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Long-term effects of different irrigation strategies on yield components, vine vigour and grape composition in *cv. Cabernet-Sauvignon (Vitis vinifera L.)*

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ABSTRACT

There are still some traditional vinegrowing areas in Spain in which water-stressed vines are considered to produce berries with the highest quality must. To assess vine response to water availability, measured in terms of crop yield, vegetative development and grape composition, five different irrigation treatments were evaluated over a five-year period in a Cabernet-Sauvignon vineyard in the Madrid region (Spain): no-irrigation (T0), water provided at 20% of the reference evapotranspiration (ET_o) (T20), water provided at 45% of the ET_o (T45), water provided at 20% of the ET_o until *veraison* and at 45% thereafter (T20-45), and water provided at 45% until *veraison* and 20% thereafter (T45-20). A yield increment was observed with increasing water volumes. The T45 vines returned a consistent yield of around 8 t/ha, and a mean shoot weight of 30-50 g. The T0 and T20 plants showed reduced yields and vegetative growth in most years (yields being the most acutely affected). Berry weight was the yield component most influenced by water availability. In years of low rainfall, fertility was markedly reduced in the T0 vines. Providing a smaller irrigation volume before or after *veraison* (the T20-45 and T45-20 treatments) led to reductions in berry weight, cluster weight and shoot weight over the last three years of the trial. Berry composition was almost unaffected by irrigation strategy. Taking into account yield, water use efficiency, and berry composition, the T45-20 treatment was the most efficient irrigation strategy.

INTRODUCTION

In Spain, the area given over to irrigated vineyards ($\approx 300,000$ ha), which has notably increased over the last ten years, represents about 30% of the total area under grapevines. Concern over the effects of irrigation regimes on vine performance arose in the 1980s. The first trials examined the relationship between the volume of water applied to vines and factors such as crop yield and grape composition. These studies led to a basic understanding of vine irrigation and, over subsequent years, it was shown that i) wine grapes are not highly demanding in their water needs (significant yields can be achieved with moderate irrigation) (García-Escudero 1991), but ii) that water supply has clear effects on both yield and fruit composition (García-Escudero 1991; Esteban et al. 2001, 2002; Intrigliolo and Castel 2009, 2010).

Early Spanish experiments on the water volumes used in vine irrigation involved mature, balanced vineyards, using the low cropping loads and the low vine densities permitted by law at the time. Under these conditions, non-irrigated vines were found to produce berries with the highest phenolic compound contents (regarded as a mark of grape quality) (García-Escudero 1991). However, later experiments, in which these legally regulated conditions were changed, returned different results. For example, Esteban et al. (2001) performed an experiment in vineyards with different cropping loads, in which vines were provided different quantities of irrigation water. Irrigation led to higher crop yields and bigger berries, but without this having any effect on must composition. Roby et al. (2004) reported that berry solute content increased with berry size, but that the correlation between these variables was not linear, with bigger berries showing lower °Brix values and anthocyanin concentrations than smaller berries. Later, Intrigliolo and Castel (2010) reported an inverse relationship between yield (which was highest in the most irrigated plants) and grape anthocyanin concentration. However, these authors indicated that this was better explained by the total leaf area/yield ratio than the effect of irrigation on the water status of the vines.

Understanding the effect of applying different volumes of water to vines, as well as the timing of irrigation, on yield and berry composition is of the greatest importance to vine growers. Only with such knowledge can the irrigation strategy most likely to guarantee the desired yield and berry quality be chosen. A moderate water deficit is normally preferred in cool areas to control shoot vigour and to allow sugars to be moved to the clusters. This results in better ripening, a higher °Brix value, lower malic acid concentrations, and more intensely coloured wines (Spring and Zufferey 2009). In warm regions with long growing seasons, however, less severe water deficits may be preferred. This encourages vegetative development and also leads to the production of larger berries and higher yields, which together may provide the advantage of delaying ripening until the autumn (Jackson and Lombard 1993). Theoretically,

