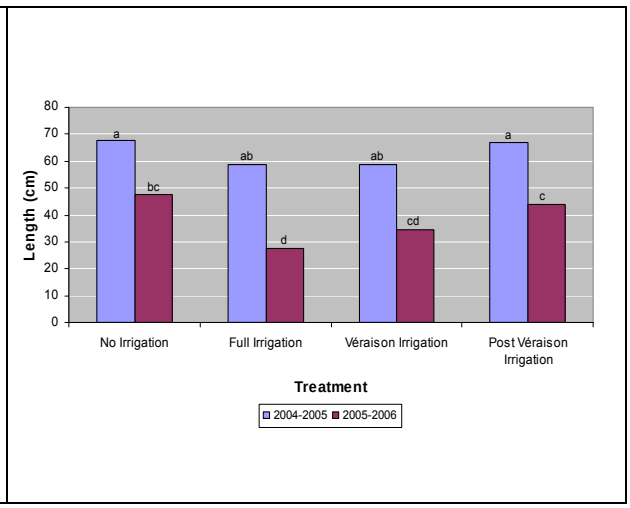

Fig. 22. Average length of secondary shoots on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

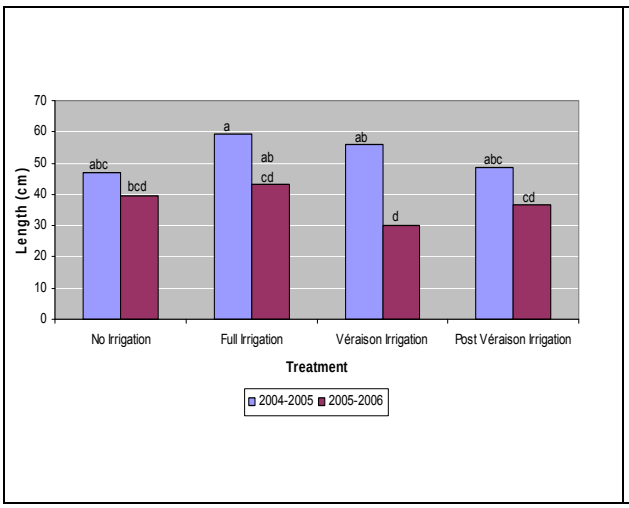

Fig. 23. Average length of secondary shoots on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

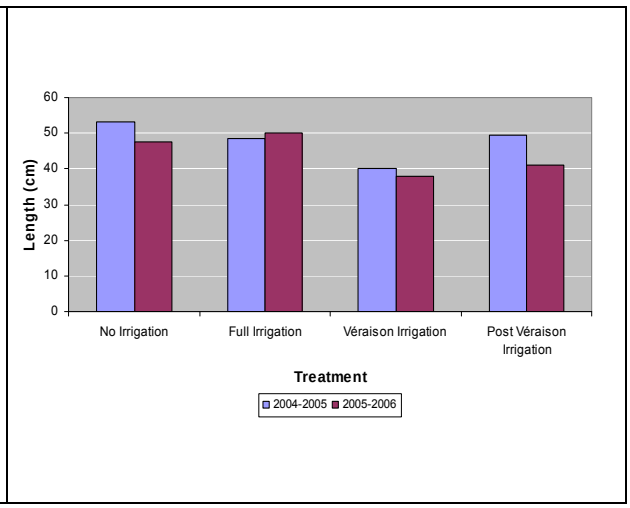

Fig. 24. Average length of secondary shoots on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

There were no significant differences in the total length of the secondary shoots between the treatments (Fig. 25). The longest secondary shoots were found for non-irrigated vines and the shortest for véraison+post véraison-irrigated vines (Fig. 25 & 26).

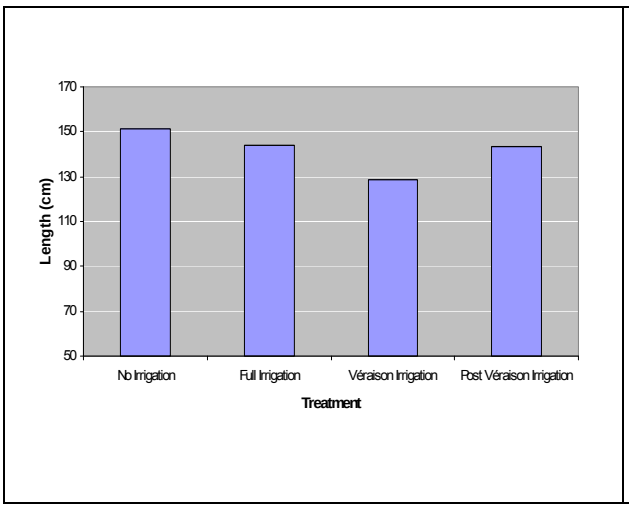

Fig. 25. Total length of secondary shoots over two seasons for different treatments.

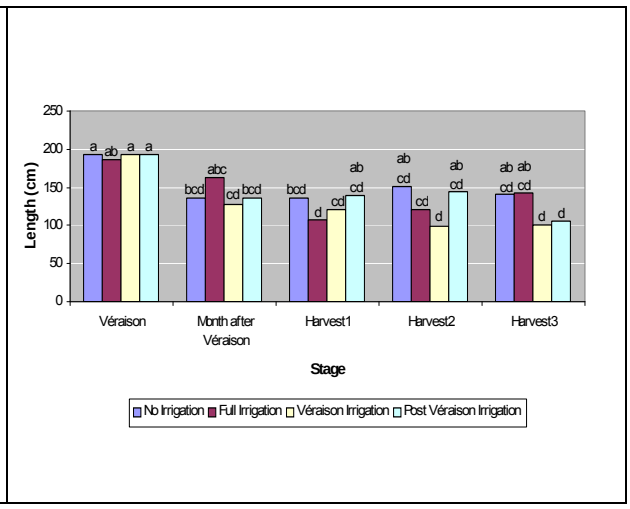

Fig. 26. Average length of secondary shoots over two seasons harvested at different stages of ripening and for different treatments.

The full irrigation treatment displayed generally higher values in the middle and apical positions on the primary shoots, whereas the deficit irrigation treatments had higher values in the basal part of the primary shoot (Figs. 27, 28, 29 & 30). The secondary shoots of middle parts of primary shoots seemed to continue to grow until the end of the ripening period when fully irrigated.

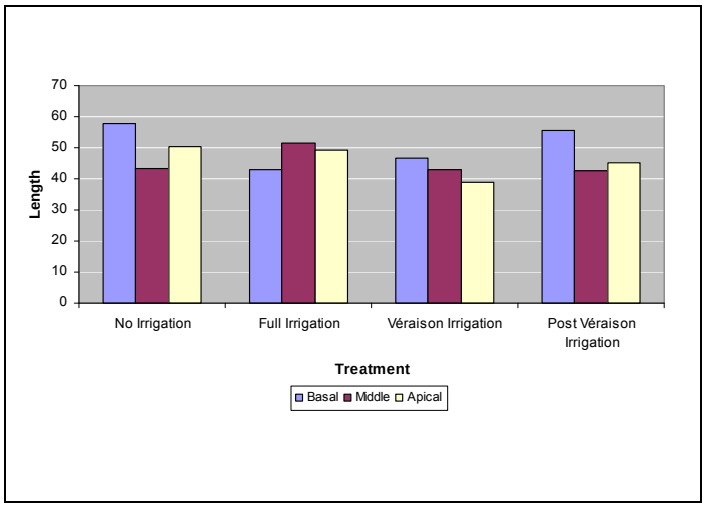

Fig. 27. Average length of secondary shoots on basal, middle and apical parts of shoots over two seasons for different treatments.

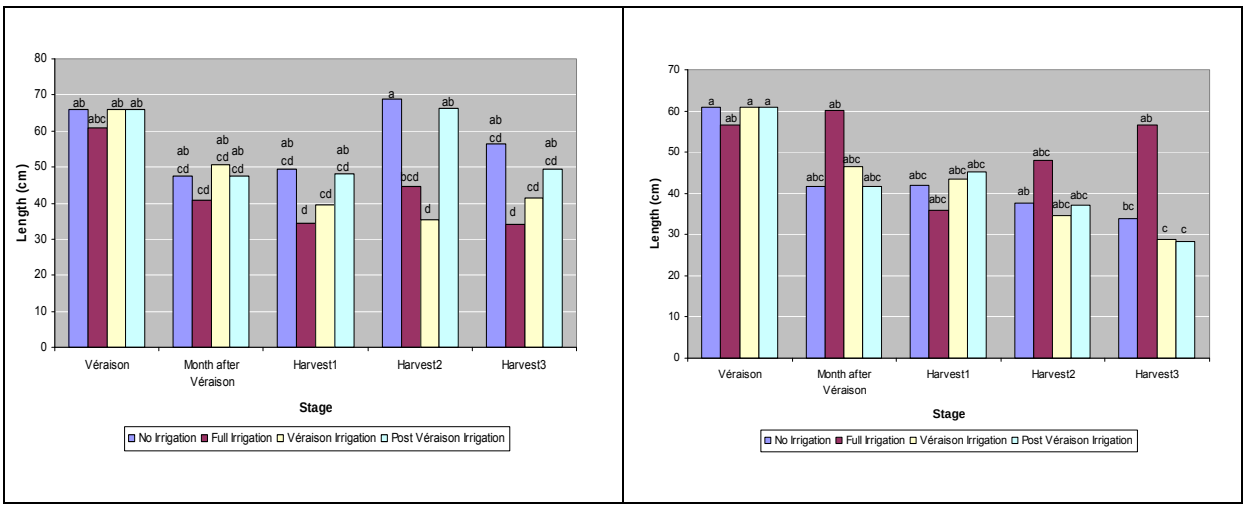

Fig. 28. Average length of secondary shoots on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Fig. 29. Average length of secondary shoots on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

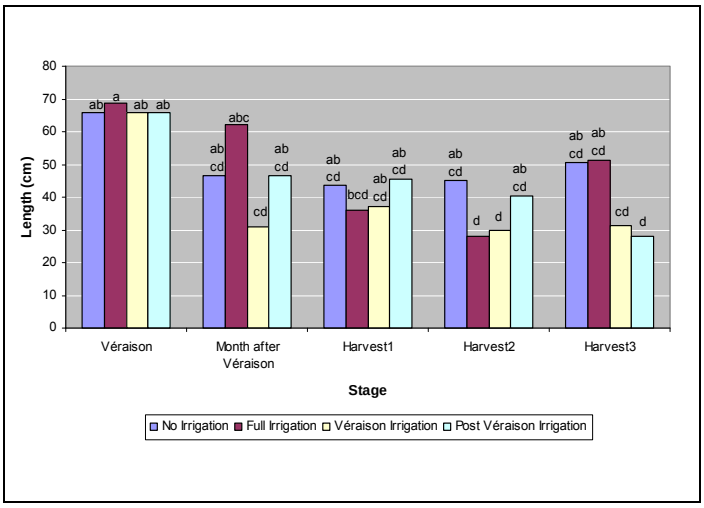

Fig. 30. Average length of secondary shoots on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Shoot mass: The secondary shoot mass on the basal, middle and apical parts of the primary shoots were statistically higher in seasons 2004-2005 (Fig. 31, 32 & 33). The full irrigation treatment seemed to have lowest overall secondary shoot mass in basal parts, but highest in middle and apical parts of primary shoots. This was also clear from the average mass over seasons (Fig. 34). Secondary shoot mass of water deficit treatments seemed restricted in the middle part of primary shoots.

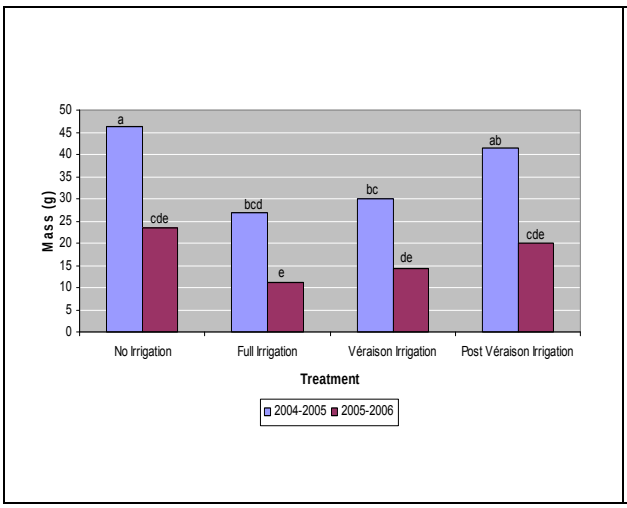

Fig. 31. Average mass of secondary shoots on basal parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

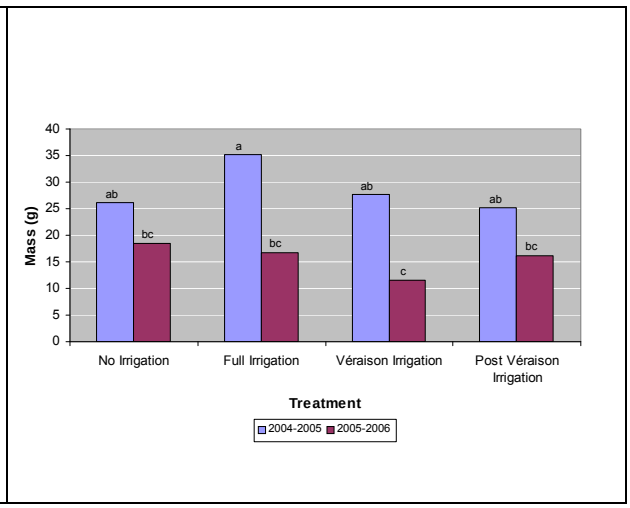

Fig. 32. Average mass of secondary shoots on middle parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

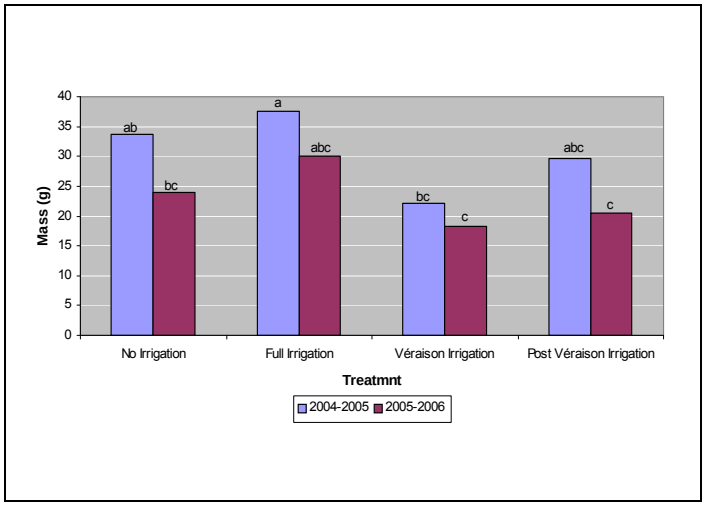

Fig. 33. Average mass of secondary shoots on apical parts of shoots for seasons 2004-2005 and 2005-2006 for different treatments.

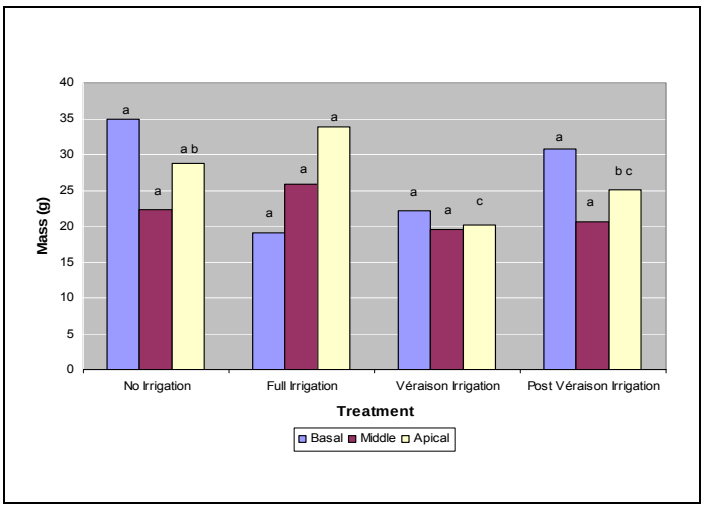

Fig. 34. Average mass of secondary shoots on basal, middle and apical parts of shoots over two seasons for different treatments.

A decreasing trend with progress of the ripening period was evident for secondary shoot mass in all areas on the primary shoot (Figs. 35, 36 & 37). Compared to the full and no irrigation treatments, the véraison+post véraison and post véraison irrigation treatments seemed to increasingly loose secondary shoot mass as the ripening period progressed. Re-growth may have occurred for the full irrigation treatment during late ripening.

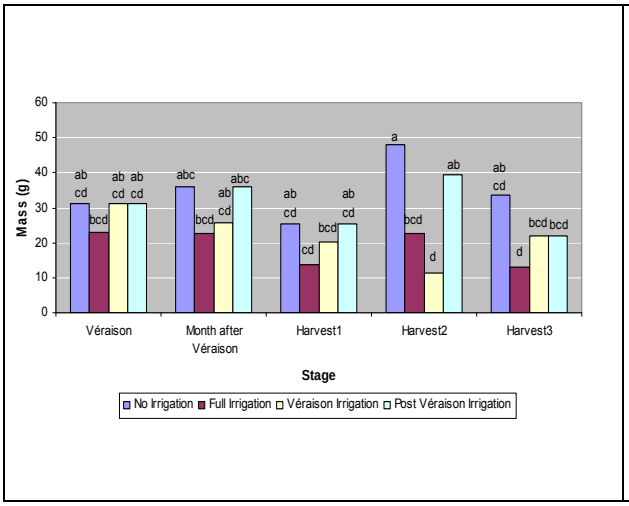

Fig. 35. Average mass of secondary shoots on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

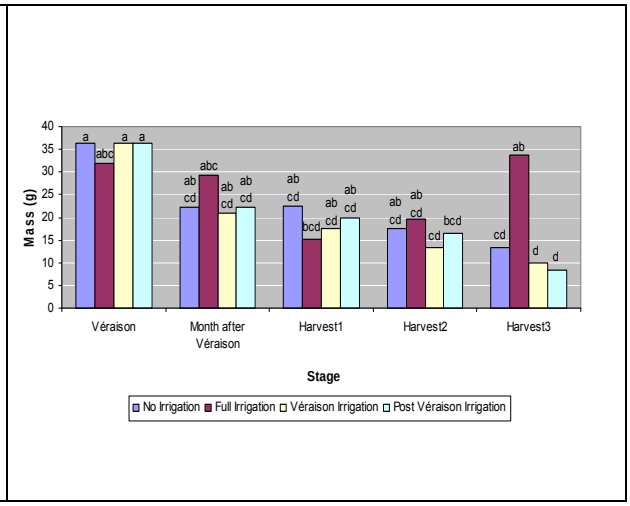

Fig. 36. Average mass of secondary shoots on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

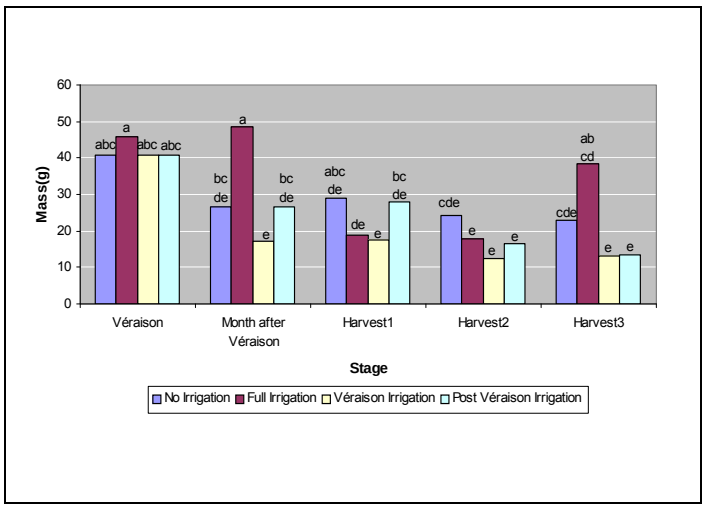

Fig. 37. Average mass of secondary shoots on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

Primary leaves

Number of leaves: In both the 2004-2005 and 2005-2006 seasons, more primary leaves were found on vines that received the full irrigation treatment (Figs. 38 & 39). No differences were found between the rest of the treatments.

