

## Grape berry metabolism in field-grown grapevines exposed to different irrigation strategies

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### Summary

The response of grape berry metabolism to vine water status was investigated in field grown grapevines (*Vitis vinifera* cv. Castelão) in southern Portugal. Water was supplied as: full irrigation (FI), to minimum water deficit corresponding to 100 % of crop evapotranspiration (Etc), partial rootzone drying (PRD) and deficit irrigation (DI), both corresponding to an irrigation amount of 50 % Etc, and a rainfed, *i.e.* no irrigation treatment (NI). In PRD, water was supplied to one side of the root system during each irrigation period, alternating sides every 15 d approximately. During the growing period, PRD and DI vines showed intermediate pre-dawn leaf water potential ( $\Psi_{pd}$ ) values (around -0.4 MPa) by the end of the growing season, FI vines -0.2 MPa and NI -0.8 MPa. Berry weight as well as the content of glucose and fructose per berry increased in irrigated vines (PRD, DI, and FI) compared to NI vines. Although both malic and tartaric acid declined in non-irrigated vines, there was no significant difference between treatments at harvest. The activities of invertase, malate dehydrogenase and malic enzyme were not affected by irrigation throughout the ripening process. The contribution of other factors involved in the reduction of sugars and organic acids in berries of non-irrigated vines are discussed. These results show that deficit irrigation, like PRD and DI, do not have any negative impact on growth and quality of grape berries compared to fully irrigated vines, but may result in improved berry quality compared to rainfed vines.

**Key words:** Berry, deficit irrigation, invertase, malic enzyme, partial rootzone drying, *Vitis vinifera*.

### Introduction

The use of irrigation in the Mediterranean viticulture has been considered as standard practice, mainly during drought periods when it has become an effective means of regulating water availability to grapevines. Maintaining the balance between vegetative and reproductive growth is, however, one of the most difficult problems in irrigated viticulture.

In recent years, different irrigation techniques have been developed, such as regulated deficit irrigation (RDI), where irrigation is reduced during a defined period of berry development and partial root-zone drying (PRD), where water is applied at alternate sides of the root system to balance vegetative and reproductive growth (McCARTHY 1997; BATTILANI 2000; LOVEYS *et al.* 2000). Previously, we showed that reduced irrigation (*e.g.* PRD and deficit irrigation, DI, where the same amount of water applied in PRD is distributed on both sides of root systems) decreased vegetative vigour and stomatal conductance compared to fully irrigated plants (FI), without altering significantly yield (SANTOS *et al.* 2003; SOUZA *et al.* 2003). The effects of these irrigation strategies on grape berry metabolism need, however, to be evaluated. Although some details of grape berry ripening have been described, the influence of some environmental factors (including water availability) on the rate of sugar import and the activities of related enzymes are still poorly understood (BOSS and DAVIES 2001).

Vacuolar acid invertase is considered the major enzyme involved in sucrose breakdown and accumulation of hexoses in grape berries (HAWKER 1969; BROWN and COOMBE 1984; DAVIES and ROBINSON 1996). The net decrease in the malic acid content during berry ripening has been attributed mainly to reduced malate synthesis by phosphoenolpyruvate carboxylase (PEP carboxylase), to its degradation by NADP-dependent malic enzyme (HAWKER 1969; LAKSO and KLIWER 1975) and to malate dehydrogenase (TAUREILLES-SAUREL *et al.* 1995).

The aim of the present study was to enlarge our knowledge regarding the effects of water availability on grape berry development and maturation in order to match physiological down-regulation by water stress with controlled irrigation programs in field-grown grapevines. Thus, the effects of different irrigation techniques on the sugar and acid content as well as on the activities of key enzymes involved in sugar and organic acid metabolism in ripening grape berries were investigated.