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3 the timing of irrigation allows growers to achieve objectives such as water saving, the control of berry
4 size and the management of berry polyphenol content (red cultivars) etc. (Ojeda et al. 2002; Roby and
5 Matthews 2003; Roby et al. 2004; Sal6n et al. 2005). However, the expected response of vines to the
6 timing of irrigation cannot be guaranteed (Intrigliolo and Castel 2010).
7

8 Since grapevines are perennial plants, their response to environmental conditions may depend on events
9 that took place during previous growing seasons. Hence, when looking for a response to different
10 irrigation strategies, it is advisable to plan at least a three-year study; some trends may only appear after
11 such a period of adaptation. The longer the experiment, the easier it should be to obtain reliable
12 information from the data. The aim of the present work was to determine the long-term effects of
13 different irrigation volumes and irrigation timing regimens on vegetative growth, yield components and
14 berry composition on initially three-year old Cabernet-Sauvignon/SO4 vines in the southeast of the
15 Madrid region (Spain).
16

17 MATERIALS AND METHODS

18
19 **The experimental vineyard and study design.** The present work was conducted between the years 2002
20 and 2006 at the “El Socorro” Experimental Vinegrowing Centre in the southeast of the Madrid region,
21 Spain (40°8’N, 3°23’W; altitude 730 m). The grapevine plants studied were Cabernet-Sauvignon (clone
22 15) vines grafted onto SO4 rootstocks (planted in 1999) oriented in a north-south direction, with 2.5 m
23 spacing between rows and 1.1 m between plants. All plants had a unilateral cordon trained at a height of
24 90 cm from the ground. All plants were spur-pruned to leave 12-14 nodes per meter. The shoots were
25 positioned vertically and thinned to 12-14 per meter at the beginning of flowering by removing non-count
26 shoots. Table 1 shows the dates and cumulative growing degree days associated with the main
27 phenological stages.
28

29 The soil at the site is a *Calcic Haploxeralf* with a clayey loam to clay texture, and a low organic matter
30 content (0.55%). According to Saxton and Rawls (2006), and taking into account the texture and organic
31 matter content, an available water capacity of around 38% (v/v) and a permanent wilting point of around
32 14% (v/v) were estimated.
33

34 The climate in the area is mild-continental Mediterranean with hot summers. The weather conditions
35 during the trial were measured by an automated meteorological station located on the plot (Table 2). The
36 reference evapotranspiration (ET_o) value was calculated using the Penman-Monteith formula, as
37 described in Allen et al. (1998).
38

39 The grapevines were subjected to one of the following treatments: no-irrigation (T₀), water provided at
40 20% of the reference evapotranspiration (ET_o) (T₂₀), water provided at 45% of the ET_o (T₄₅), water
41 provided at 20% of the ET_o until *veraison* and at 45% thereafter (T₂₀₋₄₅), and water provided at 45%
42 until *veraison* and 20% thereafter (T₄₅₋₂₀). Irrigation treatments were established with respect to ET_o
43 rather than crop evapotranspiration (ET_c) given the lack of information on crop coefficients for this
44 region. The percentage ET_o values covered by irrigation (20% and 45%) were established in accordance
45 with knowledge gained over the years at the experimental site to maintain moderate but different water
46 deficit levels. Each treatment had three replicates, the study following a randomised complete block
47 design. Each replicate consisted of four to five rows of twelve vines; the perimeter vines were used as
48 buffers. Therefore, each replicate had two to three data-providing rows with ten data-providing vines per
49 row, resulting in twenty to thirty data-providing vines for each replicate.
50

51 Plants were drip-irrigated using individual pressure-compensated drip emitters (flow rate 4 L/h). All
52 emitters were located at the midpoint between two consecutive plants. The frequency of water application
53 was set to 2 to 3 days per week.
54

55 Each season, irrigation began just after shoot growth stopped, when the pre-dawn water potential was
56 between -0.2 and -0.3 MPa. However, in 2005, irrigation was brought forward due to the extreme weather
57 conditions causing irregular shoot growth. Irrigation started on July 3rd (day of year [DOY] 184), July
58 8th (DOY 189), July 18th (DOY 200), May 18th (DOY 138) and June 22nd (DOY 163) in 2002, 2003,
59 2004, 2005 and 2006 respectively. The irrigation volume was calculated on a weekly basis by subtracting
60 the effective rainfall from the ET_o value obtained for the previous week. The irrigation period, which
finished when the effective rainfall exceeded $K (20\% \text{ or } 45\%) \times ET_o$, lasted 97, 84, 84, 145 and 85 days
in 2002, 2003, 2004, 2005 and 2006 respectively.