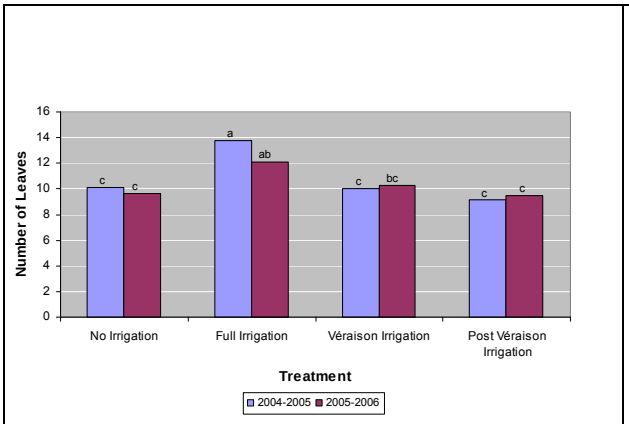

Fig. 38. Average number of primary leaves for seasons 2004-2005 and 2005-2006 for different treatments.

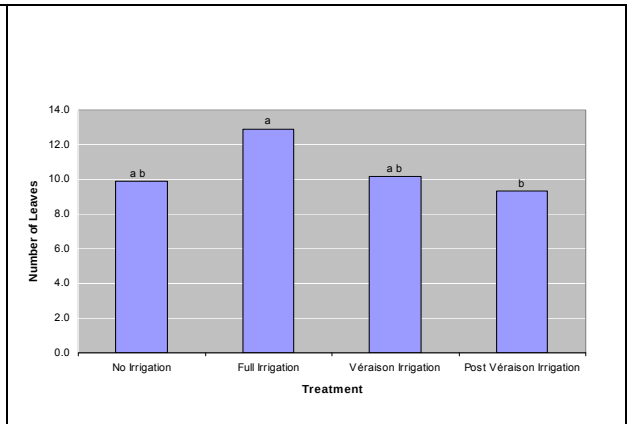

Fig. 39. Total number of primary leaves over two seasons for different treatments.

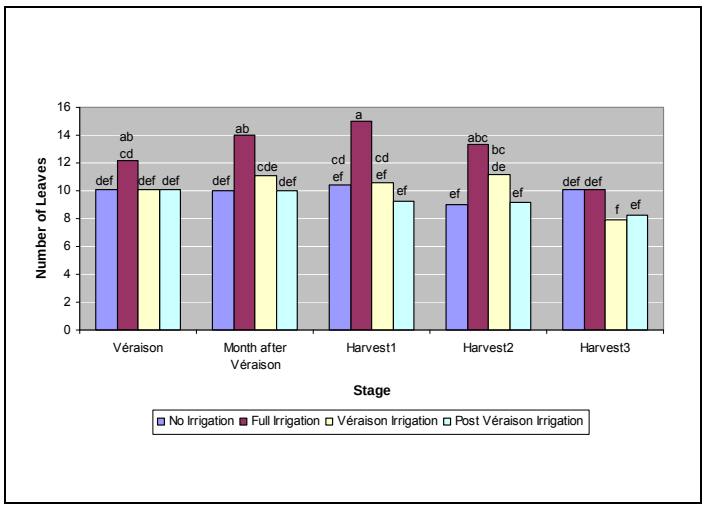

Fig. 40. Average number of primary leaves over two seasons harvested at different stages of ripening and for different treatments.

A decreasing trend was evident as the season progressed (Fig. 40). In accordance with the primary shoot length (Fig. 3), the number of primary leaves of the full irrigation treatment was highest at the first harvest stage, indicating that the shoots continued to grow well into the ripening period. Leaves, however, quickly abscised after this stage.

Leaf area: The primary leaf area of the treatments differed between seasons 2004-2005 and 2005-2006, the former resulting in significantly higher values (Fig. 41). In accordance with shoot length (Fig. 3) and number of primary leaves (Fig. 38), largest primary leaf area was found in the first season with full irrigation (Fig. 41). This was also evident from the average over seasons (Fig. 42). When the treatments were harvested at different times during ripening, a decrease in leaf area could clearly be observed during particularly the last two stages of ripening (Fig. 43). This was more severe for the véraison+post véraison and post véraison irrigation treatments. The apparent recuperation in terms of water potential during the last stage of harvest (Chapter 3), may have contributed to maintaining the leaf area for longer.

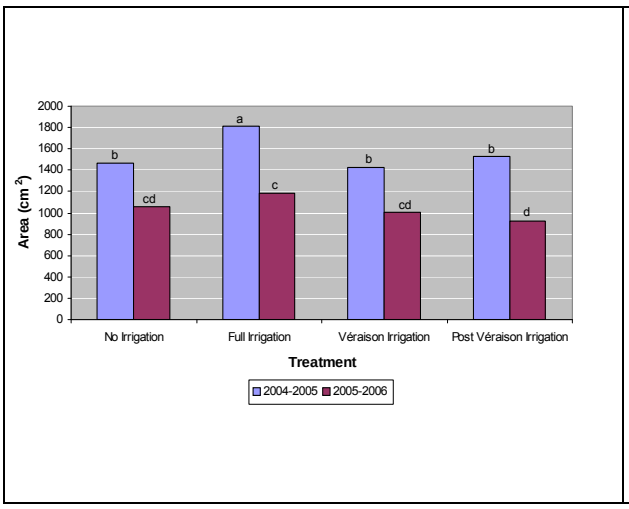

Fig. 41. Average area of primary leaves for seasons 2004-2005 and 2005-2006 for different treatments.

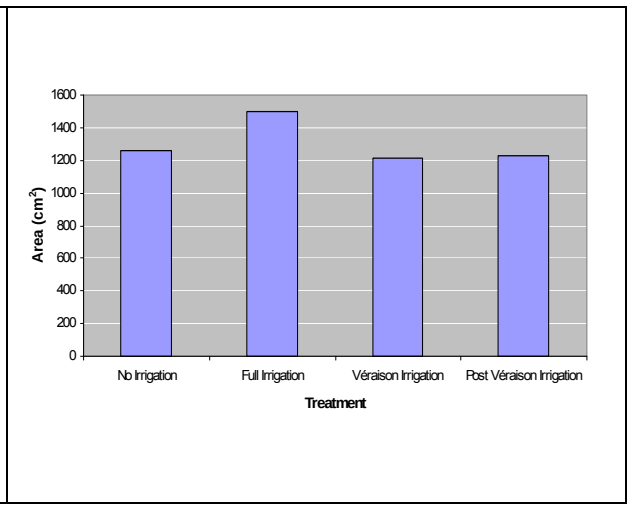

Fig. 42. Total area of primary leaves over two seasons for different treatments.

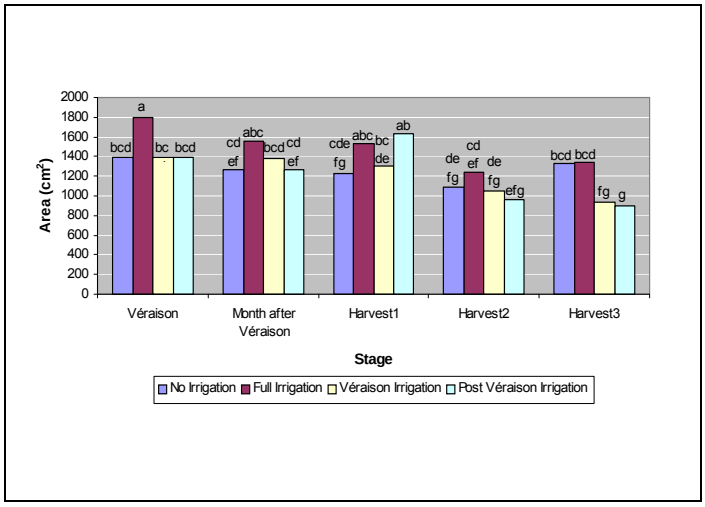

Fig. 43. Average area of primary leaves over two seasons harvested at different stages of ripening and for different treatments.

Leaf area:leaf mass ratio: There were no significant differences between treatments for the primary leaf area:leaf mass ratio (Fig. 44). The average leaf area:leaf mass ratio over seasons decreased from véraison to one month after véraison, but increased again there after and stayed high during all the harvest stages (Fig. 45). This may have occurred as a result of leaf senescence and the general reduction in leaf water content during this time (Chapter 3).

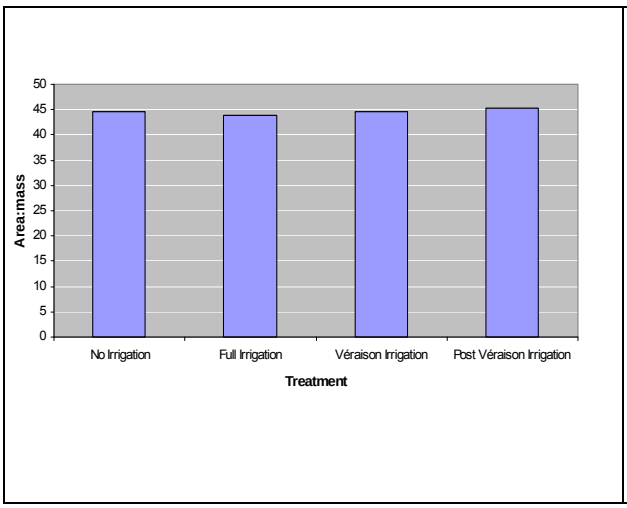

Fig. 44. Total area:mass of primary leaves over two seasons for different treatments.

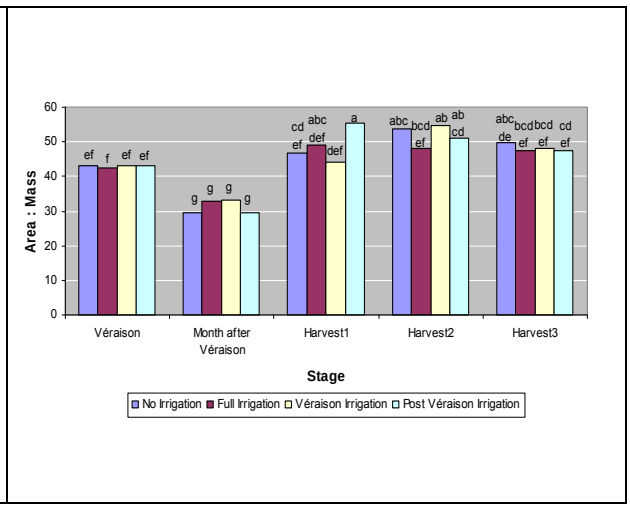

Fig. 45. Average area:mass of primary leaves over two seasons harvested at different stages of ripening and for different treatments.

Secondary leaves

Number of leaves: The number of secondary leaves differed only slightly between seasons (Fig. 46). The full irrigation treatment had significantly more secondary leaves than the other treatments (Fig. 47).

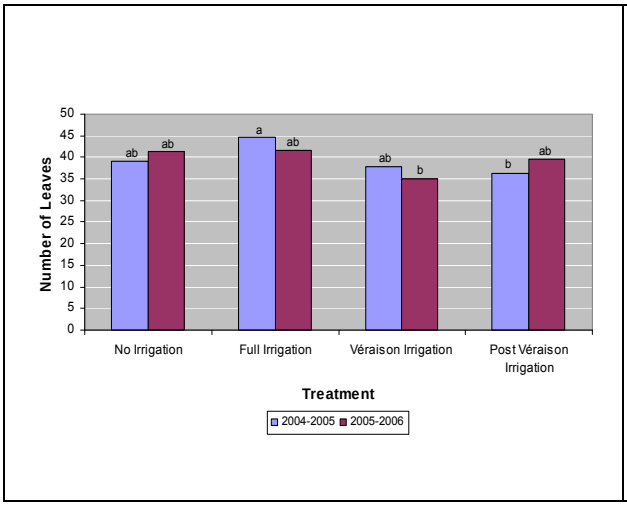

Fig. 46. Average number of secondary leaves for seasons 2004-2005 and 2005-2006 for different treatments.

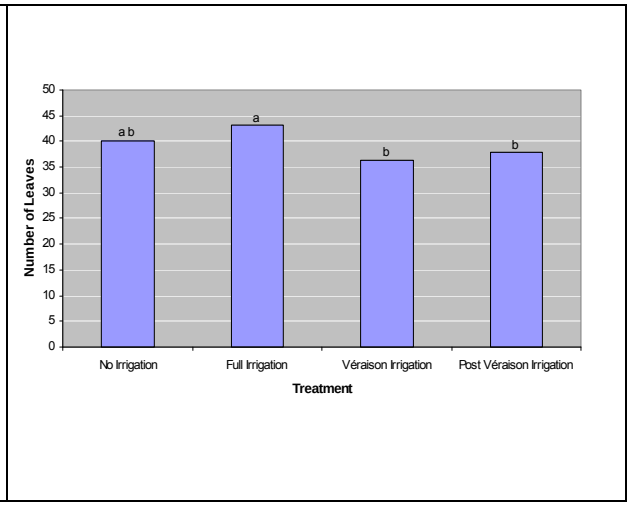

Fig. 47. Total number of secondary leaves over two seasons for different treatments.

The number of secondary leaves already showed a decline at a month after véraison (Fig. 48). The secondary shoots apparently started growing again late during ripening, although this was not evident from the length of the shoots (Chapter 3). Similar patterns were found in the basal, middle and apical regions of the primary shoots (Figs. 49, 50 & 51).

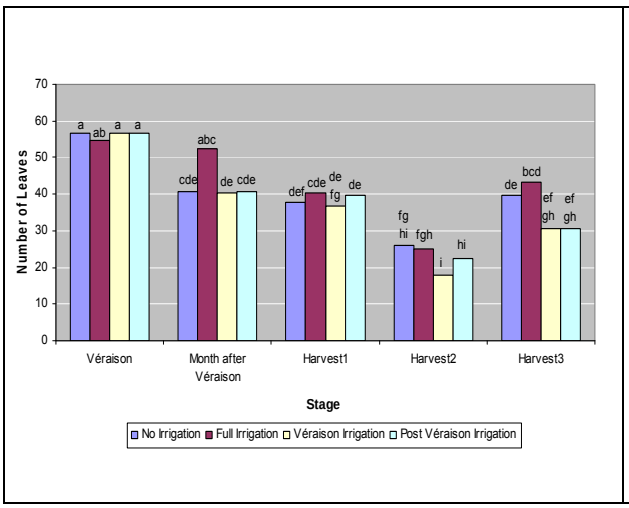

Fig. 48. Average number of secondary leaves over two seasons harvested at different stages of ripening and for different treatments.

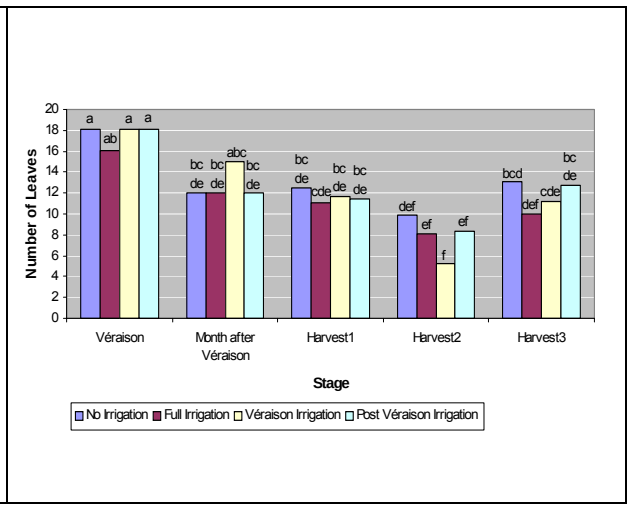

Fig. 49. Average number of secondary leaves on basal parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

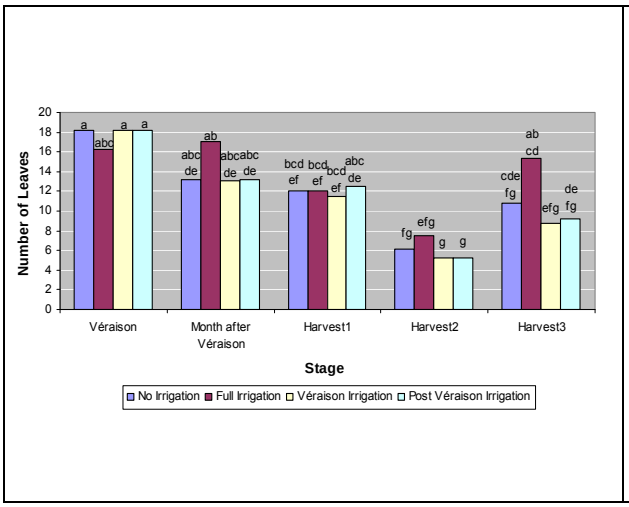

Fig. 50. Average number of secondary leaves on middle parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

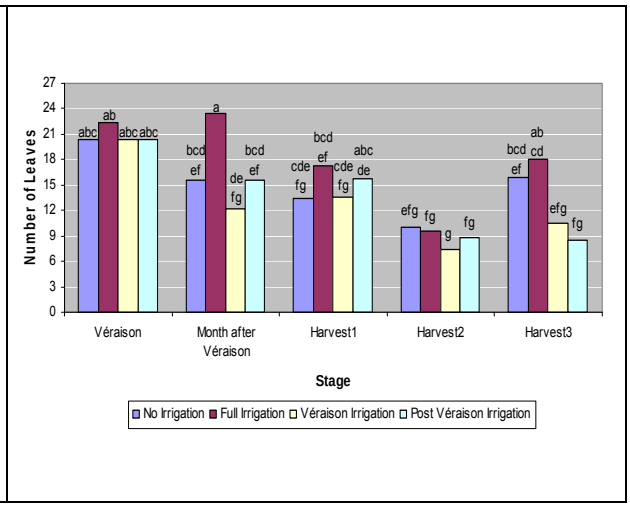

Fig. 51. Average number of secondary leaves on apical parts of shoots over two seasons harvested at different stages of ripening and for different treatments.