### Material and Methods

**Plant material and irrigation treatments:** The experiments were conducted in a commercial vineyard

located 70 km east of Lisbon, at the Centro Experimental de Pegões, 30°38' N and 8°39'W, during the 2002 season. The mediterranean climate is hot and dry in summer while winters are mild. The average annual precipitation and temperature is 694 mm and 16 °C, respectively. The soil is derived from podzols, with a sandy surface layer (0.6-1.0 m) and clay at 1 m depth. The variety used was Castelão (*Vitis vinifera* L.), an early ripening red wine variety (SANTOS *et al.* 2003), grafted on 1103 Paulsen in 1996. The grapevines were spaced 2.5 m between rows and 1.0 m within rows and trained on a vertical trellis with a pair of movable foliage wires for upward shoot positioning. The vines were spur-pruned on a bilateral Royat Cordon (approximately 16 buds per vine).

Irrigation was applied with drip emitters (4 l h<sup>-1</sup> for FI and PRD and 2 l h<sup>-1</sup> for DI), two per vine, positioned 30 cm from the vine trunk at both sides of the rows. Water was supplied according to crop evapotranspiration (ET<sub>c</sub>) calculated from the evaporation of a Class-A pan and corrected with the crop coefficients (K<sub>c</sub>) proposed by PRICHARD (1992). The treatments were: rain-fed, non-irrigated (NI); partial rootzone drying (PRD, 50 % of the ET<sub>c</sub> was supplied to only one side of the root system, alternating sides each 15 d approximately); deficit irrigation (DI, 50 % of the ET<sub>c</sub> was supplied to both sides of the vine, 25 % on each side); full irrigation (FI, 100 % of the ET<sub>c</sub> was supplied to both sides of the root system, 50 % on each side). Water was supplied twice per week from the beginning of berry development (June) until harvest (September). The total amount of water supplied to FI plants was 197 mm (493 l per vine). The PRD and DI vines received half of that amount. The total rainfall during the experimental period (mid-June until the beginning of September) was 8.8 mm and total annual rainfall in 2002 was 757 mm.

**Water status:** Pre-dawn leaf water potential was measured with a Scholander-type pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA). The measurements were done on 6 fully expanded leaves per treatment.

**Sugar and organic acid analysis:** The grapes berries used for sugar and organic acids analyses were taken during the summer of 2002, at 15-d-intervals approximately. Four replicate samples of 50 berries were harvested from different exposed bunches at random and immediately frozen in N<sub>2</sub> and stored at -80 °C until analysis. Ten frozen berries were weighed and used for sugar and organic acid determination.

After maceration of thawed berries, the samples were immediately filtered through two layers of cheesecloth and centrifuged at 27,200 g for 15 min. An anion exchange resin (Bio-Rex 5, Bio Rad Labs) was used to separate the supernatant into sugar and acid fractions. A 1 ml aliquot of the supernatant was pipetted into an Econo-column (Bio Rad Labs) containing Bio-Rex 5 resin and allowed to run freely, followed by elution with deionised water to a final volume of 10 ml. This eluate contained soluble sugars, while organic acids were retained on the column. The latter were desorbed from the resin with 1 ml of 10 % H<sub>2</sub>SO<sub>4</sub>, followed by deionised water to a final volume of 5 ml. This fraction contains the acid compounds. Each fraction was filtered

through 0.22 mm membranes (Millipore) before HPLC measurements.

Glucose and fructose as well as malic and tartaric acid were analysed using a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA) equipped with an Aminex HPX-87H column (Bio-Rad, Richmond, CA) and a differential refractometer detector (LKB, Bromma, Sweden). The column was operated at 65 °C and 5 mM H<sub>2</sub>SO<sub>4</sub> was used as the mobile phase at a 0.6 ml·min<sup>-1</sup> flow rate. The compounds were eluted in 20 min.

**Enzymes of sugar and acid metabolism in grape berries:** Five frozen berries (without seeds), taken from the samples used for sugar and organic acid analyses just before veraison (July) and at the end of the ripening period (August), were ground in a mortar containing liquid N<sub>2</sub> and 0.5-0.6 g of frozen powder was mixed with 5 ml of 100 mM Hepes-KOH (pH 8.0), 10 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 2 mM dithiothreitol (DTT), 0.1 % triton X-100, 4 % (v/v) 'Complete-protease inhibitor cocktail with EDTA', 10 % polyethyleneglycol (PEG) 4000, and 10 % glycerol. The extract was centrifuged at 27,200 g for 10 min and the supernatant was used for determination of enzymatic activities of acid invertase (EC 3.2.1.26), malic enzyme (EC 1.1.1.40) and malate dehydrogenase (EC 1.1.1.37).