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4 **Water status.** The midday leaf water potential (Ψ_l) of four leaves per treatment was measured using a
5 pressure chamber (Soil Moisture Corp., Santa Barbara, California); this was performed twice during
6 ripening, always the day after an irrigation event. All leaves selected for this purpose were mature, fully
7 expanded and at the moment of selection were exposed to direct sunlight. The leaf blades were enclosed
8 in a plastic bag just before the petiole was cut.
9

10 **Grape composition.** At harvest, for each replicate, two samples of 100 and 150 berries were collected,
11 put into tagged plastic bags, placed in a dry ice portable cooler, and taken rapidly to the laboratory for
12 analysis. The 100-berry samples were weighed, the must total soluble solids ($^{\circ}$ Brix) content determined
13 using a refractometer (PR32 Atago Co. Ltd., Tokyo, Japan), the titratable acidity determined using
14 standardized 0.1 N NaOH (end point pH 8.2), and the must pH recorded using a Metmorph 702SM
15 automatic neutralizer (Titrimo, Herisau, Switzerland). The 150-berry samples were also weighed, and
16 processed to determine their phenolic composition. The total phenolic index (TPI), total anthocyanins and
17 extractable anthocyanins (the last two variables expressed as mg malvidin/L) were determined according
18 to the method of Glories and Augustin (1993).
19

20 **Yield components.** The number of clusters and shoots, and the crop yield at harvest, were recorded for
21 all the data-providing vines of each replicate. The number of clusters per shoot was calculated as the
22 number of clusters/total number of shoots. The average cluster weight was obtained by dividing the total
23 yield by the total number of clusters. The number of berries per cluster was determined as the average
24 cluster weight/the berry weight (obtained for the berries sampled for must composition).
25

26 **Vigour.** At pruning, the number of shoots and the pruning weight were determined for all the data-
27 providing vines of each replicate. The average shoot weight (g) was obtained by dividing the total pruning
28 weight by the total number of shoots.
29

30 **Data analysis.** Two-way (irrigation x year) analysis of variance (ANOVA) was performed using SPSS v
31 15.0 statistical software (SPSS, Chicago, Illinois). Differences between treatments were assessed using
32 Duncan's multiple range test, with significance set at $P \leq 0.05$. Linear regression analysis was used to
33 explore the relationships between the different variables studied.
34

35 RESULTS

36 Meteorological conditions, irrigation periods and vine water status

37 Budbreak occurred during the second half of April, and flowering during the first half of June each year
38 (Table 1). Water requirements were higher in 2003, 2005 and 2006, with the ratio for accumulated
39 growing degree days/total days between budbreak and harvest of 11.1, 12.0 and 11.0 $^{\circ}$ C/day, and average
40 daily evapotranspiration rates of 5.0, 5.6 and 5.3 mm/day respectively. Rainfall was lower in 2005 and
41 2006, with no effective rainfall between budbreak and harvest in 2005 (Table 2). Due to these annual
42 weather variations, irrigation started at different times; in 2002, 2003 and 2004 irrigation began in July,
43 while in 2005 and 2006 it began in May and June respectively. In these two years, harvesting took place
44 earlier than in other years; the budbreak-harvest periods were therefore shorter (Table 1). The irrigation
45 doses applied until harvest varied widely (Table 2). The least watered treatment, T20, received 70 mm in
46 2002 and 155 mm in 2005, while the most irrigated treatment, T45, received 156 mm in 2002, and 339
47 mm in 2005. After the experiment, the irrigation treatments could be divided into three groups according
48 to the water status of the plants: rain-fed (T0), with $\Psi_l < -1.4$ MPa during ripening; water restriction until
49 *veraison* (T20 and T20-45), with $\Psi_l \approx -1.3$ MPa during ripening; and water requirements satisfied over the
50 season (T45-20 and T45), with $\Psi_l > -1.2$ MPa (Table 3).
51
52