The full irrigation treatment showed a higher concentration of secondary leaves in middle and apical parts of primary shoots, whereas the other treatments had higher numbers in apical parts (Fig. 52).

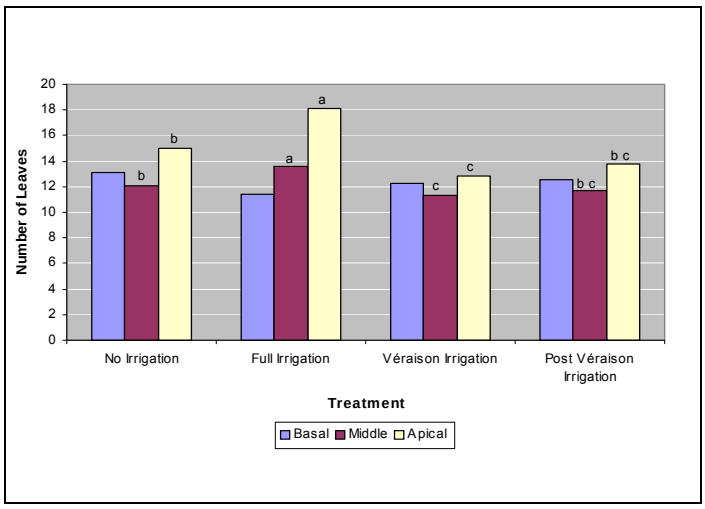

Fig. 52. Average number of secondary leaves on basal, middle and apical parts of shoots over two seasons for different treatments.

Leaf area: The secondary leaf area of the treatments differed between seasons 2004-2005 and 2005-2006 (Fig. 53). The secondary leaf area of the full irrigation treatment and no irrigation treatment was significantly higher than that of the other treatments (Fig. 54).

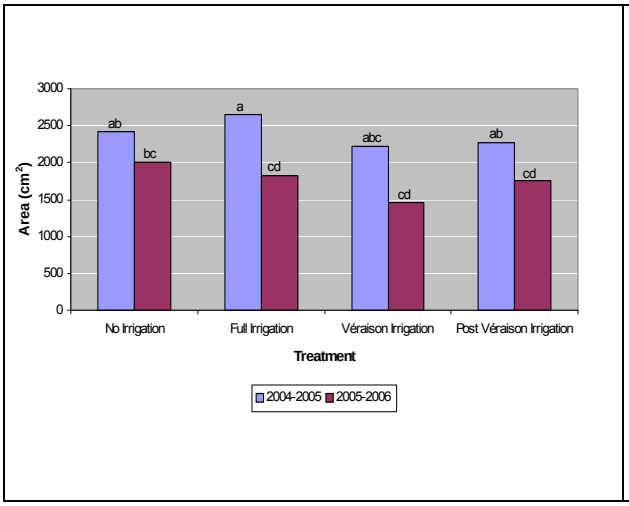

Fig. 53. Average area of secondary leaves for seasons 2004-2005 and 2005-2006 for different treatments.

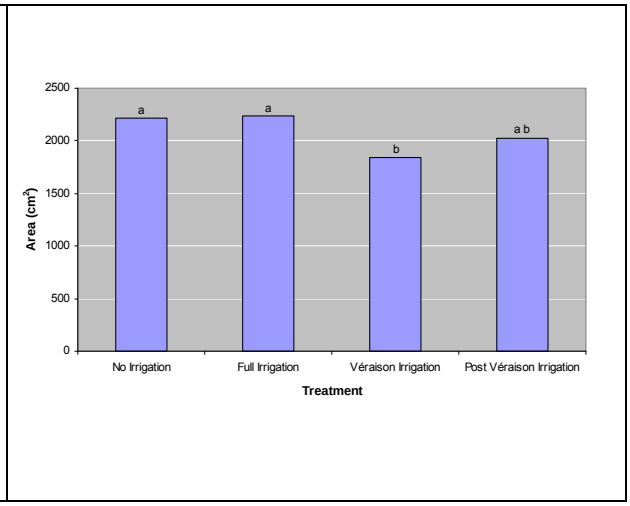

Fig. 54. Total area of secondary leaves over two seasons for different treatments.

Similar patterns to those for the number of secondary leaves were found at different stages of ripening (Fig. 55). Less secondary leaf area occurred on middle and apical regions of primary shoots when vines were deficit irrigated (Fig. 56).

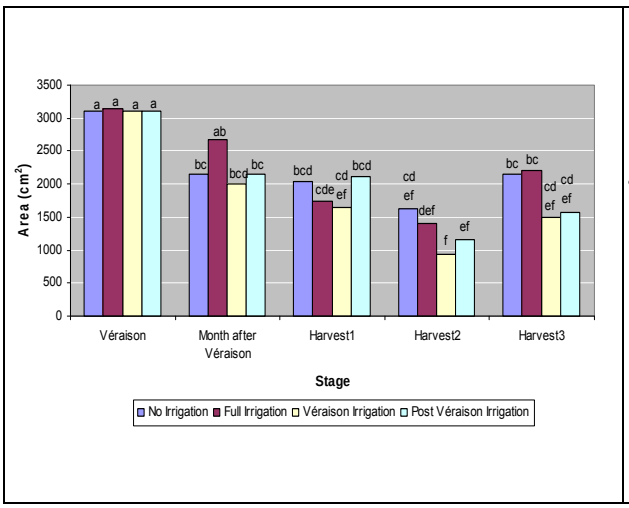

Fig. 55. Average area of secondary leaves over two seasons harvested at different stages of ripening and for different treatments.

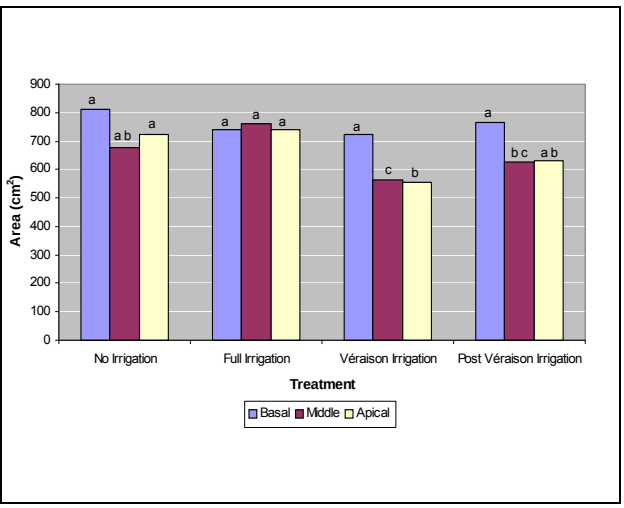

Fig. 56. Average area of secondary leaves on basal, middle and apical parts of shoots over two seasons for different treatments.

Leaf area:leaf mass ratio: The average leaf area:leaf mass ratio of secondary leaves of the treatments differed between seasons 2004-2005 and 2005-2006, the former having had higher values (Fig. 57); similar trends were found between treatments, the no and full irrigation treatments showing slightly higher values (Figs. 57 & 58). No particular trend was found over the ripening period and the different harvest stages (Fig. 59).

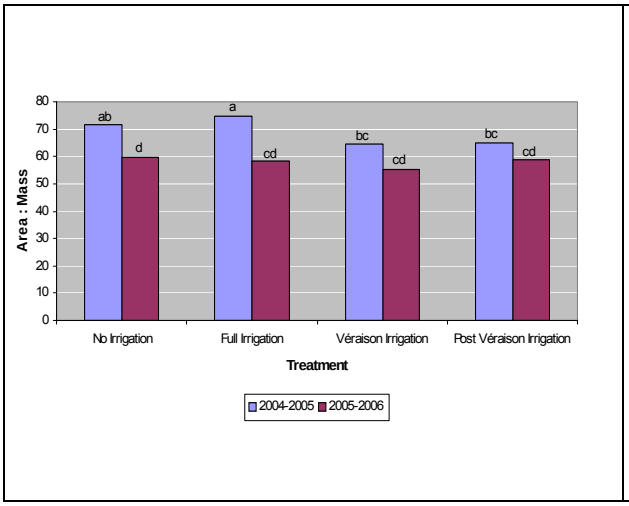

Fig. 57. Average leaf area:leaf mass of secondary leaves for seasons 2004-2005 and 2005-2006 for different treatments.

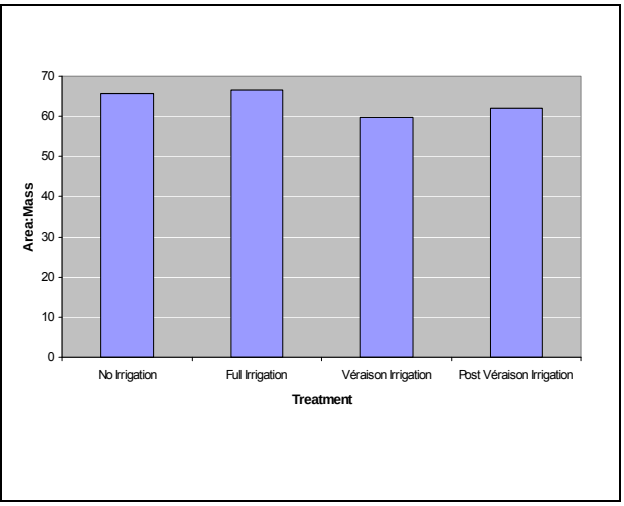

Fig. 58. Total leaf area:leaf mass of secondary leaves over two seasons for different treatments.

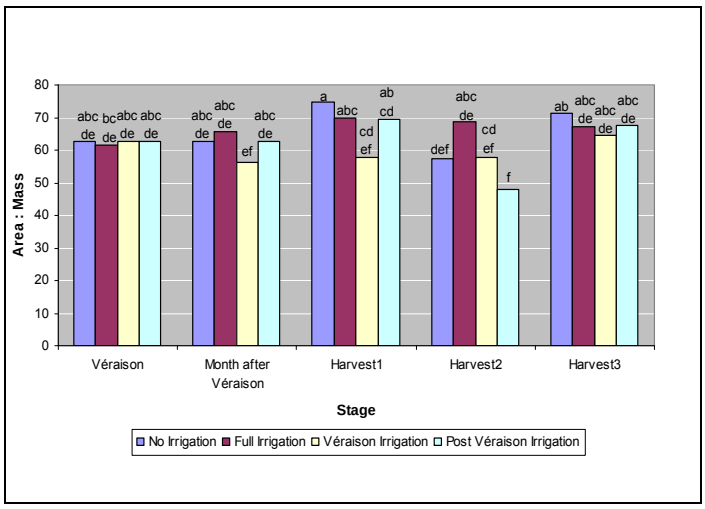

Fig. 59. Average leaf area:leaf mass of secondary leaves over two seasons harvested at different stages of ripening and for different treatments.

Reproductive growth

Number of bunches: The number of bunches per shoot were statistically different between seasons 2004-2005 and 2005-2006 (Fig. 60). No significant differences were found in the number of bunches between the treatments (Fig. 61).

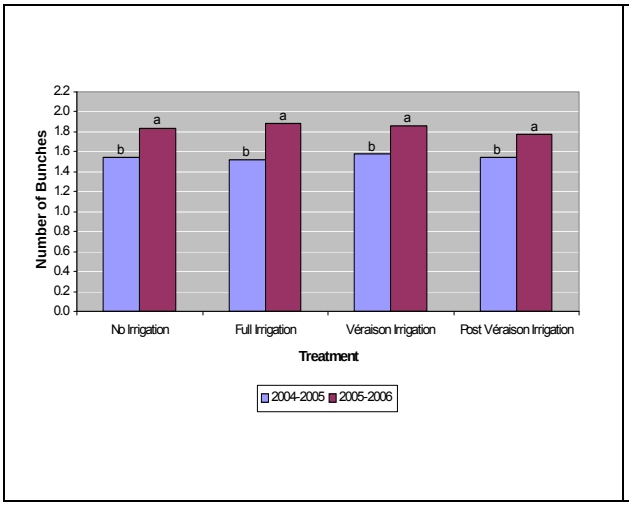

Fig. 60. Average number of bunches for seasons 2004-2005 and 2005-2006 for different treatments.

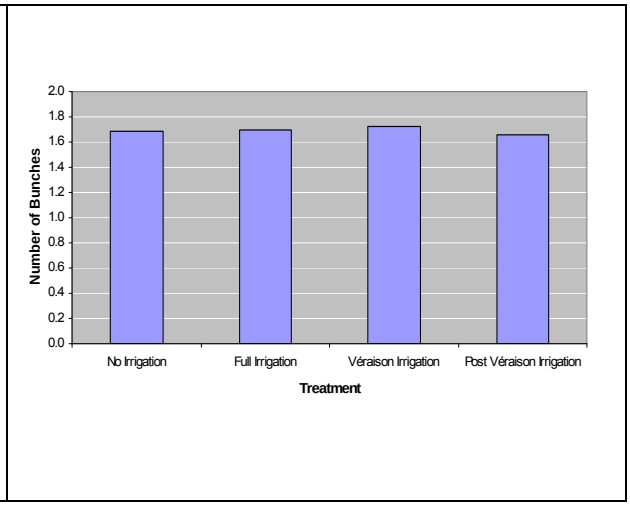

Fig. 61. Total number of bunches over two seasons for different treatments.

Bunch mass: Statistical differences in bunch mass were found between seasons 2004-2005 and 2005-2006 (Fig. 62). Although no statistical significant differences were found in total bunch mass over two seasons for the different treatments, the véraison+post véraison and post véraison irrigation treatments tended to have lower bunch mass compared to that of the rest (Fig. 63).

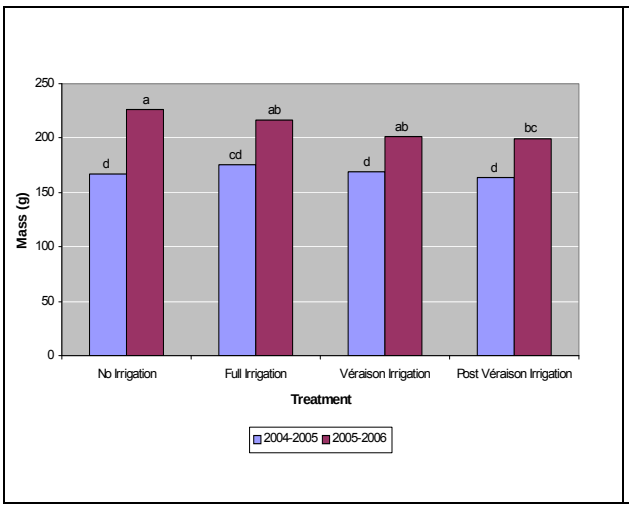

Fig. 62. Average bunch mass for seasons 2004-2005 and 2005-2006 for different treatments.

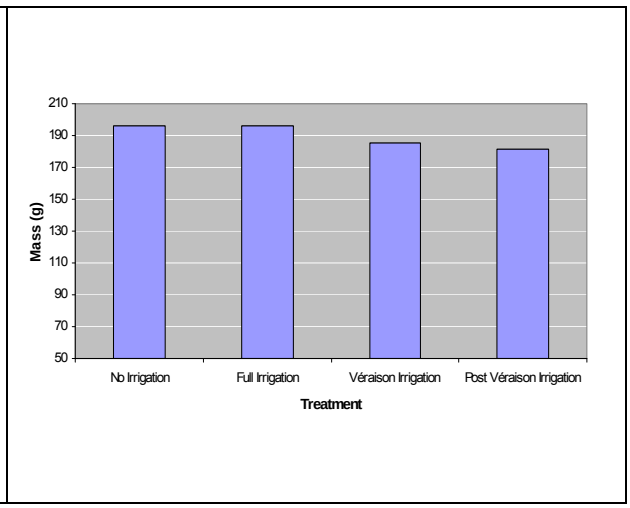

Fig. 63. Total bunch mass over two seasons for different treatments.

The development of the bunches over two seasons, harvested at different times of ripening, indicated a steady decrease in mass for all the treatments up until the third stage of harvest (Fig. 64). The largest decrease in bunch mass occurred between the first and second harvest stages (Fig. 64). Bunch mass of the no irrigation treatment seemed to keep stable after that, whereas the other treatments continued to loose mass.

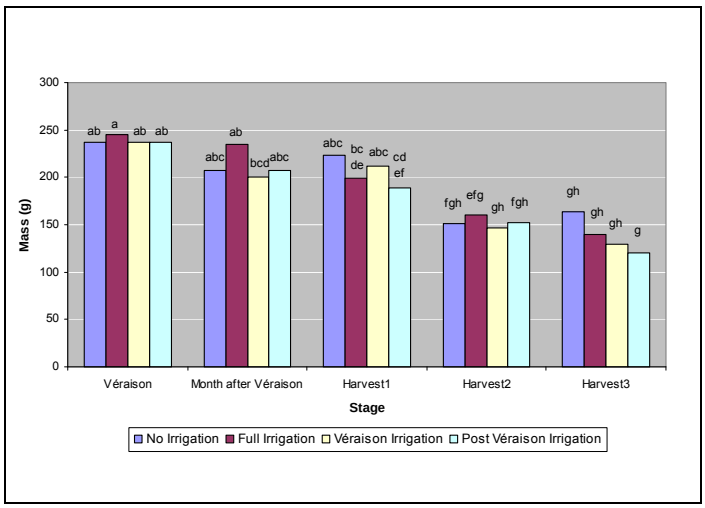

Fig. 64. Average bunch mass over two seasons harvested at different stages of ripening and for different treatments.

Number of berries: Higher numbers of berries for all treatments were found in 2005-2006 (Fig. 65). Generally higher numbers of berries apparently occurred for the no irrigation treatment (Fig. 66). It seemed largely the result of a maintenance of

berry numbers, especially during late ripening (Fig. 67). Berry attachment seemed to be affected by irrigation after an extended dry period.