The activity of invertase was determined as described by HUNTER *et al.* (1994) with some modifications. The extract was incubated in a mixture containing 100 mM NaOAc pH (4.0), 100 mM sucrose for 15 min at 30 °C. The reaction was stopped by addition of DNSA-reagent (1 % 3,5-dinitrosalicylic acid (w/v) in 0.5 M KOH and 1 M potassium sodium tartarate, thus shifting the pH far into the alkaline range). The mixture was kept in boiling water for 10 min, cooled to room temperature and colour intensity was read at 540 nm against a blank with zero s reaction time.

The activity of malic enzyme was measured by following reduction of NADP at 340 nm using a modified method of LAKSO and KLIEWER (1975). The assay mixture contained 100 mM Hepes-KOH (pH 8.0), 1 mM MgCl<sub>2</sub> and 0.13 mM NADP. The reaction was started by addition of 4 mM of L-malate and the activity was measured by following the reduction of NADP at 340 nm.

The activity of malate dehydrogenase was assayed using a modified method from LOPEZ-MILLÁN *et al.* (2000). The assay mixture contained 50 mM Hepes-KOH (pH 8.0), 0.1 mM NADH and 0.4 mM oxalacetate. The reaction was started by addition of oxalacetate and the activity was measured by following the oxidation of NADH at 340 nm.

**Statistical analysis:** Statistically significant differences between factor groups were evaluated with Tukey's HSD for a = 0.05 using the "Statistica" software (ver 5.0 StatSoft, Tulsa, OK, USA). All measurements shown are means ± SE. Linear regressions were obtained using Sigma Plot software (vers. 7.0, SPSS Science, Chicago).

## Results

The seasonal evolution of pre-dawn leaf water potential ( $\Psi_{pd}$ ) showed significant differences among treatments

(Fig. 1). As expected, the full-irrigated treatment (FI) maintained the highest values of  $\Psi_{pd}$  (about -0.2 MPa) throughout the season. In contrast,  $\Psi_{pd}$  was significantly lower in non-irrigated vines (NI) as compared to other treatments and at harvest reached values at about -0.8 MPa. PRD gave values higher than DI throughout most of the season, but both treatments showed similar values at harvest.

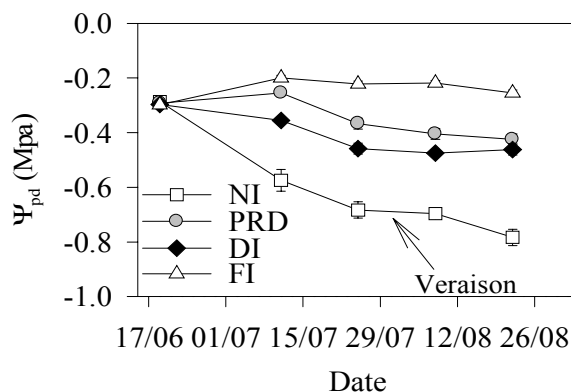


Fig. 1: Seasonal evolution of pre-dawn leaf water potential ( $\Psi_{pd}$ ). Values of  $\Psi_{pd}$  are means  $\pm$  SE. NI: non-irrigated, PRD: partial rootzone drying, DI: déficit irrigation and FI: full irrigated.