53 Effect of water supply on crop yield and its components.

54 Water availability substantially affected crop yield (Table 4), with significant differences recorded for the
55 last three years of the study. Berry and cluster weights increased with increasing water availability in four
56 out of the five years of the study (Table 4). In 2005 and 2006, the number of clusters per shoot and the
57 number of berries per cluster were significantly lower in the T0 treatment compared to the irrigated ones
58 (Table 4). While berry and cluster weights were affected by water availability from the second year of
59 study, an effect on fertility was observed only from the fourth year, and only in the T0 treatment.
60 Differences in berry weight, cluster weight, and yield increased from 2003 to 2005, and remained
constant in 2006.

Effect of water supply on vegetative growth

Vegetative growth, estimated via the shoot weight, was less sensitive than yield components to variations in water availability (Table 5). Differences were observed after the third year of study. From 2003 to 2006, the shoot weight progressively decreased for all treatments, although the fall was more drastic for the lower water availability treatments. Thus, the differences between treatments increased over the years. Only the most strongly irrigated treatment (T45) maintained a shoot weight of about 30 g or higher over the entire study period.

Effect of water supply on grape composition

Berry composition was barely affected by water availability (Table 6). Differences were only observed in 2006, resulting in i) a higher sugar concentration as water availability diminished (due to berry weight loss through dehydration, and ii) a higher total acidity and lower pH for the higher irrigation rate treatments (T45, T45-20 and T20-45). Differences in the phenolic composition of the berries were only observed in 2005, the driest season, when the higher irrigation rate treatments reached the highest concentrations of extractable anthocyanins.

Relationship between water availability, yield and berry composition

Data on the response of the vineyard to water availability (rainfall plus irrigation) during different periods defined by the main phenological stages were analysed by linear regression.

Yield increased significantly with increasing water availability between budbreak and harvest (Fig. 1). The number of clusters per shoot did not correlate significantly with water availability in any of the periods studied (data not shown). However, as mentioned above, the fertility of the T0 treatment was lower than that of the irrigated treatments in 2005 and 2006, when the weather conditions were more adverse (Table 2) and an accumulated effect of water restriction from previous years was apparent. This cumulative effect was highlighted by the significant effect observed for 'year' in ANOVA (Table 5). Cluster weight depended significantly on the availability of water between budbreak and harvest (Fig. 2). The number of berries per cluster and berry weight - the two components that determine cluster weight - were dependent on water availability between flowering and *veraison* (Fig. 3), and between *veraison* and harvest (Fig. 4), respectively. The differences in berry weight were associated with water availability between budbreak and harvest, although the correlation coefficient was lower ($R^2 = 0.53$) (data not shown).

Throughout the different periods studied, no significant relationship was seen between water availability and any of the variables chosen to determine the composition of the berries ($^{\circ}$ Brix, total acidity, pH, TPI, total or extractable anthocyanins).

DISCUSSION

Until the third year of the experiment, no significant differences in yield were seen between the various irrigation strategies examined. This may have several explanations. Certainly, in 2002, the first year of the trial, the number of potential clusters per shoot was naturally determined during the previous year, i.e., before the experiment began. Further, it should be remembered that carbohydrate held in reserve can be mobilized under high plant demand, thus the reserve from the previous year might be called upon in any growing cycle, masking the effects of the conditions reigning in that cycle. Moreover, under field conditions, rainfall events as well as the volume and characteristics of the soil explored by the roots, may buffer the yield response under irrigation shortage. The ratio of the water volume provided in the T20 treatment to that applied in T45 ranged between 44% (2006) and 61% (2003), highlighting the influence of the weather conditions in different years.