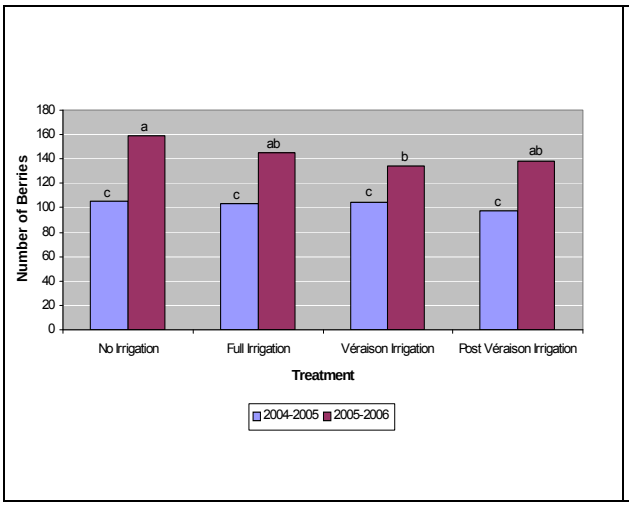

Fig. 65. Average number of berries for seasons 2004-2005 and 2005-2006 for different treatments.

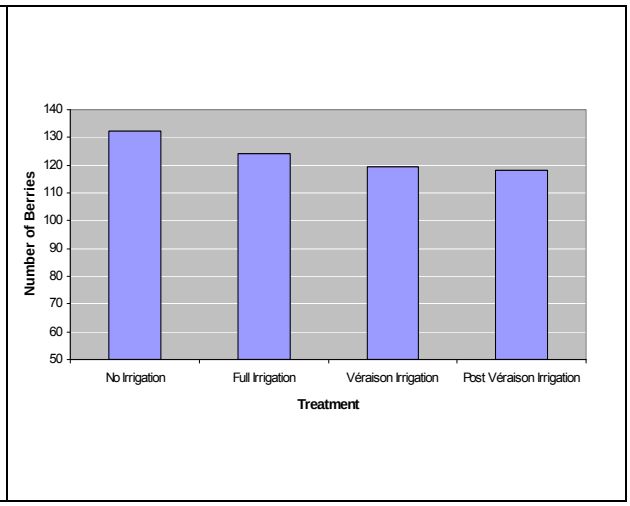

Fig. 66. Total number of berries over two seasons for different treatments.

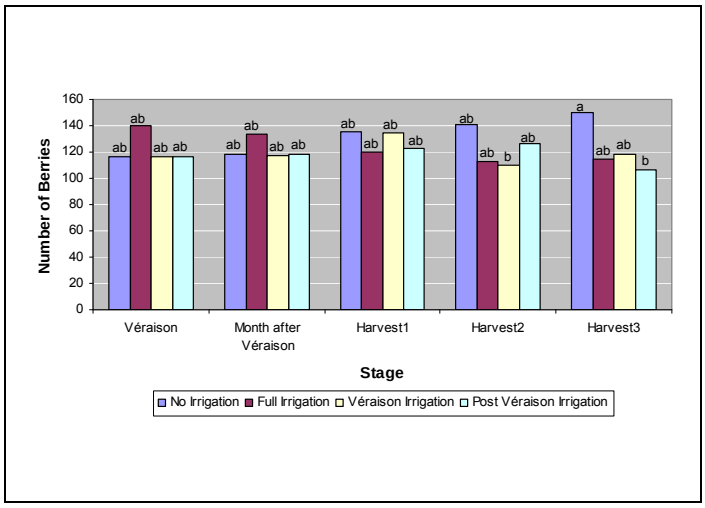

Fig. 67. Average number of berries per bunch over two seasons harvested at different stages of ripening and for different treatments.

Total mass of berries: Similar trends to what were found for bunch mass and berry numbers, occurred for berry mass (Figs. 68, 69 & 70).

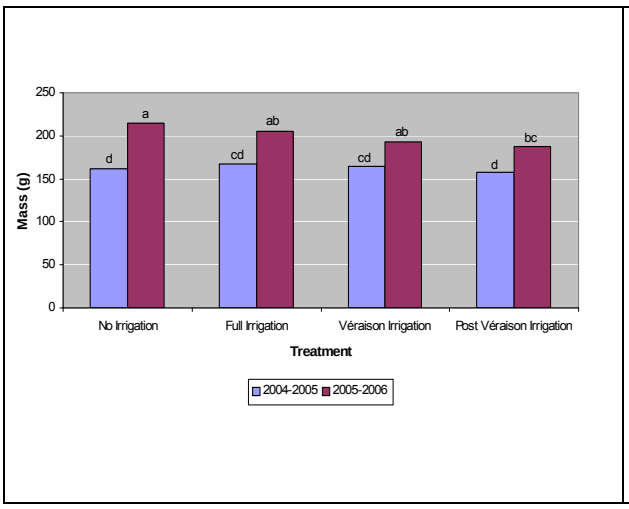

Fig. 68. Average mass of berries for seasons 2004-2005 and 2005-2006 for different treatments.

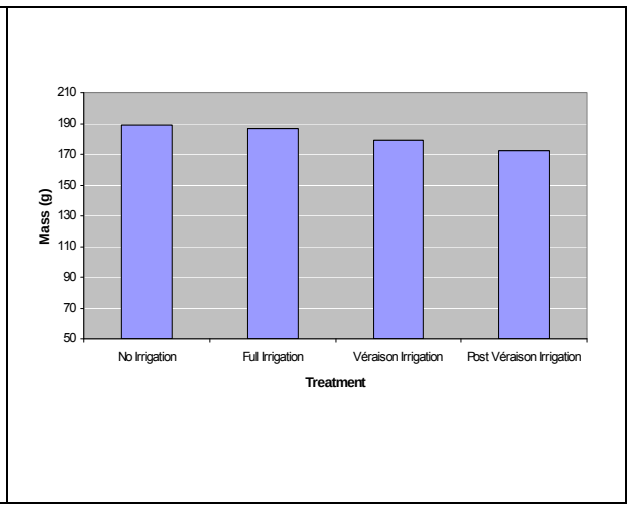

Fig. 69. Total mass of berries over two seasons for different treatments.

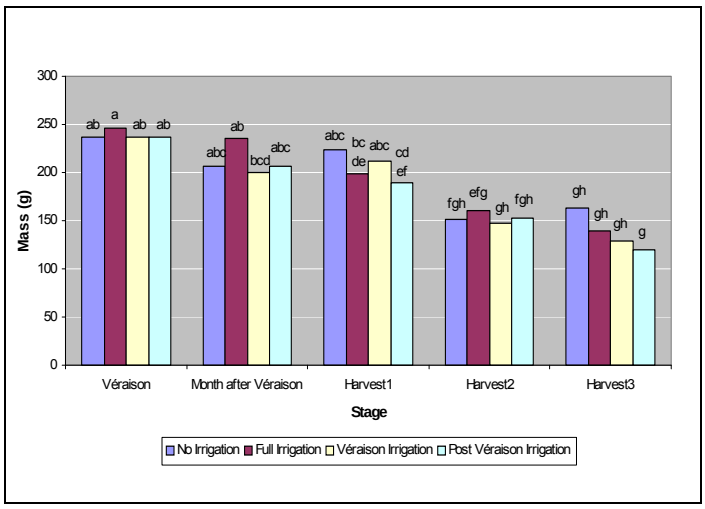

Fig. 70. Average mass of berries over two seasons harvested at different stages of ripening and for different treatments.

Average mass per berry: Significantly higher berry mass was found in season 2004-2005 (Fig. 71). Berry mass was only slightly lower for the post véraison irrigation treatment (Fig. 72). From one month after véraison, the mass per berry progressively decreased until the last harvesting stage (Fig. 73). The berries seemed to loose more water the later irrigation was applied and the longer the ripening period.

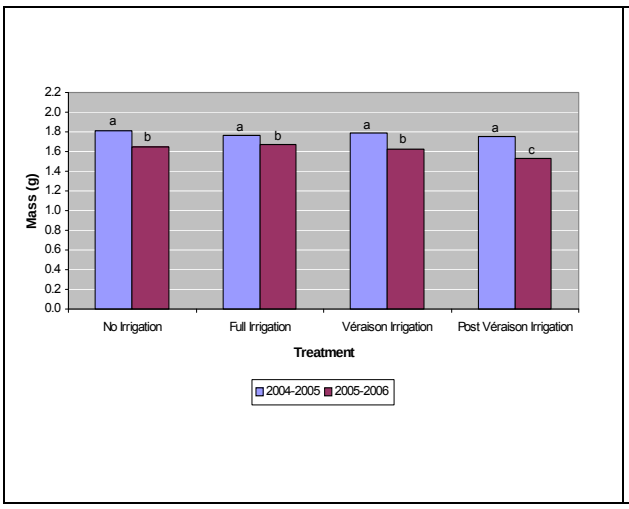

Fig. 71. Average mass per berry for seasons 2004-2005 and 2005-2006 for different treatments.

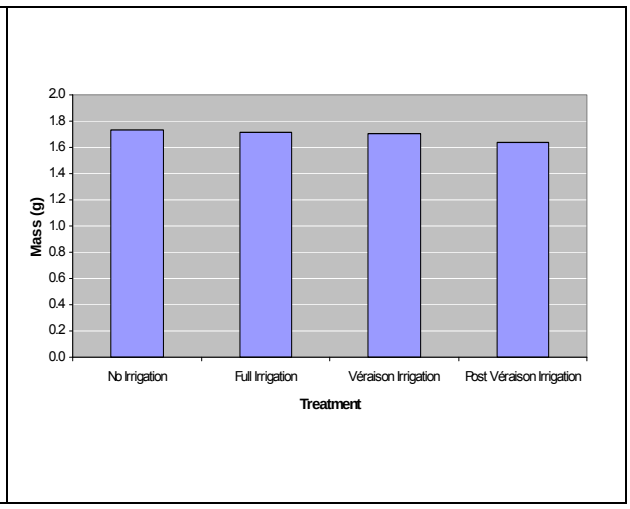

Fig. 72. Total mass per berry over two seasons for different treatments.

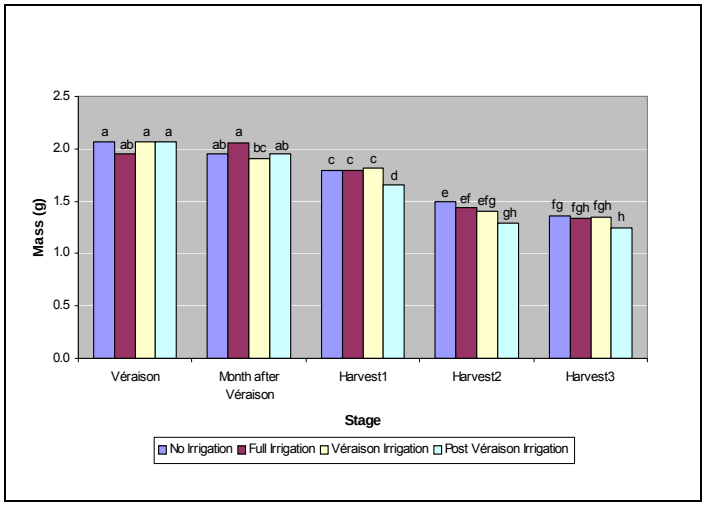

Fig. 73. Average mass per berry over two seasons harvested at different stages of ripening and for different treatments.

Skin area per berry: The skin area per berry was significantly higher in season 2004-2005 (Fig. 74). The full irrigation treatment had the largest skin area per berry (Figs. 74 & 75). The skin area seemed to have increased until the second harvest stage, after which it decreased. The latter decrease most probably resulted from berry shrinkage that occurred with longer hang time (Fig. 76).

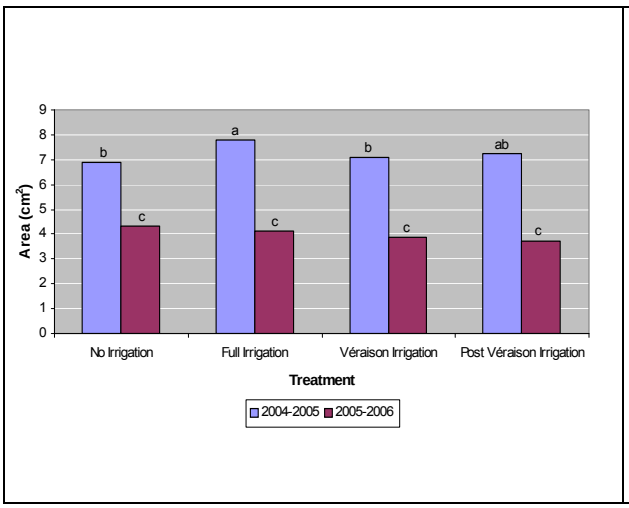

Fig. 74. Average skin area per berry for seasons 2004-2005 and 2005-2006 for different treatments.

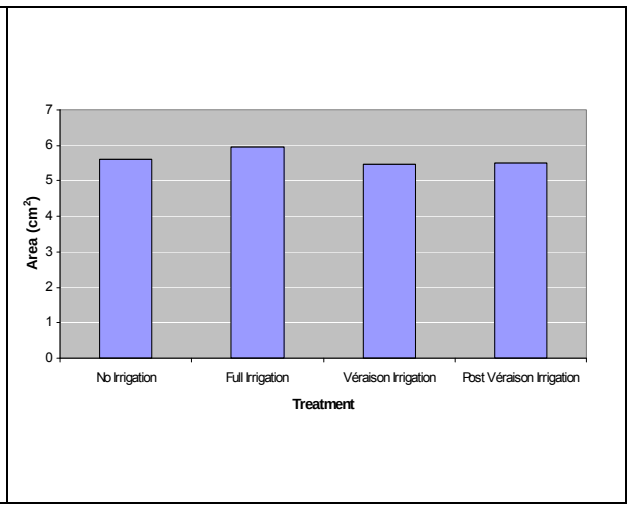

Fig. 75. Total skin area per berry over two seasons for different treatments.

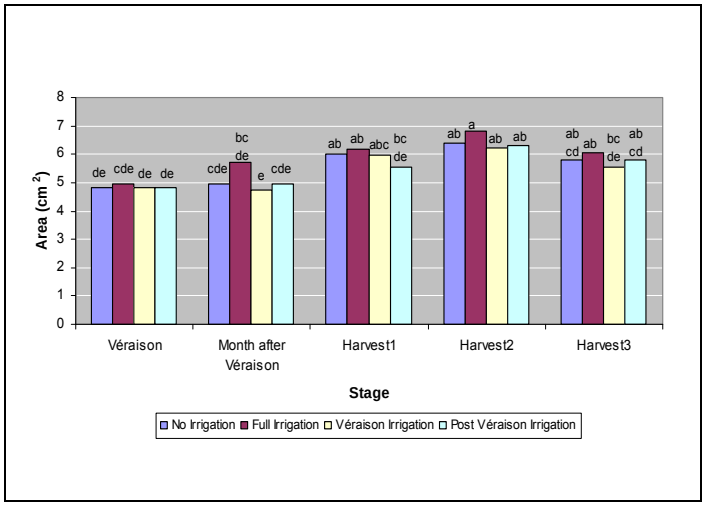

Fig. 76. Average skin area per berry over two seasons harvested at different stages of ripening and for different treatments.

Skin:pulp ratio: In accordance with the smaller berries found in the 2004-2005 season, the skin:pulp ratio was higher during this season, compared to the 2005-2006 season (Fig. 77). Highest average skin:pulp ratio occurred for the post véraison irrigation treatment (Fig. 78).

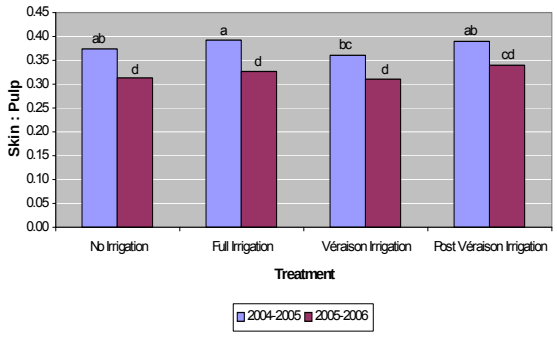

Fig. 77. Average skin:pulp ratio for seasons 2004-2005 and 2005-2006 for different treatments.

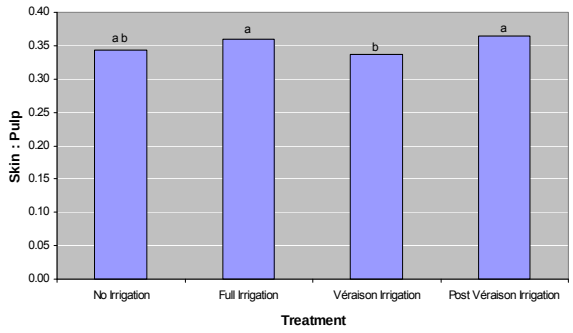

Fig. 78. Total skin:pulp ratio over two seasons for different treatments.

It is interesting to note that the skin:pulp ratio generally decreased from the second to the third harvest stage (Fig. 79). Given the decrease in berry size during this time, the decrease in ratio is unexpected, but may point to berry shrivelling being the overriding factor in the reduction of skin surface area.

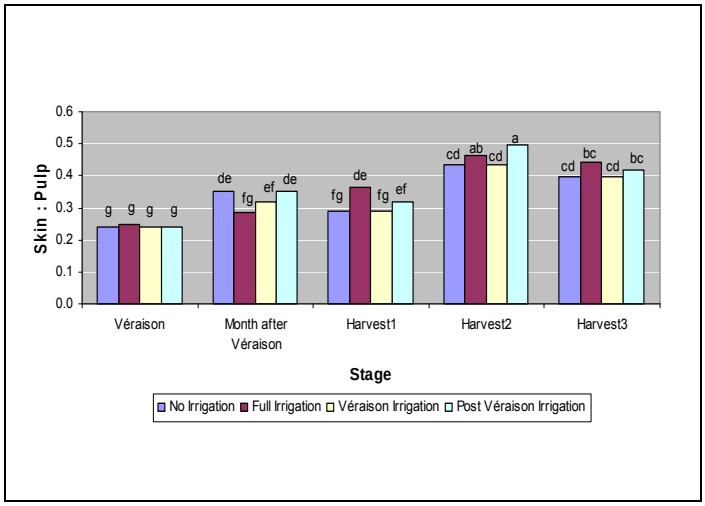

Fig. 79. Average skin:pulp ratio over two seasons harvested at different stages of ripening and for different treatments.

DISCUSSION

Vegetative growth

Primary shoots: The primary shoots of the full-irrigated vines were longer than those of the deficit irrigated and non-irrigated vines. These findings are in accordance with those found by Matthews *et al*. (1987) and Lebon *et al*. (2001), who noted an increase in shoot length with irrigation. Between treatments, no significant

differences in mass of the primary shoots of the basal, middle, and apical parts were found. The basal parts were clearly heavier than the other shoot parts. Irrigation during the whole season apparently led to longer, but lighter shoots, whereas irrigation at véraison+post véraison and post véraison apparently led to heavier basal parts and seasonal water deficit to heavier apical parts of shoots. The sudden increase in primary shoot mass of the fully irrigated vines after véraison may be ascribed to a delay in shoot maturation and reserve accumulation, which may have started earlier in the deficit irrigation treatments. The results point to a redistribution in water and re-allocation of reserve build-up in the shoot according to the time of irrigation and the requirements of the bunches in terms of berry growth and water loss.