Berry weight of the irrigated treatments (FI, PRD and DI) were significantly higher (30 %) as compared to NI at harvest (Fig. 2). The concentration of glucose and fructose in the berries increased during the growing season (Fig. 3), whereas malic and tartaric acid decreased (Fig. 4). In general, berry composition was not significantly affected by the irrigation techniques when the results were expressed on a concentration basis ( $\text{g}\cdot\text{l}^{-1}$ ) (Fig. 3 a, b). However, in order to eliminate the dilution effect due to changes of berry size, sugars expressed on a per berry basis ( $\text{mg}\cdot\text{berry}^{-1}$ ) showed statistically significant differences among treatments

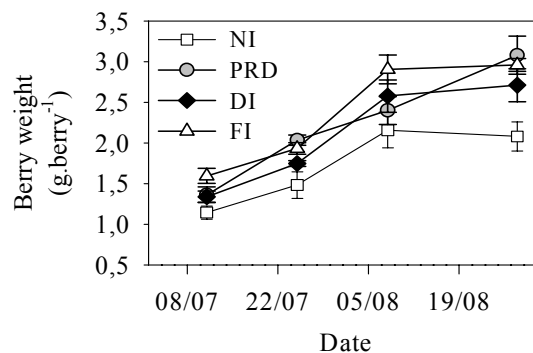


Fig. 2: Seasonal trends of berry weight for various irrigation treatments. Values are means  $\pm$  SE. (For abbreviations see Fig. 1).

(Fig. 4 c, d). Glucose and fructose accumulation was significantly higher in irrigated treatments than in NI vines. No significant differences were observed among treatments with respect to the evolution of organic acid concentration (Fig. 4 a, b). However, on a per berry basis malic acid was significantly higher in FI vines compared to NI vines (Fig. 4 c). PRD and DI vines showed no significant differences compared to FI or NI vines. Tartaric acid remained high during most of the experimental period in irrigated treatments, the differences being only statistically significant between FI and NI berries (Fig. 4 d). At the last sampling date, there were no significant differences in tartaric acid between treatments.

The glucose/fructose ratio was high (about 5) at the beginning of July, however after veraison the ratio decreased to 1.1 in all treatments (Fig. 5 a). There were no significant differences between treatments in the evolution of glucose/fructose ratio. The tartrate/malate ratio, increased from veraison until harvest, the only significant differences between treatments occurring by the end of August, when NI berries showed significantly higher ratios than FI and PRD

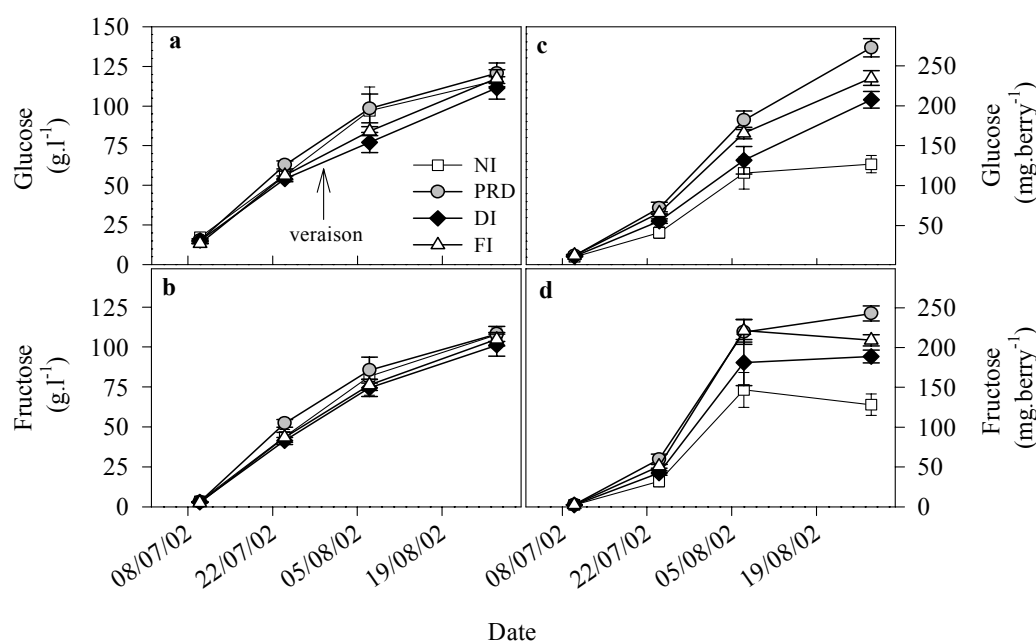


Fig. 3: Sugar accumulation in berries subjected to different irrigation treatments. Values are means  $\pm$  SE. (For abbreviations see Fig. 1).

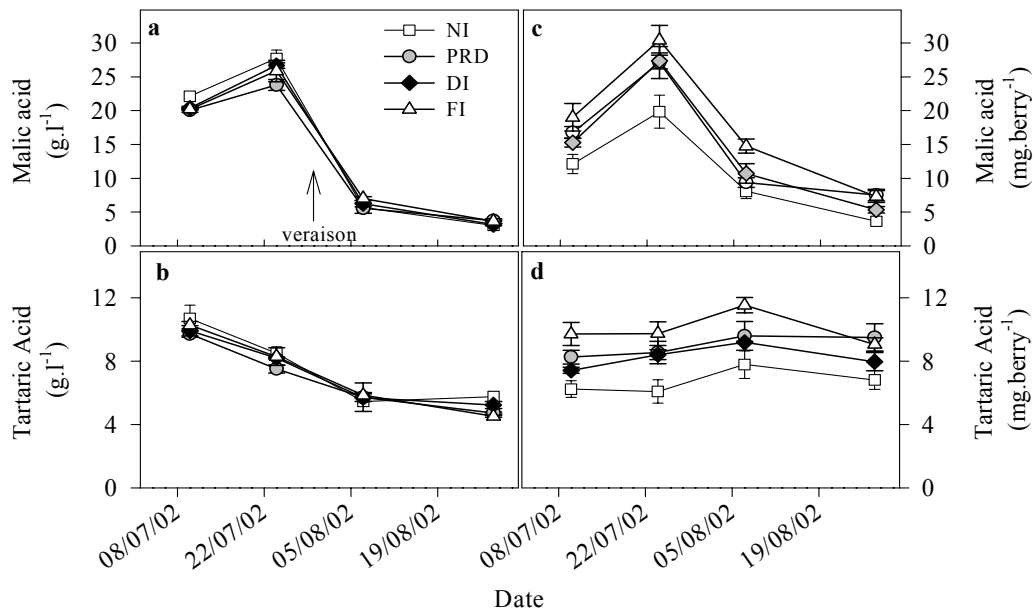


Fig. 4: Organic acid pattern in berries subjected to different irrigation treatments. Values are means  $\pm$  SE. (For abbreviations see Fig. 1).

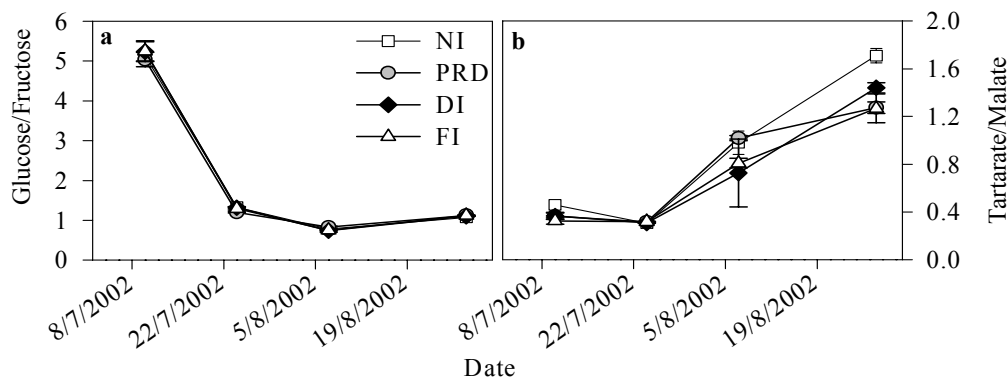


Fig. 5: Effects of irrigation treatments on the glucose/fructose ratio (a) and tartarate/malate ratio (b). Values are means  $\pm$  SE. (For abbreviations see Fig. 1).

(Fig. 5 b). However, there were no differences between irrigated treatments.