Secondary shoots: According to Lebon *et al*. (2001) secondary shoot growth is the main contributor to the shoot dimension in response to different irrigation treatments. Williams & Grimes (1987) also noted a greater secondary shoot sensitivity to water stress, compared to primary shoots. Although there were no significant differences in the total number of the secondary shoots in this study, secondary shoots on fully irrigated vines seemed to develop faster than those on deficit-irrigated treatments. Secondary shoots on fully irrigated vines also seemed to concentrate in the middle parts of primary shoots.

The full irrigation and véraison+post véraison irrigation treatments tended to have more secondary shoots than the other two treatments. As canopy management practices were applied (removal of leaves and shoot tips), the vines may also have compensated for that by forming secondary shoots (Hunter, 2000; Hunter & Visser, 1990). For example, topping of the shoots after full canopy development usually stimulates secondary shoot growth on the primary shoot, due to the removal of apical dominance caused by the inhibitory effect of growth regulators such as auxin (Hunter, 2000).

The secondary shoot length on the basal parts of the primary shoots of non-irrigated and post véraison irrigated vines was longer than that of the other treatments. Fewer, but longer, secondary shoots therefore developed on the late deficit irrigated and the non-irrigated vines. The secondary shoots of the deficit-irrigated vines seemed to grow longer in the basal primary shoot region. According to Hunter &

Visser (1988), the apical, middle and basal leaves translocate their photosynthetates mainly to the bunches from berry set until véraison. After véraison, the bunches were mostly nourished by the basal leaves and retranslocation occurred in the shoot. The presence of longer secondary shoot length in basal regions may therefore nourish the bunches more efficiently and for longer during the ripening period.

Leaves: Vigorous vines can often continue to actively develop leaf area after fruit set (Miller *et al*., 1996a). The number of primary leaves on the fully irrigated vines was higher than on the vines of the other treatments. The longer shoot lengths of the full irrigation treatment probably contributed to this.

The primary leaves of the fully irrigated vines comprised a larger area per shoot than that of the other treatments, but with no statistical differences. According to Gomez-del-Campo *et al*. (2002) water stress does not significantly modify the distribution of primary and secondary leaf area development. This study showed that re-distribution of leaf area may indeed occur when vines are subjected to water deficit treatment.

Noticeably more secondary leaves occurred on the fully irrigated vines, compared to the other treatments. These results are in agreement to those found by Ginestar *et al*. (1998) and Reynolds & Naylor (1994). The full irrigation treatment also seemed to develop more secondary leaves in middle and apical parts of the primary shoots. The deficit-irrigated vines, however, concentrated secondary growth in the apical parts. This is difficult to explain, but may indicate a delay in secondary shoot initiation and development under water deficit conditions, thereby forcing the secondary shoots towards apical primary shoot regions. The larger primary and secondary leaf areas of the full irrigation treatment may also be due to leaf area expansion caused by low light intensities in vigorous canopies (Keller & Hrazdina, 1996).

Reproductive growth

Bunches: During the different stages of harvesting, the number of bunches and bunch mass did not differ significantly between treatments. This is in agreement with results found by Ginestar *et al*. (1998), suggesting that differences in yield found with deficit irrigation were not due to differences in bunch number. In this study, véraison+post véraison and post véraison irrigation treatments tended to have lower

bunch mass. Bunches of irrigated vines seemed to lose mass continually, whereas those of vines that received no additional irrigation seemed to stabilise at the second harvest stage.

Although berry set can be compromised by water stress over the flowering and setting period, the average number of berries over two seasons showed no significant differences between treatments as no obvious water stress occurred during flowering and berry set. The higher number of berries on the non-irrigated vines seemed to be the result of the maintenance of berry numbers, especially during late ripening. The pedicel attachment may have been affected by irrigation after an extended dry period, most probably due to swelling of the berry central tissue after shrinkage.

Berry size, skin area and skin:pulp ratio: According to Hunter (1991; Coombe, 1992b), the grape berry is a non-climacteric fruit with a double sigmoid growth curve. The increase in mass of the berry can be divided into three growth phases (Pratt, 1988), the first being a period of rapid growth and cell division until the seeds reach their mature size (Staudt *et al*., 1986); a period of slow growth because of cell expansion ending with the beginning of véraison; and a period of rapid growth ending immaturity and during which carbohydrates accumulate (Alleweldt, 1977). The latter is associated with berry softening and colouring (Hunter, 1991).

The concentration of the must is indirectly affected by the final size of the grape berry, in that this concentration depends on the skin surface:berry volume ratio (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004). According to Hardie & Considine (1976), Van Zyl (1984), and Sipiora & Gutiérrez-Granda (1998), the supply of water to the grapevine is an environmental factor affecting berry size. In this study, berry mass seemed to be slightly lower for the post véraison irrigation treatment. The study indicates that berry water content was negatively affected the later irrigation was applied during the ripening period and the longer the ripening period. The reason for the former is not clear, but may point to water potential gradients driving a faster loss of water from the berry, when receiving water after an extended relatively dry period.

The reason for the relatively high berry mass of the non-irrigated vines is not clear as yet, but could be due to rain that was received during the season and/or due to the maintenance of water because of stomatal closure reducing the impact of the water deficit. On the contrary, the fully irrigated vines had sufficient water to absorb and to buffer the loss of water through transpiration. Cell division of the berry pericarp occurs only during the first growth phase (Ojeda, 1999) and cell volume is thus reduced by water deficit during this period. Water deficit occurring between véraison and maturity may also reduce cell expansion (Ojeda *et al*., 2001), but cell volume may recover partially or totally if water is available (Van Zyl, 1984; McCarthy, 1997). A reduction in berry size because of water deficit during the first growth phase is often irreversible even when there is no water shortage after the beginning of ripening (Hardie & Considine, 1976; Van Zyl, 1984; McCarthy, 1997). Higher water contents do not necessarily lead to heavier berries. The efficiency of the canopy and solute transport to the berry may also affect berry mass (Alleweldt, 1977) by means of a more balanced distribution of water and carbon in the plant. The decrease in berry mass during late ripening accompanied by berry shrinkage can be ascribed to the loss of water (Reynolds & Naylor, 1994; McCarthy, 1999; McCarthy & Coombe, 1999).

The full irrigation treatment had the largest berry skin surface and points to cell expansion forced by the high water potential of this treatment (Ojeda *et al*., 2001). Skin area enlargement could be observed through the different stages of harvesting up to the second harvest, after which it decreased, probably due to berry water loss and shrivelling.

The highest skin:pulp ratio was found for the post véraison treatment. The ratio generally decreased from the second to third harvest stage. Given the decrease in berry size during this time, the decrease in the ratio is surprising and seems to point to skin shrivelling as the overriding factor determining the ratio at this time. The skin:pulp ratio had a direct correlation with berry mass and water for all the treatments. As the berry mass and water content increased, the skin:pulp ratio decreased. McCarthy (2000) found that the mass ratio of skin:pulp increased with water deficit.

CONCLUSIONS

Full irrigation stimulated primary shoot length compared to that of deficit-irrigated vines. Despite this, the shoots seemed of lower mass. Basal parts of shoots were clearly higher in mass than middle and apical parts for all treatments. Irrigation during ripening seemed to induce higher basal shoot mass, whereas seasonal water deficit seemed to stimulate apical shoot mass. In general, the results seem to indicate earlier and more complete shoot maturation (reserve accumulation) with longer water deficit. Timing of irrigation affects the distribution of water and the build-up of reserves in the shoot. The rate of development and position of occurrence of secondary shoots were affected by irrigation. Seasonal irrigation seemed to accelerate development and stimulate occurrence of secondary shoots in middle parts of primary shoots. In contrast, longer water deficit (seasonal and post véraison irrigated) seemed to induce fewer, but longer, secondary shoots in basal parts of primary shoots. This may be beneficial regarding the nourishing of the bunches during ripening, because of maturity of leaves on these shoots and closeness of bunches. The results show that a re-distribution of leaf area on the shoot may occur when vines are subjected to water deficit.

Bunch and berry mass were affected by water deficit and the timing of irrigation. Irrigation during ripening seemed to induce a continuation of berry water loss, whereas extended water deficit seemed to induce earlier and restricted water loss. Berries also seemed to maintain berry attachment during ripening better under restricted soil water conditions; late irrigation, after an extended dry period, seemed to affect berry pedicel attachment. Late ripening irrigation apparently stimulated further berry water loss under seasonal water deficit conditions. The latter may have also been affected by a re-distribution of carbon and water in the plant under drier conditions.

The full irrigation treatment during the season induced larger berry skin surface and probably represents cell expansion forced by high water potential. Skin area enlargement continued until late during ripening, after which berry shrivelling occurred. Berry shrivelling during this time seemed to be the overriding factor determining a reduction in skin:pulp ratio at the last harvest stage. The highest skin:pulp ratio nonetheless occurred for the post véraison irrigation treatment.

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Chapter V

RESEARCH RESULTS

GRAPE COMPOSITION OF SHIRAZ/RICHTER 99 GRAPEVINES AS AFFECTED BY WATER STATUS AND RIPENESS LEVEL

ABSTRACT

In this study, grapevine water relations during the berry ripening period, under the influence of various irrigation strategies, were investigated in an attempt to quantify the effect of water status on the grape composition of a Shiraz/Richter 99 vineyard. Comparisons based on certain berry parameters during ripening were made between different irrigation strategies (no irrigation; and irrigation at all phenological stages; at véraison and post véraison; and at post véraison). Water deficit seemed to have enhanced the sugar accumulation. The post véraison irrigation in particular seemed to favour a wide window for harvesting. Irrigation treatments did not seem to affect the titratable acid of the berry must. The relatively high titratable acid of the post véraison irrigation treatment, despite high soluble solid concentrations, is noticeable. The pH of the must was not affected by the different irrigation treatments. Irrigation at post véraison, and especially véraison+post véraison, seemed to have a greater effect on the synthesis of the phenolic and anthocyanin content, as it reached higher values than in the berry skins of the fully irrigated and non-irrigated vines. Fully irrigated vines showed higher initial tannin values, decreased sharply, and then increased to highest concentrations. The generally higher phenolic, anthocyanin and tannin contents and higher colour density of the véraison+post véraison irrigation berries may increase the potential structure and complexity of wine made from these berries. The style of wine may be affected by the timing of irrigation.

INTRODUCTION

Grape composition parameters such as pH, titratable acid and sugar levels, together with berry mass just prior to harvest, give an indication of potential wine quality (Jones & Davis, 2000). Berry size at harvest, for especially red grape varieties, is considered a very important component of determining wine grape quality all over the world. Smaller berries produce wine with more complexity, aroma and colour (McCarthy, 2000). There is an increasing need to manipulate berry size in the vineyards and to obtain optimum levels of ripeness regarding sugar, pH, titratable acid and phenolic compounds to produce wine of high quality. According to Bravdo *et al*. (1985) severe water stress reduces soluble solids and total acidity and increases pH. An oversupply of water can result in similar effects for soluble solids, total acidity and pH (Neja *et al*., 1977).

Rainfall and soil moisture play an integrated role in the composition of grapes. Rainfall can be supplemented by irrigation. Water availability may have effects on grape composition and maturity levels. Vigorous growth and dense canopies are common features of South African grapevines (Hunter *et al*., 1991). Smart & Coombe (1983) noted that excessive irrigation delayed ripening, increased the yield due to berry enlargement, and reduced anthocyanins due to shading caused by excessive shoot growth. Water stress, on the other hand, altered fruit composition (Schultz & Matthews, 1993; Roby & Matthews, 2004), reduced shoot growth (Smart & Coombe, 1983; Schultz & Matthews, 1993; Roby & Matthews, 2004) and enhanced early ripening, but reduced the yield and berry mass due to excessive exposure (Smart & Coombe, 1983).

The grape berry has a double sigmoid growth curve (Hunter, 1991; Coombe, 1992b). According to Coombe (1992a,b), berry fruit development has two cycles: the first phase of berry growth begins with cell division in the pericarp tissue, which largely determines the final shape and size of the berries. Berry ripening occurs during the second cycle, beginning at véraison. The start of the ripening stage at véraison is associated with cell enlargement, change in berry colour from green, berry softening and sugar accumulation (Hunter, 1991; Coombe, 1992a), with a decrease in acidity and astringency, loss in chlorophyll and an increase in aroma (Hunter, 1991).

Wang *et al.* (2003) found that the sugar concentration of the berries was not modified by water stress during the early stages of the second phase of berry growth. The size of the berries of the water-deficit and irrigated vines was different. During the later stages of the second phase of berry growth, the sugar concentration of the two treatments was significantly different, being higher in the normally watered vines than in the water stressed vines.

According to Ojeda *et al.* (2002) and Castellarin *et al.* (2005) no significant differences were found in the final sugar concentration between irrigated and deficit treatments. The total soluble solids per berry were proportional to berry size as indicated by berry mass (Ojeda *et al.*, 2002). Roby *et al.* (2004) stated that the total soluble solids per berry increased linearly with berry size and the concentration of soluble solids in each berry was dependent on size. According to Matthews & Anderson (1988), the amount of sugar was higher in continually irrigated vines than

in water stressed vines. Ginestar *et al*. (1998) also noted that the sugar in water stressed treatments was lower than in watered treatments. Hardie & Considine (1976) found a reduction in total sugar accumulation for stressed vines and the berries had a later maturity date. On the other hand, Morris & Cawthon (1982) noted that excess water would normally reduce sugar, but with moderate irrigation, during dry years, would increase it.

The organic acid content of grape berries consists mainly of tartaric, malic and citric acids and can be measured by titration and expressed as total titratable acids (Ribéreau-Gayon *et al.*, 1998). Acid is a very important quality factor, wine with too much acid is tart in taste, whereas wine with low acid levels may produce a bland wine. High pH levels reduce wine quality (Boulton, 1980) and increase the probability of micro-organism activity; it also has a negative effect on the colour intensity of red wines and the aging ability of the wine (Ribéreau-Gayon *et al.,* 1998).

Increased water availability often causes an increase in the potassium and pH levels in the berry and wine (Freeman & Kliewer, 1983). The presence of potassium in the berries and wine appears to be linked to pH and acidity (Boulton, 1980; Freeman & Kliewer, 1983). Musts with a high potassium concentration tend to have high pH and malate. According to Hunter *et al*. (1991) and Hunter & Ruffner (2001), berries reached the highest malic and tartaric concentrations at pea size. From véraison to ripeness malic acid decreased (Iland & Coombe, 1988; Hunter, 1991; Hunter *et al*., 1991; Coombe, 1992b), due to malic acid metabolism during ripening (Iland & Coombe, 1988). The tartaric acid content in the berries changed very little from véraison to ripening (Iland & Coombe, 1988; Hunter, 1991; Hunter *et al*., 1991; Coombe 1992b). Smart & Coombe (1983) noted that excessive irrigation slows ripening, increases yield partially by berry enlargement, and elevates must pH and acidity from shading due to continuous and excessive shoot growth. An increase in shading within the canopy was associated with an increase in the must malic acid content (Coombe, 1987; Archer, 1988; Smart *et al*., 1988; Archer & Strauss, 1989) and a decrease in tartaric acid (Smart *et al*., 1985; Archer, 1988; Archer & Strauss, 1989). Water stress leads to early ripening, but reduces yield, berry mass and malic acid by excessive exposure (Smart & Coombe, 1983; Iland & Coombe, 1992). According to Ginestar *et al*. (1998), increased bunch exposure might lead to an

increase in berry temperature causing an increase in respiration of malic acid and leading to higher pH values.

Excessive amounts of potassium in the berries are mostly because of excessive soil moisture and the availability of potassium in the soil. According to Gladstones (1992), the effects of irrigation or excessive soil moisture on must and wine pH are primarily because of impaired canopy light conditions and thus accumulation of potassium. Yuste *et al.* (2004) noted a higher acidity level in berries of non-irrigated vines. The lower acidity in the berries of irrigated vines resulted from the increase of berry size that contributed to a reduction in concentration. Authors like Mullins *et al.* (1992) suggested that the decrease in tartaric acid concentration could be due to dilution resulting from the increase in berry size. The low acidity and high pH values in the berries of water stressed vines could be related to potassium accumulation in the berries (Boulton, 1980; Jackson & Lombard, 1993). According to Boulton (1980), the reduction of photosynthetic activity of the leaves is related to potassium transport from the leaves to the berries. Thus water stress and a decrease in photosynthetic activity could have caused a higher potassium accumulation in the berries and also a higher pH (Yuste *et al.*, 2004).

Phenolic compounds play an important role in the flavour of red wines (Ribéreau-Gayon *et al.*, 1998). Phenolic compounds are responsible for positive tasting characteristics, but are also responsible for unpleasant negative characteristics (Ribéreau-Gayon *et al.*, 1998). In red wines; body, structure, fullness and roundness are quality characteristics. Negative aspects such as bitterness, roughness, harshness, astringency and thinness should be prevented, as they are incompatible with quality. The overall organoleptic impression is based on a harmonious balance between these two groups of sensations. These sensations are directly related to the type and concentration of the various molecules, such as anthocyanins and especially tannins (Ribéreau-Gayon *et al.*, 1998).

Phenolic compounds are mainly localised in the skin and seeds of the grape berry (Ojeda *et al*., 2002). In the case of red grape varieties, the skin is particularly rich in flavonols and anthocyanins. The composition of phenolics depends on the cultivar and is influenced by viticultural and environmental factors (Brossaud *et al*., 1999). Since phenolic concentration depends on the skin surface:berry volume ratio, the

final size of the berry affects the phenolic concentration (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004).

Previous work on water deficit treated vines done by Ojeda (1999), showed that the pericarp cellular volume, independently of period and intensity of water deficit, causes berry size and mass reduction due to deficit treatments. Cell multiplication and indirectly cell numbers per berry pericarp were not affected. The biosynthesis of phenolic compounds was followed by their content expressed in terms of the skin mass per single berry (concentration of phenolics was expressed in mg/g of fresh skin mass) and the results indicated that the phenol content of the berries was dependent on total skin mass, which was affected by water deficit, primarily when it was applied during the green growth stages of the berry, from anthesis to véraison.

According to Freeman & Kliewer (1983) and Hardie & Considine (1976), wine colour was reduced due to irrigation, by reducing the proportion of pigments in the coloured form. This was due to an increase in pH, which was associated with larger berries and higher yields, produced due to irrigation (Hardie & Considine, 1976). These differences have been related to greater skin area:volume ratio of smaller, non-irrigated berries.