Fig. 6 shows the impact of soil water availability on enzymes involved in sugar and acid metabolism of grape berries analysed before veraison (July) and at the end of the ripening period (August). When the enzyme activities were expressed on a per gram fresh weight basis, only invertase activity was reduced in irrigated treatments by the end of August (Fig. 6 a, b, c). However, there were only statistically significant differences between NI and FI vines. This suggests that the decline in activity per gram fresh weight in FI vines was the result of increased berry weight. The activity of invertase, malate dehydrogenase and malic enzyme was not affected by irrigation treatments when the activities were expressed on a per berry basis (Fig. 6 d, e, f). However, the activities of invertase and malic enzyme were significantly higher in August than in July. Moreover, invertase activity was positively related to the concentration of glucose and fructose during ripening (Fig. 7 a, b) while the reduction of malic acid was negatively related with the increase in activity of malic enzyme (Fig. 7 c).

## Discussion

Vine water status was clearly affected by the different treatments as shown by the seasonal evolution of  $\Psi_{pd}$  (Fig. 1). The deficit irrigation treatments, PRD and DI, resulted in intermediate values of  $\Psi_{pd}$  between FI and NI, although PRD had higher values than DI on most sampling dates. The reduced leaf area and/or decreased stomatal conductance of PRD as compared to DI observed previously (SANTOS *et al.* 2003; SOUZA *et al.* 2003) may explain the high values of pre-dawn leaf water potential observed in PRD vines throughout the season.

Several studies have shown that irrigation delays sugar accumulation and increases berry size, both generally considered detrimental to wine quality (FREEMAN and KLIEWER 1983; JACKSON and LOMBARD 1993). In our study, although berry weight increased in the irrigated treatments (Fig. 2), the amount of glucose and fructose per berry was also increased by irrigation (Fig 3 c, d) indicating a proportional increase in berry sink strength and/or increased sugar availability.