Objectives of the study were to determine the effect of varying vine water status, in combination with ripeness level, on grape composition. The significance of vine water status on the length of the ripening period and the composition of the grapes as related to optimum grape ripeness, was investigated.

MATERIALS AND METHODS

Experimental vineyard

A seven-year-old *Vitis vinifera* L. cv. Shiraz (clone SH1A), grafted onto Richter 99 (*Vitis Berlandieri x Vitis rupestris*) (clone RY2A) was used for this study. The experimental vineyard is situated on the Experiment farm of ARC Infruitec-Nietvoorbij in the Stellenbosch Region, Western Cape. The area is characterised by a Mediterranean climate. The vines are spaced 2.75 m x 1.5 m on a Glenrosa soil with a western aspect (26º slope) and orientated in a North-South direction. The vines are trained onto a 7-wire lengthened Perold trellising system (VSP) of which three sets of wires are movable. Vines were pruned to two-bud spurs with a spur spacing

of approximately 15 cm. Canopies were suckered, shoot positioned and tipped/topped during the pre-véraison period. Irrigation was applied through a micro-sprinkler system.

Treatments and layout

Four treatments, comprising irrigation combinations to field water capacity at different stages, were applied (field water capacity of the soil was determined before the start of the experiment). The treatments were completely randomised in two blocks, representing two replications. Thirty vines were used per replication. The treatments were: (i) seasonal irrigation from berry set with further irrigation at pea size, (ii) véraison and one month post véraison, (iii) irrigation at véraison with further irrigation at post véraison, (iv) irrigation at post véraison and (v) no irrigation. The sampling of the treatments was split into five stages: véraison, one month after véraison, and three times during the ripening period.

Measurements

Determination of acid, pH and sugar: Soluble solids (ºB), total titratable acidity (G/L) and pH were determined on the berry must by using standard laboratory methods.

Determination of colour, phenolics and tannins: [Described in Hunter *et al*. (1991)] Fifty berries were randomly sampled and stored at -20ºC until analyses. Skins were separated from the pulp by gently squeezing it between thumb and forefinger. Skins were then blotted dry and the fresh mass (g) determined. Skins were frozen at -20ºC prior to freeze-drying with a Christ Alpha freeze-drying unit. Dried skins were weighed, ground in a Sorvall Omni-mixer, and stored in the dark at room temperature until further use.

One gram of freeze-dried skin tissue was extracted in 30 mL methanolic 0.1% HCl (pH 3.5) solution at room temperature using a Janke & Kunkel horizontal shaker (model HS 500), operating at 250 rpm for 15 minutes. The extract was then centrifuged at 27138 g for 15 minutes, the supernatant decanted and the process repeated twice. The supernatants were combined and acidified to pH 1.0 using 1 M HCl. The solution was then made up to 100 mL with extraction solvent (pH 1.0) and left in the dark for approximately one hour at room temperature. After dilution (1:4),

absorbancies of anthocyanins (at 420 nm and 520 nm) and total phenolics (at 280 nm) were determined with a LKB Biochrom Ultrospec spectrophotometer (ll E) using 2 mm quartz cells.

Tannins were measured by using the same 1:4 diluted supernatants that were used to determine the absorbancies of the anthocyanins and phenolics. A aliquot of 250 µL of the 1:4 diluted supernatants was taken and combined with 2.5 mL dimethylaminocinnamaldehyde (DMAC). The absorbance of the tannins was measured at 640nm with a LKB Biochrom Utrospec spectrophotometer (ll E) using a 2mL cuvet after 10 minutes of colour development at room temperature.

Statistical procedures

A random split-plot experiment was performed with main plot treatments as four irrigations (full irrigation; véraison and at post véraison irrigation; post véraison irrigation; and no irrigation), replicated randomly within each of the two blocks. The sub-plot treatments were five different stages of ripening (véraison, one month after véraison, and three further stages approximately two weeks apart). The whole experiment was repeated over two seasons on the same experimental plots. The repeated measurements for the two seasons were considered as sub-sub-plot treatments (Little & Hills, 1972). The appropriate analyses of variance were performed on all the variables measured (SAS Institute, Inc., 1999).

Shapiro-Wilk test was performed to test for non-normality of the residuals (Shapiro & Wilk, 1965). Deviation from normality was mainly due to kurtosis and not skewness; the data were therefore considered as reliable (Glass *et al*., 1972). Students' t-LSD (least significant difference) was calculated at a 5% significance level to compare means of significant effects (Snedecor & Cochran, 1967).

RESULTS

Soluble solids: The soluble solid content of the berries for different treatments differed between seasons 2004-2005 and 2005-2006, the latter displaying lowest values (Fig. 1). It seemed that the soluble solid content of the berries under full irrigation was lower than that of the rest for both seasons (Figs. 1 & 2). The average soluble solid content measured over two seasons and harvested at different times during ripening indicated a steady rise in ºBalling from véraison onwards (Fig. 3). At

each stage of ripening the soluble solid content in the must of the full irrigation treatment was less than that of the other treatments. Irrigation at véraison+post véraison and post véraison stages apparently favoured soluble solid accumulation. The latter treatment in particular seemed to have led to an extended ripening, increasing the window for harvesting.

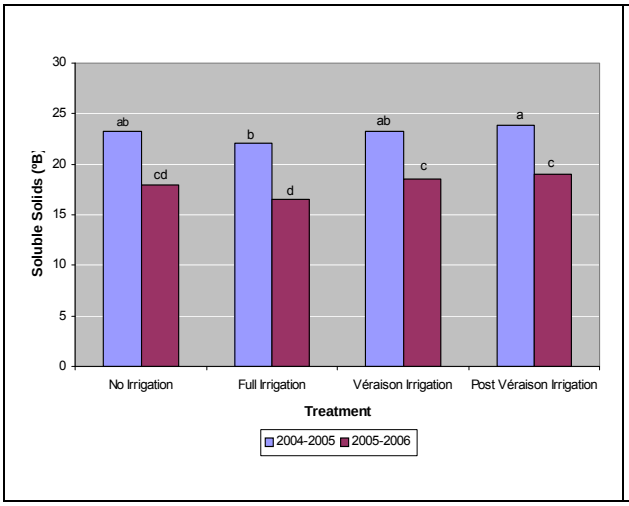

Fig. 1. Soluble solid content of berry must for seasons 2004-2005 and 2005-2006 for different treatments.

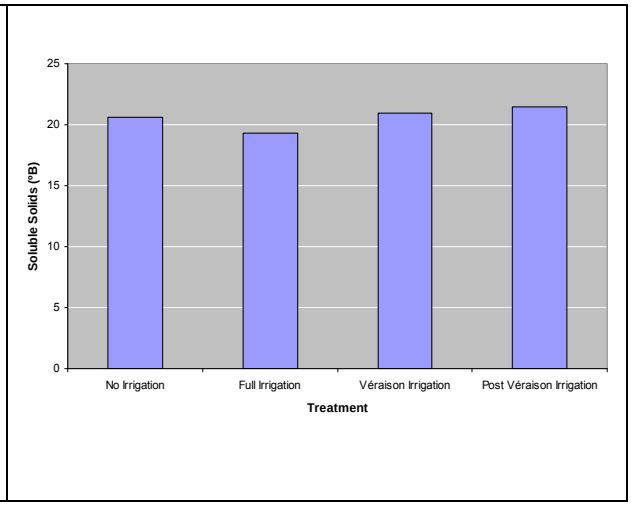

Fig. 2. Average soluble solid content of berry must over two seasons for different treatments.

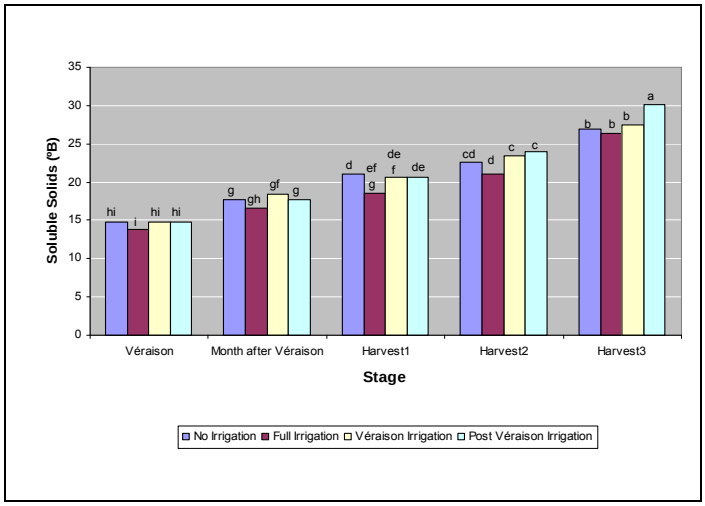

Fig. 3. Average soluble solid content of berry must over two seasons harvested at different stages of ripening and for different treatments.

Titratable acidity and pH: In accordance with the lower soluble solid values, higher must titratable acidity was found in the 2005-2006 season (Fig. 4). Only slightly higher titratable acidity was found for the full irrigation treatment (Fig. 5).

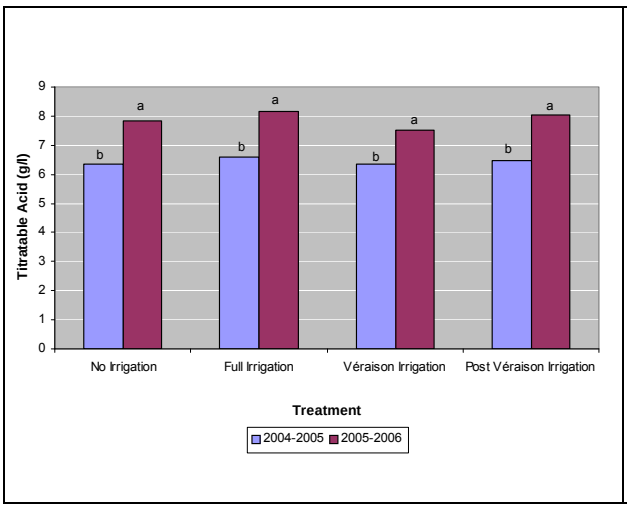

Fig. 4. Titratable acid of berry must for seasons 2004-2005 and 2005-2006 for different treatments.

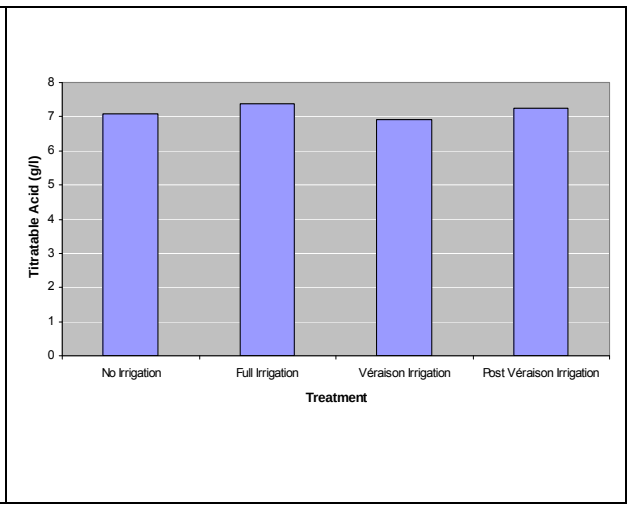

Fig. 5. Average titratable acid of berry must over two seasons for different treatments

The average titratable acid of the berry must measured over two seasons and harvested at different times during ripening indicated a steady decrease in acid from véraison onwards (Fig. 6). Although no statistical significant differences between the treatments occurred (except at véraison), the véraison+post véraison and particularly the post véraison treatment showed surprisingly high titratable acidity in view of the relatively high soluble solid contents.

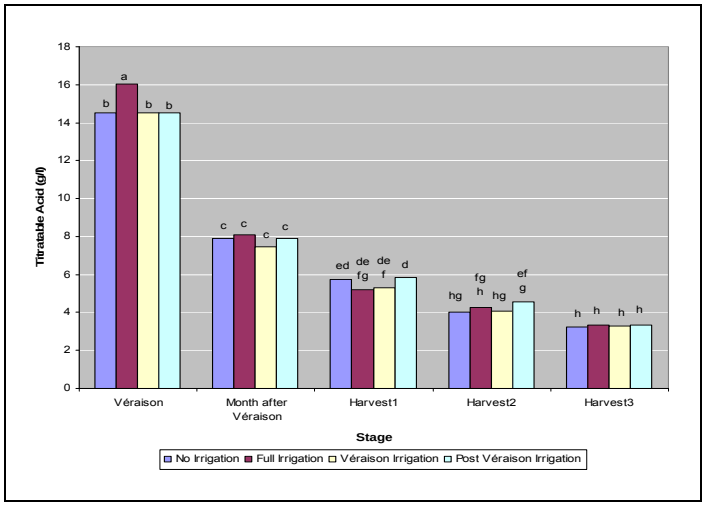

Fig. 6. Average titratable acid of berry must over two seasons harvested at different stages of ripening and for different treatments.

In concurrence with the titratable acid of the berry must, the pH of the must of the treatments decreased in the 2005-2006 season (Fig. 7). No difference in the average pH of the berry must was found between the treatments (Fig. 8).

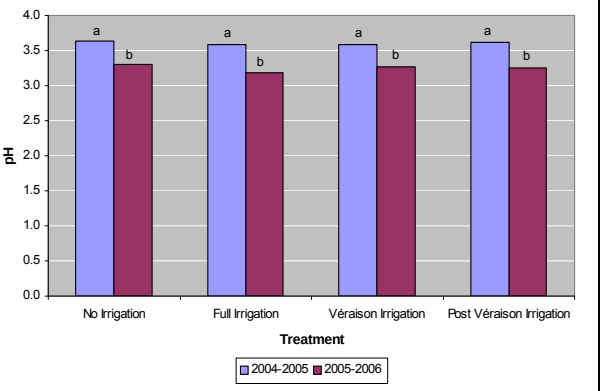

Fig. 7. Berry must pH for seasons 2004-2005 and 2005-2006 for different treatments.

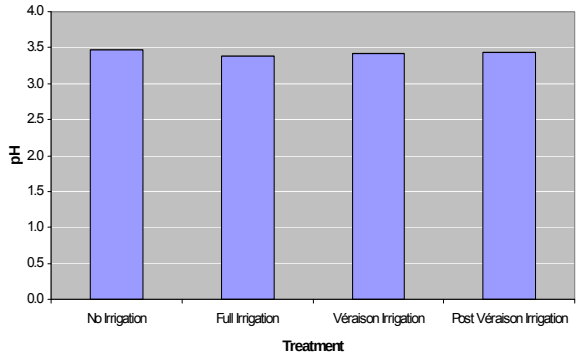

Fig. 8. Average pH of berry must over two seasons for different treatments.

The average pH of the berry must measured over two seasons and harvested at different times during ripening indicated a steady increase in pH from véraison onwards (Fig. 9). No differences occurred between treatments.

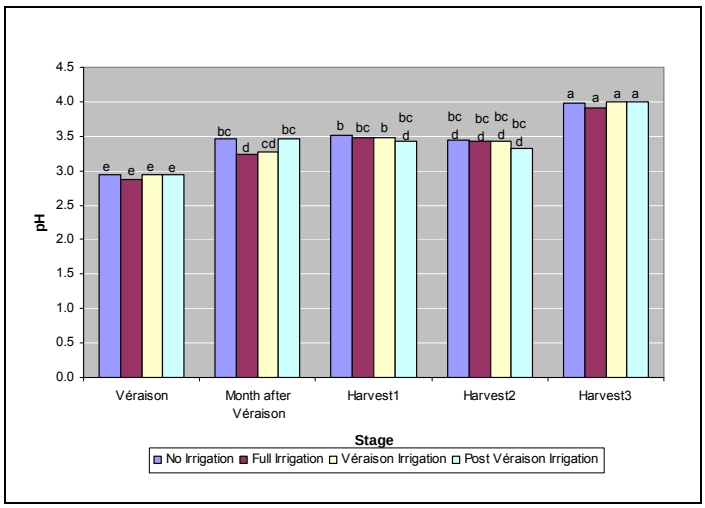

Fig. 9. Average pH over two seasons harvested at different stages of ripening and for different treatments.

Phenols: Higher phenolic contents were found in the berry skins during the 2005-2006 season (Fig. 10). Although a steady pattern occurred during this season, this was not the case in the 2004-2005 season. The fully irrigated treatment nonetheless showed lower average values (Fig. 11). The average skin phenolic content of the berries measured over two seasons and harvested at different times during ripening indicated a steady increase in the phenolic content from véraison until the first harvest stage (Fig. 12). This occurred for all treatments, except for the post véraison treatment in which case the phenolic content of the berries decreased from one

month after véraison to the first harvest stage. The latter probably resulted from the irrigation at one month after véraison. From the first to the second harvest stage the phenolic contents of the post véraison treatment increased, whereas those of the other treatments decreased. From the second to the third harvest stage the phenolic content of the no irrigation treatment continued to decrease, that of the post véraison treatment increased, and that of the fully irrigated and véraison+post véraison treatment kept virtually stable.

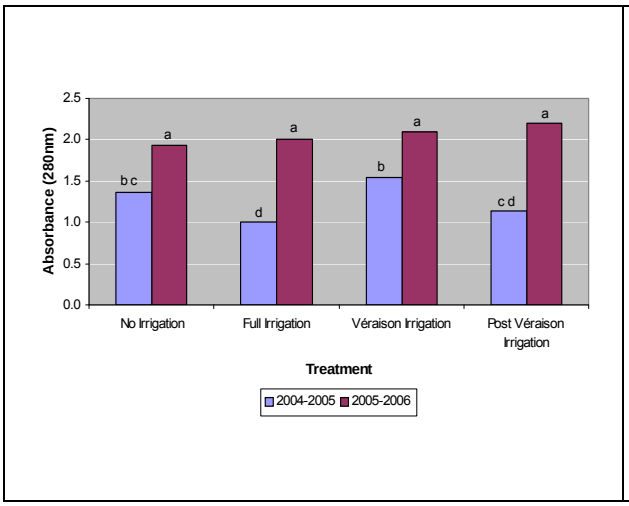

Fig. 10. Phenolic content (absorbance at 280 nm) for seasons 2004-2005 and 2005-2006 for different treatments.

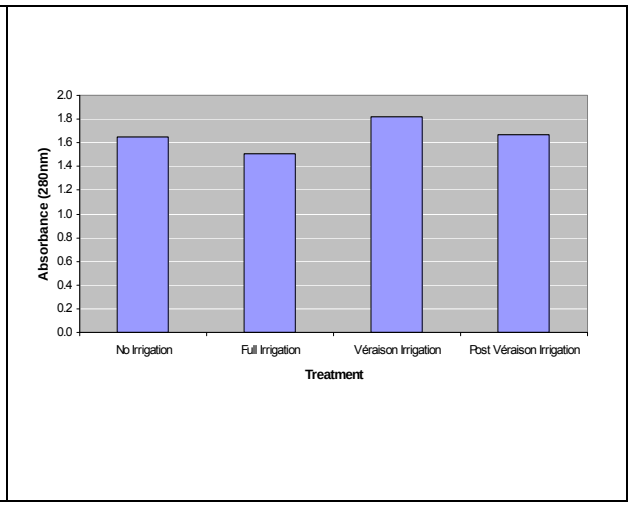

Fig. 11. Average phenolic content (absorbance at 280 nm) over two seasons for different treatments.

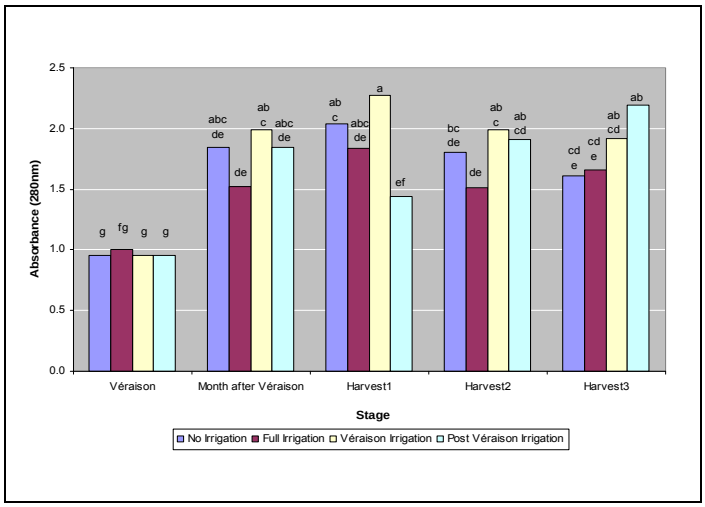

Fig. 12. Average phenolic content (absorbance at 280 nm) over two seasons harvested at different stages of ripening and for different treatments.

Anthocyanins and colour density: Similar to the phenolic content, the anthocyanin content of the berry skins of the different treatments was also higher in the 2005-

2006 season (Fig. 13). The trends were similar to those found for the phenolic content and showed lower average values for the full irrigation treatment (Fig. 14)

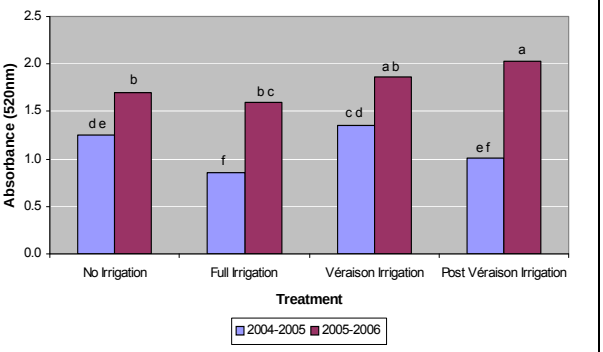

Fig. 13. Anthocyanin content (absorbance at 520 nm) for seasons 2004-2005 and 2005-2006 for different treatments.

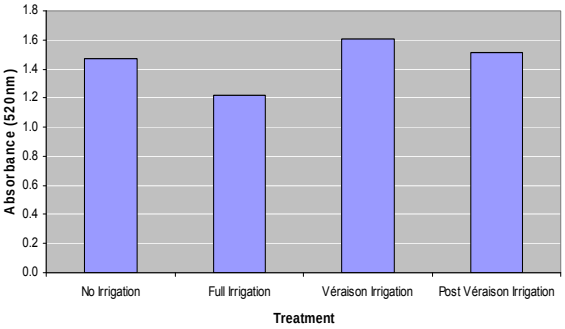

Fig. 14. Average anthocyanin content (absorbance at 520 nm) over two seasons for different treatments.

The average anthocyanin contents of the berry skins measured over two seasons and harvested at different times of ripening followed the same trends than those of the total phenolic contents (Fig. 15). A longer deficit period, followed by irrigation during the ripening period seemed to be favourable to the maintenance of phenolics in the skin for a longer period. Trends of the berry skin colour density of the different treatments followed similar trends to those of the anthocyanins (Figs. 16, 17 & 18).

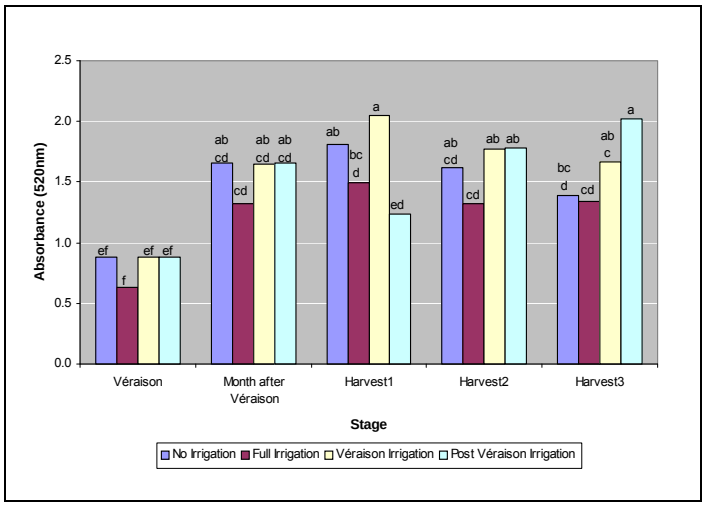

Fig. 15. Average anthocyanin content (absorbance at 520 nm) over two seasons harvested at different stages of ripening and for different treatments.

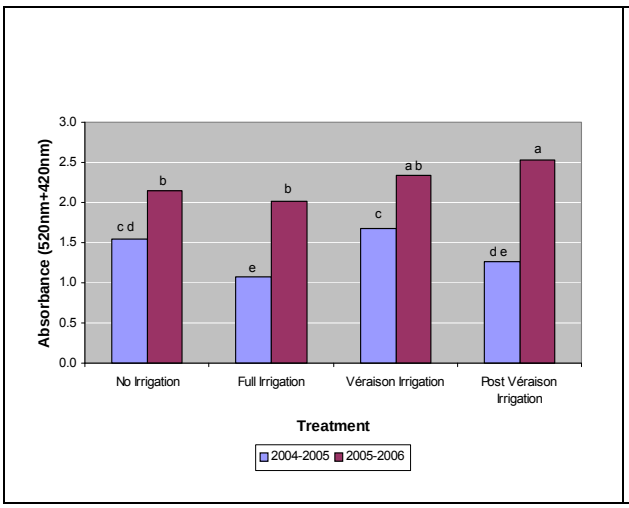

Fig. 16. Colour density (absorbance at 520 nm and at 420 nm) for seasons 2004-2005 and 2005-2006 for different treatments.

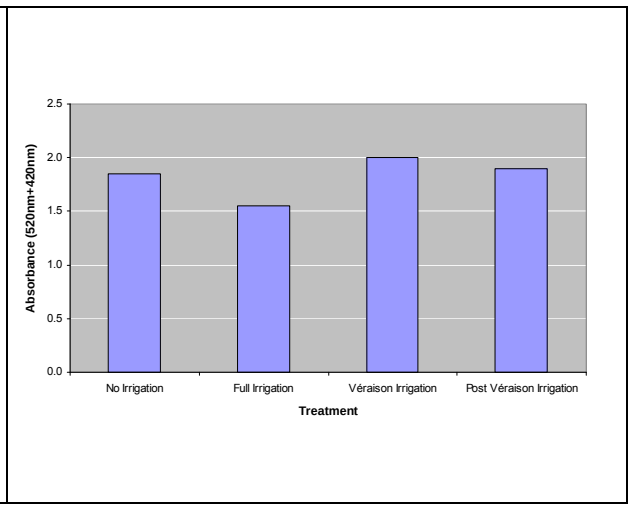

Fig. 17. Average colour density (absorbance at 520 nm and at 420 nm) over two seasons for different treatments.

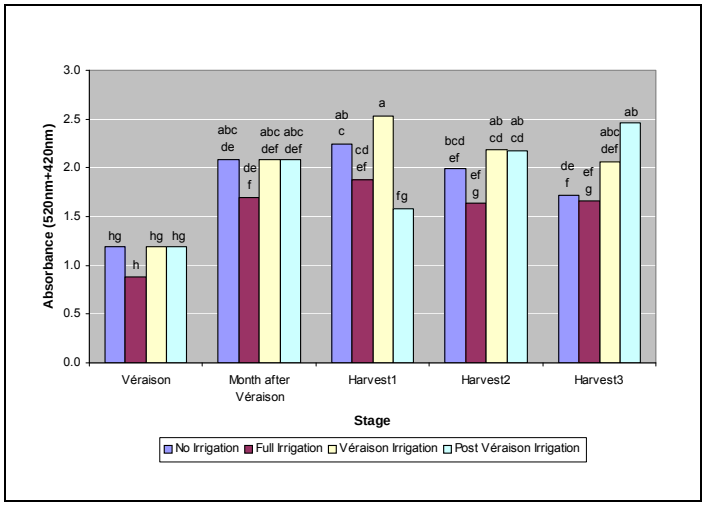

Fig. 18. Average colour density (absorbance at 520 nm and at 420 nm) over two seasons harvested at different stages of ripening and for different treatments.

Tannin: The tannin contents of the berry skins followed opposite seasonal trends to those of the total phenolics and colour expression, being lower during the 2005-2006 season (Fig. 19). The seasonal differences were in agreement with those found for soluble solids. The fully irrigated and véraison+post véraison irrigated treatments seemed to have highest average skin tannin contents (Fig. 20).

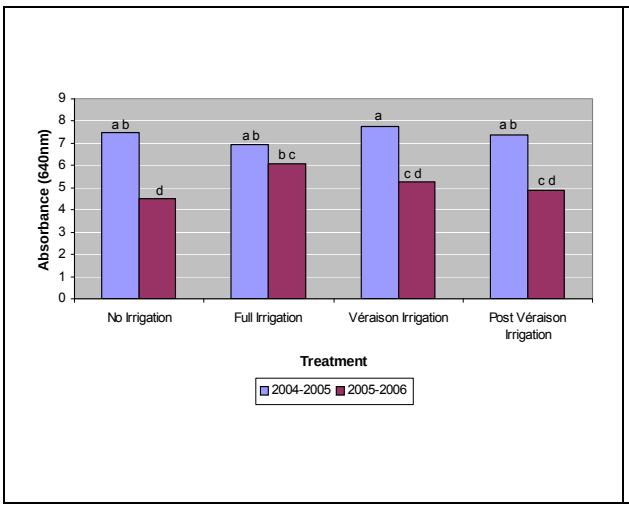

Fig. 19. Tannin content (absorbance at 640 nm) for seasons 2004-2005 and 2005-2006 for different treatments.

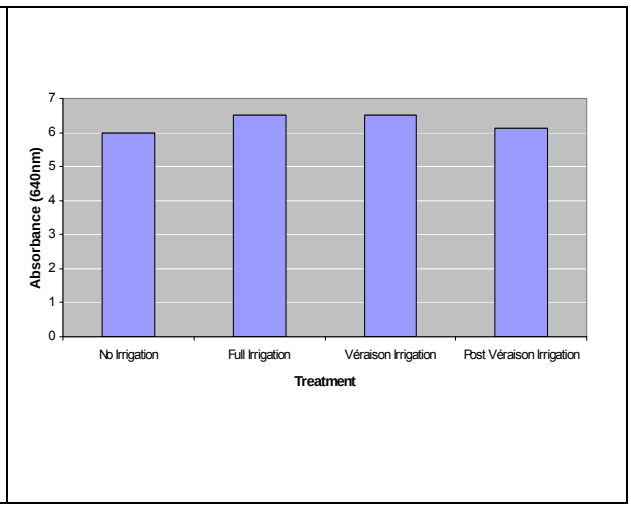

Fig. 20. Average tannin content (absorbance at 640 nm) over two seasons for different treatments.

The average tannin content of the berry skins measured over two seasons and harvested at different times during ripening indicated a steady increase in the tannin content from véraison until a month after véraison, where it seemed to have reached a maximum (Fig. 21). It then decreased for all the treatments, except for the véraison+post véraison irrigation and the full irrigation treatments, in which cases it increased again at the last harvest stage.

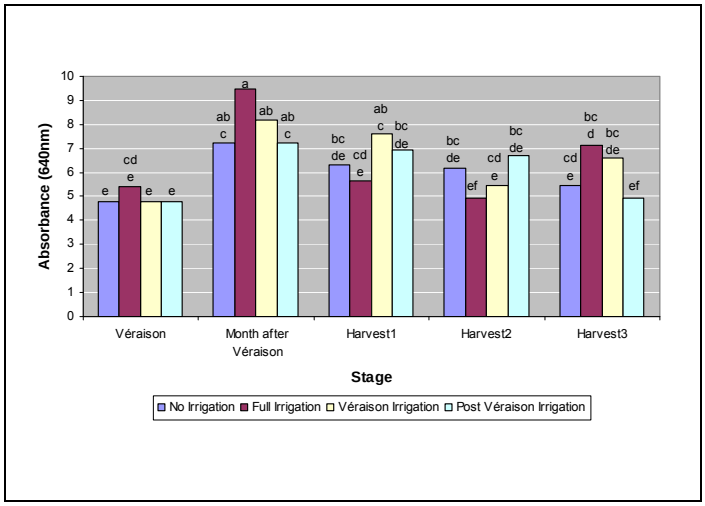

Fig. 21. Average tannin content (absorbance at 640 nm) over two seasons harvested at different stages of ripening and for different treatments.

The tannin content of the fully irrigated berries started off at higher values than the other treatments, but ended up having the lowest contents at the second harvest stage, where after it increased again. A similar trend occurred for the véraison+post

véraison irrigation. Further ripening than the second harvest stage therefore did not favour tannin contents in the skins and might have led to astringency on taste.

DISCUSSION

Wine quality is primarily dependent on the composition of the grapes and the ripeness level at harvest. Sugar play a vital role in the ripeness of wine grapes, together with pH and acidity. Wine producers most commonly use these indicators for identifying the ripeness level and optimal time of harvesting. Therefore an understanding of the development of these parameters during berry ripening is important. With an increase in ripening, sugars and pH increase and acidity decreases. Sugar levels also indicate the potential alcohol content after fermentation and the likelihood of residual sugar. Wines made from grapes with high concentrations of sugar usually have a higher alcohol content.

Water deficit may influence grape composition directly by modifying the physiological processes or indirectly by modifying the physical canopy environment and therefore fruit exposure (Ginestar *et al*., 1998). Smart & Coombe (1983) noted that excessive irrigation delayed ripening, increased the yield due to berry enlargement, and reduced anthocyanins due to shading that was caused by excessive shoot growth. Water stress, on the other hand, altered fruit composition, reduced shoot growth (Smart & Coombe, 1983; Schultz & Matthews, 1993; Roby & Matthews, 2004), enhanced early ripening, but reduced the yield and berry mass due to excessive exposure (Smart & Coombe, 1983). According to Hasselgrove *et al*. (2000), berries developing in well-exposed canopies, as opposed to those developing in heavily shaded canopies, have higher must sugar concentration, lower pH, higher titratable acidity and less incidence of unripe flavours.

Soluble solids: During the different stages of ripening (after véraison) the soluble solid concentration of all the treatments increased linearly with a linear decrease in berry mass (Chapter 4) and water content (Chapter 3). This is similar to the pattern described by Coombe (1992b), who stated that an increase in ºBalling of ripening grapes is usually associated with a loss of berry water, without a loss in the mass of the solutes in the berries. Water stress induced a faster increase in berry sugar than in the case of well watered vines, which had a slow increase in sugar (Smart & Coombe, 1983). Water stress enhanced early ripening due to excessive exposure.

During the different stages of ripening, the berries of the full irrigation treatment had less soluble solid contents than the deficit treatments, being significantly lower than the no irrigation treatment at the first harvest, and significantly lower than the véraison+post véraison and post véraison irrigation treatments at the second harvest. At the third harvest, the full irrigation treatment had the least soluble solid contents in the berries. It seemed as if the soluble solid contents of the berries of the deficit-irrigated vines initially increased faster than those of the full irrigation treatment. From the second harvest onwards, the soluble solid concentration apparently benefited from the additional irrigation at véraison+post véraison and post véraison, respectively. In water stressed vines, photosynthetic activity is reduced because of stomatal closure (Dűring, 1990; Schultz, 1996) and this may lead to lower carbohydrate production and thus translocation, compared to vines not subjected to water deficit (Gomez-del-Campo *et al*., 2002).

Although a decrease in ºBalling can usually be explained as being due to an increase in berry water following irrigation or rain, Coombe (1992b) also stated that there are indications that a decline in ºBalling might not always be associated with a change in water per berry. Production as well as translocation of carbohydrate to the berry may also be impaired. The lower soluble solid content in the berries of the full irrigation treatment, compared to the véraison+post véraison irrigation and post véraison irrigation treatment, may be explained by the findings of Morris & Cawthon (1982), who noted that excess water would normally reduce sugar, but with moderate irrigation, during dry years, it may be increased. The late ripening irrigation (post véraison) in particular seemed to favour a wider harvesting window.

Titratable acidity and pH: During the different stages of ripening (after véraison) the titratable acidity of the must of all the treatments decreased as the soluble solids increased up to the final stage of harvest. Freeman & Kliewer (1983) found that water stress or irrigation had no effect on the decline of the must titratable acidity. No significant differences in the titratable acid of the berry must were found between the treatments. The relatively high titratable acid of the post véraison irrigation treatment, despite the high soluble solid content, is still remarkable. This result is in concurrence with other studies on water stress and its effect on berry composition (Hardie & Considine, 1976; Reynolds & Naylor, 1994; Matthews & Anderson, 1988; Ginestar *et al.*, 1998). However, it is possible that the composition of the organic

acids may have changed, especially the tartaric:malic acid ratio; this was not determined in the study.

The pH of the must of all the treatments increased up to the third stage of harvest. This is in agreement with results of Iland & Coombe (1988) and Hunter (1991). No differences between treatments occurred. It may have been expected that the translocation of potassium to the berries may have increased for the water deficit treated vines due to a possible decrease in photosynthetic activity (not determined). The accumulation of potassium in the berries is one of the factors with the greatest impact on the must pH (Boulton, 1980; Ginestar *et al.*, 1998, Yuste *et al.*, 2004). The relatively high pH of the full irrigation treatment may have resulted from a dense canopy caused by increased growth, leading to overshadowing (Smart & Coombe 1983). It is also possible that the ratio of tartaric:malic acid may have changed in favour of malic acid and therefore leading to a organic acid composition with lower buffering potential.