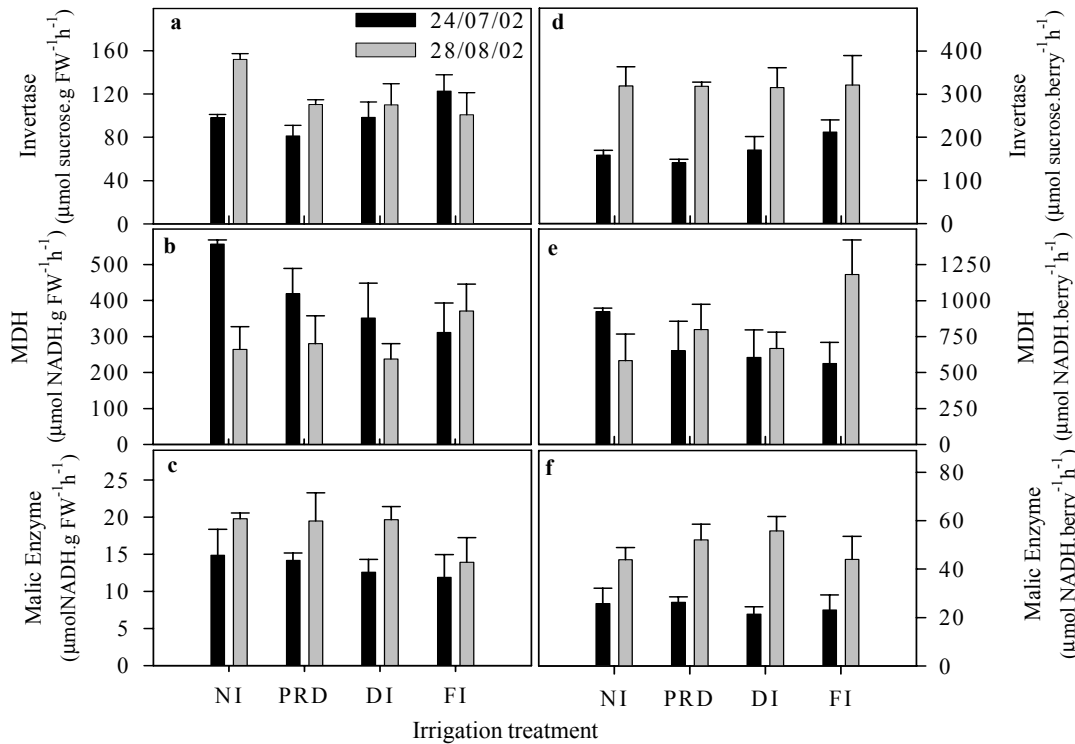


Fig. 6: Effects of different irrigation treatments on the activity of invertase, malate dehydrogenase (MDH) and malic enzyme. Values are means  $\pm$  SE.

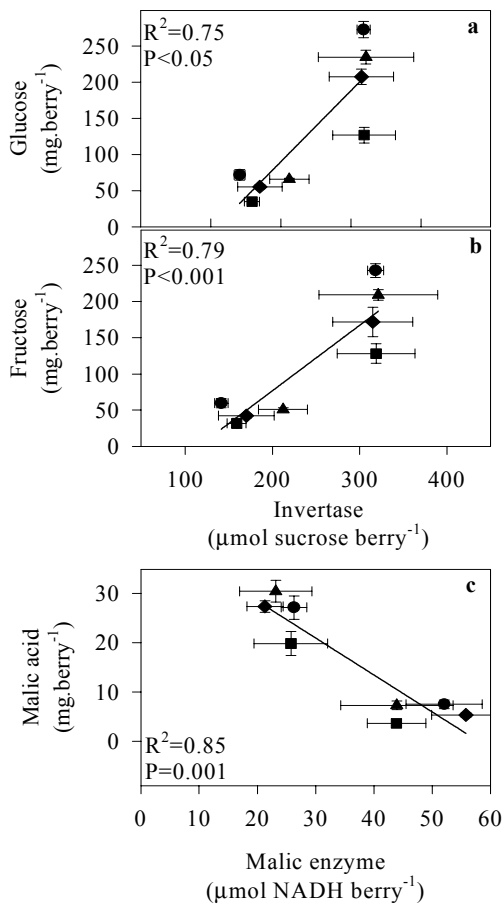


Fig. 7: Relation between glucose and fructose with invertase activity (a, b) and malic acid with malic enzyme activity (c). On two dates all treatments were included. Values are means  $\pm$  SE.

The higher sugar levels per berry in the irrigated treatments relative to NI were not accompanied by an increase in invertase activity per berry, which could account for increased sink strength. Most of the sugar in the berries is synthesized in leaves by photosynthesis and transported to the berries via the phloem, mainly as sucrose (RUFFNER *et al.* 1995; DAVIS and ROBINSON 1996). The high accumulation of sugars per berry in irrigated vines could result from increased photosynthesis measured in these treatments as compared to non-irrigated treatment in the same experiment (SOUZA *et al.* 2004). Although the invertase activity was not affected by the watering regimes, there was a significant increase in enzyme activity at the end of the ripening period, in proportion to the increase in berry size (Fig. 6 d). The high levels of hexoses accumulating in the berries were strongly associated with the rise in invertase activity as was also shown by HAWKER (1969) and PEREZ and GOMEZ (2000). The sugar accumulation process in grape berries is still not well understood, and although soluble invertase may be important in the accumulation of hexoses in the vacuole, their synthesis does not seem to be the trigger for its accumulation (DAVIS and ROBINSON 1996; DREIER *et al.* 1998). Other mechanisms must be involved in the regulation of sugar accumulation, such as differences in water potential between source and sink, which favours the movement of phloem sap into the berries (LANG and DÜRING 1991). Moreover, phloem unloading of sugar could have been affected by water stress, as was recently demonstrated by WANG *et al.* (2003).