Phenols, skin colour and tannins: The composition of phenolics depends on the cultivar, and is influenced by viticultural and environmental factors (Brossaud *et al*., 1999). Since phenolic concentration depends on the skin surface:berry volume ratio, the final size of the berry affects the phenolic concentration (Singleton, 1972; Matthews & Anderson, 1988; Ojeda *et al*., 2002; Roby & Matthews, 2004; Roby *et al.*, 2004). Work done by Ojeda (1999) showed that the pericarp cellular volume, independently of period and intensity of water deficit, causes berry size and mass reduction due to deficit treatments. Cell multiplication and indirectly cell numbers per berry pericarp were not affected. The biosynthesis of phenolic compounds may be followed by their content expressed in terms of the skin mass per single berry. The results indicated that the phenol content of the berries was dependent on total skin mass, which was affected by water deficit. The phenolic concentration would therefore be affected by the water content of the skins of the berries.

Although there were no statistical differences in the phenolic content of the skins over two seasons, the phenolic content of the full irrigation treatment was less than that of the other treatments. Significant differences in the phenolic content were observed only during the 2004-2005 season between treatments. Perusal of the different stages of measurement showed that the phenolic content of the berry skins

increased steadily from véraison until the first harvest stage. The formation of phenolics was therefore not affected by the dilution effect of an increasing berry volume. This was, however, not the case for the post véraison irrigated vines, in which case the long deficit period probably played a role in the effect of irrigation, resulting in a decrease in values from the post véraison to the first harvest stage. The skin phenolic content of the post véraison treatment continued to increase from the first to the last harvest stage, whereas that of the fully irrigated and véraison+post véraison irrigated treatments decreased from the first to the second harvest stage and kept virtually stable after that. The skin phenolic content of the no irrigation treatment steadily decreased from the first harvest to the last harvest stage. During this time, the skin surface area followed a similar pattern for all the treatments, increasing from the first to the second harvest stage and decreasing thereafter (Chapter 4). The skin surface area seemed not to have played a significant role in skin phenolic contents. This appears to be in contrast to results found by others (Singleton, 1972; Matthews & Anderson, 1988; Roby *et al.*, 2004) who noted a positive relationship between phenolic content and skin surface area. It is possible that the ripening period between the studies differed, this study having a longer ripening period.

The level of shade in the canopies and the degree of bunch exposure could also have affected the total phenol content of the berries, as the full irrigation treatment in particular had a denser canopy than the other treatments. According to Smart *et al*. (1985) and Hunter (1991), vigorous growth conditions with lower canopy light intensities, may induce lower phenol contents in berries as opposed to bunches with better sunlight exposure.

The trends found for skin anthocyanin content were similar to those found for phenolic content. The anthocyanin content of the full irrigation treatment was lower than that of the deficit treatments. This is in agreement with results found by Freeman & Kliewer (1983). For both anthocyanin and skin colour density, it seemed that a longer deficit period, followed by irrigation during ripening, was favourable to the maintenance and or continued formation of phenolic compounds in the skin for longer and effectively contributed to a wider harvesting window. These results concur with other findings (Freeman & Kliewer, 1983; Kennedy *et al*., 2002; Ojeda *et al*., 2002; Roby *et al*., 2004; Sivilotti *et al*., 2005), where treatments under mild water

stress showed higher anthocyanin concentrations, most probably due to improved bunch exposure (Ginestar *et al*., 1998).

The berry skin anthocyanin content of the no irrigation treatment decreased quickly after the first harvest stage. A lack of continued water flow to the berries may have led to the degradation of anthocyanins.

The fully irrigated and véraison+post véraison irrigated treatments seemed to have highest average skin tannin contents. Seasonal trends were opposite to those of phenolics and anthocyanins, but similar to those found for soluble solids. Tannin contents seemed to reach maximum values at one month after véraison, where after it decreased for all but the full and véraison+post véraison irrigated treatments, in which cases it increased again at the last harvest stage. In the case of these treatments further ripening than the second harvest stage may not have been favourable to softer mouth-feel, but may have increased astringency. According to Roby *et al.* (2004) the concentration of skin tannin was unchanged with berry size. Thus the concentration of the tannins increased by an effect of vine water status restricting degradation, which was independent of the role of water status on the size of the berry, because all the berries in comparison were of similar size.

In accordance with Kennedy *et al.* (2002), the development of the average tannin content of the berries measured over two seasons harvested at different times of ripening indicated a steady increase in the tannin content from véraison up until a month after véraison where it seemed to have reached a maximum, and then decreased for all the treatments. The véraison+post véraison irrigation treatment and the full irrigation treatment, however, indicated an irregular increase at the third harvest stage.

CONCLUSIONS

Different irrigation treatments seemed to have affected the soluble solid accumulation in the grape berries. No irrigation, véraison+post véraison irrigation and post véraison irrigation seemed to have enhanced the sugar accumulation. No irrigation ostensibly delayed soluble solid accumulation in the berries, probably due to insufficient photosynthetic carbohydrate production and translocation. Soluble solid concentration apparently benefited from additional irrigation at véraison+post

véraison and post véraison, after an extended deficit period. The post véraison irrigation in particular seemed to favour a wider window for harvesting.

Although the irrigation treatments did not seem to affect the titratable acid of the berry musts, the relatively high titratable acid of the post véraison irrigation treatment, despite high soluble solid concentrations, is noticeable. The pH of the musts was not affected by the different irrigation treatments. Previous studies showed increased (Freeman & Kliewer, 1983) and decreased (Neja *et al*., 1977) must pH due to supplementary irrigation, but the effects were always marginal. In this study it could be argued that the full irrigation treatment had higher than expected pH, possibly due to unfavourable canopy conditions.

Differences in phenolic content were observed during the different harvest times. Véraison+post véraison irrigation and post véraison irrigation seemed to have the greatest influence on the skin phenolic contents as it developed to a higher extent. The average anthocyanin content and the colour density followed the same trend than the phenolic content. The full irrigation treatment seemed to have restricted the development of the anthocyanins and decreased the colour density in the berry skins. It seemed as if irrigation treatments had direct and indirect effects on the biosynthesis of colour components in the berry skins. The production of photosynthetates may have been negatively affected due to excessive water deficit, whereas overshadowing may have been caused by vigorous vegetative growth due to too much water.

The average tannin content in the berry skins did not differ between irrigation treatments. The tannin content in the fully irrigated vines increased to a higher initial level than that of the deficit treatments and seemed to have decreased more rapidly thereafter. At the first two harvest stages, the tannin content was higher in the treatments with water deficit, whereas at the last harvest stage, the berry skins of vines that received water at all stages (fully irrigated) and at véraison+post véraison had higher tannin contents. Irrigation at post véraison and especially véraison+post véraison seemed to have a greater effect on the synthesis of the phenolic, anthocyanin and tannin content as it reached higher values than in the berry skins of the fully irrigated and non-irrigated vines.

Berry size and thus skin:pulp ratio must be considered before quality assessment based on phenol, colour and tannins, can be made. Small berries are considered a key component of grape quality (Bravdo *et al*., 1985; McCarthy, 2000; Kennedy *et al*., 2002) for red cultivars such as Shiraz (McCarthy, 2000). According to Freeman & Kliewer (1983) and Hardie & Considine (1976), wine colour was reduced due to irrigation, because of reduction in the proportion of pigments in the coloured form. Hardie & Considine (1976) noted a decrease in colour where yields have been increased by irrigation. These differences have been related to greater skin area:volume ratio of small, non-irrigated berries.

Up until the first harvest stage, the skin total phenolics, anthocyanins and tannins have been reasonably concerted with berry mass in terms of patterns among the treatments. However, from the second harvest, opposite patterns to berry mass emerged, this being particularly noticeable at the last harvest stage with smaller (lighter) berries apparently leading to higher extraction from skins. It is interesting to note that tannin contents, except for the no irrigation treatment, did not follow the expected smaller berry, higher values, and pattern at the last harvest stage, but rather changed parallel to berry mass changes. An exception to the latter occurred for the no irrigation treatment.

The higher phenolic, anthocyanin and tannin content and higher colour density of the véraison+post véraison irrigation berries may increase the potential structure and complexity of wine made from these berries. It is interesting to note that the style of wine may be affected by the timing of irrigation. Fully irrigated vines may lead to more green and astringent wine flavours, non-irrigated vines may lead to soft wines with reasonable colour and structure when harvested earlier, véraison+post véraison-irrigated vines may lead to well-structured and coloured wines, and post véraison irrigated vines may lead to well-structured and coloured wines that may either be high in tannin (earlier harvesting) or with softer mouth-feel (late harvesting).

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Chapter VI

GENERAL DISCUSSION AND CONCLUSIONS

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National as well as international competition between wine producing countries forces grape growers to produce grapes of the best possible quality for the production of outstanding wines that would satisfy the requirements of the consumer.

Water is an important factor influencing vegetative and reproductive growth. Vines with unrestricted growth may lead to increased berry size and reduced wine quality. Water deficit may reduce the functioning of grapevine canopies. Berry size at harvest, for especially red grape varieties, is considered a very important component of determining wine grape quality all over the world (Bravdo *et al*., 1985; McCarthy, 2000; Kennedy *et al*., 2002). It is assumed that smaller berries may deliver wine with more complexity, aroma and colour. There is an increasing need to manipulate berry size in the vineyards and to obtain optimum levels of ripeness regarding sugar, pH, titratable acid and phenolic compounds to produce wine of higher quality. The potential for extracting higher amounts of phenolic compounds from the skin increases with smaller berries. In warmer wine producing countries, smaller berries may facilitate the buffering of pH increases and resulting negative effects on wine quality.

Manipulation of vegetative or reproductive growth to maintain or to enhance wine grape quality without adversely affecting yield would be beneficial to many viticulturists and winemakers (McCarthy, 1997). A possible method of manipulating berry size is by controlling the soil water availability during berry development. Water deficit has inhibitory effects on vegetative and reproductive growth and alters the phenology (Coombe, 1992). According to Smart (1974), water deficit has many effects on the grapevine, which involve reactions at intercellular, cellular and tissue levels. A decrease in stomatal opening is one of the most common responses. This enables the plant to alleviate unfavourable conditions of water status and environmental stress, but it reduces the uptake of CO_2 and hence photosynthesis (Dűring, 1990; Schultz, 1996).

Water stressed plants with lower photosynthesis, together with reduced leaf area, result in lower production compared to vines not subjected to water deficit (Gomez-del-Campo *et al*., 2002).

Soil water status may lead to leaves and bunches developing in different conditions, varying from heavily shaded to exposed canopies. According to Hasselgrove *et al*. (2000), berries developing in well-exposed canopies, as opposed to those developing in heavily shaded canopies, have higher must sugar concentration, lower pH, higher titratable acidity and less incidence of unripe-like flavours. Strong water deficit reduced shoot growth, altered fruit composition (Smart & Coombe, 1983; Schultz & Matthews, 1993; Roby & Matthews, 2004), and enhanced ripening, but reduced yield and berry mass due to excessive exposure (Smart & Coombe, 1983). By reducing berry size, bunches would be less compact with a more open bunch framework that would expose a greater berry surface area of such berries to sunlight. Higher sunlight levels within and around the bunch may improve the colour of red-skinned grape berries (Smart, 1982).

The purpose of this study was to determine the effect of varying vine water status on vegetative and reproductive growth as well as grape composition in a Shiraz/Richter 99 vineyard as well as the significance of vine water status in the length of the ripening period and in grape composition, as related to the identification of different ripeness levels. The study was done on a seven-year-old *Vitis vinifera* L. cv. Shiraz (clone SH1A), grafted onto Richter 99 (*Vitis Berlandieri x Vitis rupestris*) (clone RY2A) in the Stellenbosch region. The vines are spaced 2.75m x 1.5m on a Glenrosa soil with a western aspect (26º slope) and orientated in a North-South direction. The vines are trained onto a 7-wire lengthened Perold trellising system (VSP) of which three sets of wires are movable. Vines were pruned to two-bud spurs with a spur spacing of approximately 15 cm. Canopies were suckered, shoot positioned and tipped/topped during the pre-véraison period. Irrigation was applied through a micro-sprinkler system.

Four treatments, comprising irrigation combinations to field water capacity at different stages, were applied (field water capacity of the soil was determined before the start of the experiment). The treatments were completely randomised in two blocks, representing two replications. Thirty vines were used per replication. The treatments were: seasonal irrigation from berry set with further irrigation at pea size; véraison and one month post véraison; irrigation at véraison with further irrigation at post véraison; irrigation at post véraison; and no irrigation. The sampling of the

treatments was split into five stages: véraison, one month after véraison, and three times during the ripening period.

The predawn leaf water potential clearly showed the differences between the irrigation treatments. Water deficit vines experienced basically similar diurnal plant water status due to efficient control of water loss by the stomata. As water stress intensified during the morning, the stomata closed, preventing an excessive drop in the leaf water potential during the day. Lower leaf water potentials indicated lower water contents in the vegetative and reproductive tissue. The water potential of irrigated vines seemed to remain more constant than that of the water deficit vines. Non-irrigated vines nonetheless seemed to maintain higher diurnal leaf water potentials, compared to the véraison+post véraison and post véraison irrigated vines. In general, higher water contents were observed in the basal parts of the primary shoots, in the secondary shoots in this region, and in leaves in this region. The vegetative organs experienced water loss through the season, but the water content increased at the end of the season at an advanced stage of berry ripening, probably due to a lower demand for water from the largely senescing canopy and changing environmental conditions (such as lower temperatures). The vine therefore recuperated in terms of water relations, seemingly irrespective of soil water availability.

Irrigating vines strongly affected vegetative development, changing the canopy dimensions. Full irrigation stimulated primary shoot length, compared to those of deficit-irrigated vines. It would seem that earlier and more complete shoot maturation (reserve accumulation) was obtained with longer water deficit. The rate of development and position of occurrence of secondary shoots was affected by irrigation. Seasonal irrigation seemed to accelerate development and stimulate occurrence in middle parts of primary shoots. In contrast, longer water deficit (seasonal deficit and post véraison irrigated) seemed to induce fewer, but longer, secondary shoots in basal parts of primary shoots. The presence of secondary shoots in this region may be beneficial regarding the ripening of the bunches under water deficit conditions. The results show that a re-distribution of leaf area on the shoot may occur when vines are subjected to water deficit. Secondary leaves in the basal position in particular, clearly had a significant role in water and photosynthetate translocation to the berries, particularly during late ripening.

Bunch and berry mass were affected by water deficit and the timing of irrigation. Berries of véraison+post véraison and post véraison irrigated vines seemed to accumulate water rapidly after irrigation. The berries seemed more sensitive to irrigation after a water deficit period. The fully irrigated and non-irrigated vines apparently managed their water accumulation; berry water content was kept constant through the season before water loss and berry shrinkage occurred. The water relations were reflected in the berry size. Transpiration losses were probably much higher in fully irrigated vines, whereas extended water deficit seemed to induce earlier and restricted water loss through efficient stomatal control, thereby maintaining water relations.

The full irrigation treatment during the season induced larger berry skin surface and probably represents cell expansion forced by high water potential. Skin area enlargement continued until late during ripening, after which berry shrivelling occurred. Berry shrivelling during this time seemed to be the overriding factor determining a reduction in skin:pulp ratio at the last harvest stage.

Irrigation treatment seemed to have affected the soluble solid accumulation in the grape berries. No irrigation, véraison+post véraison irrigation and post véraison irrigation seemed to have enhanced the sugar accumulation, whereas full irrigation reduced accumulation. No irrigation apparently delayed soluble solid accumulation in the berries, probably due to insufficient photosynthetic carbohydrate production and translocation. Soluble solid concentration apparently benefited from additional irrigation at véraison+post véraison and post véraison after an extended deficit period. The post véraison irrigation in particular seemed to favour a wide window for harvesting.

Although the irrigation treatments did not seem to affect the titratable acid of the berry musts, the relatively high titratable acid of the post véraison irrigation treatment, despite high soluble solid concentrations, is noticeable. The pH of the musts was not affected by the different irrigation treatments. In this study it could be argued that the full irrigation treatment had higher than expected pH, possibly due to unfavourable canopy conditions.

Véraison+post véraison irrigation and post véraison irrigation seemed to have the greatest influence on the skin phenolic contents, anthocyanin contents and the colour density, as it developed to a higher extent. The full irrigation treatment seemed to have restricted the development of the anthocyanins and decreased the colour density in the berry skins. It seemed as if irrigation treatments had direct and indirect effects on the biosynthesis of colour components in the berry skins. The production of photosynthetates may have been negatively affected due to excessive water deficit, whereas overshadowing may have been caused by vigorous vegetative growth due to too much water. Either way would have imposed a stress condition in the vine, affecting grape composition.

The tannin content in the fully irrigated vines increased to a higher initial maximum than the deficit treatments and seemed to have decreased more rapidly there after. At the first two harvest stages, the tannin content was higher in the treatments with water deficit, whereas at the last harvest stage, the berry skins of vines that received water at all stages (fully irrigated) and at véraison+post véraison had higher tannin contents. Irrigation at post véraison and especially véraison+post véraison, seemed to have a greater effect on the synthesis of the phenolic, anthocyanin and tannin content as it reached higher values than in the berry skins of the fully irrigated and non-irrigated vines.

Berry size and thus skin:pulp ratio must be considered before quality assessment based on phenol, colour and tannins, can be made. Up until the first harvest stage, the skin total phenolics, anthocyanins and tannins have been reasonably concerted with berry mass in terms of patterns among the treatments. However, from the second harvest, opposite patterns to berry mass emerged, this being particularly noticeable at the last harvest stage with smaller berries apparently leading to higher extraction from skins. It is interesting to note that tannin contents, except for the no irrigation treatment, did not follow the expected smaller berry, higher values, pattern at the last harvest stage, but rather changed parallel to berry mass changes. An exception to the latter occurred for the no irrigation treatment.

The higher phenolic, anthocyanin and tannin content and higher colour density of the véraison+post véraison irrigation berries may increase the potential structure and complexity of wine made from these berries. The style of wine may be affected by

the timing of irrigation. Fully irrigated vines may lead to more green and astringent wine flavours, non-irrigated vines may lead to soft wines with reasonable colour and structure when harvested earlier, véraison+post véraison-irrigated vines may lead to well-structured and coloured wines, and post véraison irrigated vines may lead to well-structured and coloured wines that may either be high in tannin (earlier harvesting) or with softer mouth-feel (late harvesting).

Irrigation strategies need to be judiciously planned and applied in order to maintain a balance between vegetative growth, reproductive development and grape composition. Véraison+post véraison and post véraison irrigated vines seemed to have the most positive effects regarding vegetative growth and berry ripening. By improving the vine water relations, a wider window for harvesting was created due to extended berry ripening and intact berry condition. The timing of irrigation may therefore lead to the production of different styles of wine, appealing to a wider range of consumers.

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