Before veraison, there was a decrease in the organic acid content in NI compared to FI vines, when the results were expressed on a per berry basis (Fig 4 c, d). Malate, the

major organic acid in grape berries, has been proposed to be degraded by NADP-malic enzyme and also by malate dehydrogenase (MDH) (HAWKER 1969; LAKSO and KLIEWER 1975). The reduction of malic acid in non-irrigated vines could not be explained by changes in the activity of these enzymes because there were no significant differences between irrigation treatments in the activity of both enzymes, either before veraison or at the end of ripening. The increased temperature of sun-exposed berries of non-irrigated vines, could also contribute to reduce malate content due to reduced synthesis by PEP carboxylase which has lower thermal stability compared to malic enzyme (LAKSO and KLIEWER 1975). The decrease in malic acid in all treatments throughout the season could be attributed, in part, to an increase in activity of the malic enzyme (Fig. 5 c, 6 f, 7 c), unlike other studies where no direct correlation was found between the change in malic acid concentration and the activity of malic enzyme in the berry tissue during ripening (HAWKER 1969; RUFFNER 1982; GUTIÉRREZ-GRANDA and MORRISON 1992). The intracellular compartmentation, rather than enzyme activity, has also been invoked to regulate the malic acid metabolism during the ripening process (RUFFNER 1982; KANELIS and ROUBELAKIS-ANGELAKIS 1993; FAMIANI *et al.* 2001).

In contrast to malic acid, the content of tartaric acid per berry was almost constant during the growing season, as has been observed in other studies with grape berries (KANELIS and ROUBELAKIS-ANGELAKIS 1993). However, like malic acid the greatest reductions over time were observed in NI berries. In general, the low organic acid concentrations in berries of NI vines may be related to the effect of water stress on carbon assimilation in leaves and/or translocation of photoassimilates to the berries. It is widely accepted that most organic acids are formed at the expense of sucrose imported from the leaves (DI MARCO *et al.* 1977; TERRIER and ROMIEU 2001) and in this experiment photosynthesis was depressed in non-irrigated vines relative to irrigated treatments (SOUZA *et al.* 2004).

The ratios of glucose/fructose and tartarate/malate may be used as quality indices to define the optimal harvest date. There was no effect of various irrigation techniques on the pattern of the glucose/fructose ratio during the season (Fig. 5 a). The highest ratio measured at the beginning of July was due to the low levels of fructose present in the green berries. During the early stages of berry development, glucose accounts for 85 % of sugar content, but in ripe berries the ratio of glucose to fructose is almost equal, as has been reported by KLIEWER (1967) and KANELIS and ROUBELAKIS-ANGELAKIS (1993). The irrigation treatments had a more pronounced effect on the tartarate/malate ratio (Fig. 6 b), which increased in non-irrigated vines compared to irrigated treatments. The values of this ratio were out of the range considered optimal (1.2-1.6) according by RIBÉREAU-GAYON (1975). This increase may have been the result of malic acid degradation associated with the rise in temperature that usually occurs in berries of sun-exposed clusters. In fact, a higher percentage of exposed clusters were observed on NI vines compared to the other treatments (SANTOS *et al.* 2003), and this was accompanied by higher berry temperatures (SANTOS *et al.*, unpubl.).

In conclusion, the deficit irrigation treatments, PRD and DI, had no negative impact on growth and quality of grape berries as compared to the FI treatment, but resulted in an improvement of berry quality compared to NI vines. In viticulture, deficit irrigation may help to regulate yield and quality in addition to its role in optimizing of crop water use efficiency. The lower sugar content in berries of non-irrigated vines may have resulted from less carbon assimilated by foliage whereas higher cluster exposure in NI plants may have resulted in a higher tartrate/malate ratio.

### Acknowledgements

We gratefully acknowledge the use of an HPLC equipment and the technical assistance in sugar and organic acid analyses enabled by Professor Isabel Spencer and César Fonseca (Universidade Nova de Lisboa). The financial support from the EU project IRRISPLIT (IAC3-CT-1999-00008) is acknowledged. C.R.S received a doctoral fellowship by Fundação para Ciência e Tecnologia (PRAXIS XXI/BD/21856/99). We also thank A. Rodrigues and E. Breia for technical support in laboratory and field experiments.

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Received November 25, 2004