The response of the plants to water supply variations was confirmed to be slow; it should thus be assessed only in the long-term context (Table 4). The average five year yields in T20, T20-45, and T45-20 were 81%, 81% and 94% of the yield of the most irrigated treatment (T45) respectively (Table 4). The proportion of water applied in T20, T20-45 and T45-20 compared to T45 was 48%, 71% and 74% respectively. Thus, in T45-20, a water saving of 26% was made compared to the T45 treatment, with no significant difference in yield (Table 4). García-Escudero (1991) found no differences in yield between irrigated treatments, and Reynolds et al. (2005) obtained only around 10% yield increases for Concord grapes, and 29% for Niagara grapes, when studying the effects of irrigation and non-irrigation treatments. These results underline the importance of taking water use efficiency (yield/mm applied) into consideration when planning the irrigation strategy for a vineyard.

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4 When rainfall was considered as part of the water available to the plants, a significant linear relationship
5 was observed between the amount of water received (irrigation plus rainfall) and the yield response
6 (Figure 1). This shows that, for yield boosting under deficit irrigation, the total water volume is more
7 critical than the irrigation strategy chosen. Marsal et al. (2008) found a significant linear relationship
8 between irrigation water applied and yield, while Myburgh (2011) observed the same correlation when
9 considering water supply as irrigation plus rainfall. Trends were similar in all these experiments but
10 yields differed according to experimental conditions (cultivar, bud load, vine spacing). Finally, some
11 irrigation studies comparing the partial rootzone drying technique (PRD) to conventional deficit irrigation
12 failed to find any significant differences either in yield or in terms of yield components for the same
13 amount of water applied (Gu et al. 2004; Santos et al. 2007; Marsal et al. 2008; Intrigliolo and Castel
14 2009). However, significant differences were seen for different volumes of water within treatments,
15 highlighting the greater influence of water volume than irrigation strategy.

16
17 In the present work, and in relation to water restriction and irrigation timing, the lack of major differences
18 in yield between treatments T45-20 and T45 (Table 4) indicates that optimal water availability before
19 *veraison* is more critical for yield than the amount of irrigation applied during ripening. Acevedo et al.
20 (2004) also reported different irrigation treatments established after *veraison* to have no effect on yield.
21 However, these authors did see differences when treatments were established after berry setting. Shellie
22 (2006) saw no differences in yield when comparing water restriction before *veraison* (35-70% ETc) with
23 no restriction over the growing season (70-70% ETc). This agrees with the present comparisons between
24 T20-45 and T45. Again, it seems that irrigation is not as influential on yield during ripening as it is when
25 applied earlier in the season.

26
27 Figure 1 shows that the independent analysis of one year trials could lead to mistaken results; true
28 outcomes can only be appreciated when data from different years are plotted together - the greater the
29 quantity of water used for irrigating the plants over the growing season, the higher the yield. Seasonal
30 variability may mask differences between treatments that are only observed when long-term studies are
31 undertaken.

32
33 Berry weight was the yield component most sensitive to water restriction. Differences were seen from the
34 second season onwards and widened over the years (Table 4). Acevedo et al. (2004), Sal6n et al. (2005)
35 and Reynolds et al. (2007) also reported that the differences in yield between different water availability
36 treatments were due primarily to berry weight. Intrigliolo and Castel (2009) obtained differences in berry
37 weight for the years with higher yields, but found no differences when yields were low. In the present
38 trial, no differences were obtained between the T20, T20-45 and T45-20 treatments, which indicates that
39 both cell multiplication (phase I) and cell expansion (phase I and phase III) were equally affected by
40 water restriction. Berry weight reduction due to water restriction in phase I was not totally offset by
41 increasing water during phase III. This finding is in agreement with that reported by Ojeda et al. (2002)
42 and Acevedo et al. (2004), which showed that water deficit during post-setting is more critical than during
43 post-*veraison*. However if the rainfall is sufficient from budbreak to *veraison*, small berry sizes can be
44 achieved by restricting the water supply during ripening, as suggested by the smaller berry weight in T45-
45 20 compared to T45 (Table 5). Ezzahouani and Williams (2007) obtained their highest berry weights with
46 an all-season-long irrigation treatment, followed by a treatment with irrigation cut-off at *veraison*, and
47 finally a treatment with irrigation cut-off at berry set. Thus, water restriction after *veraison* would appear
48 to have a milder effect on berry weight than an early cut-off.

49
50 In the present work, fertility, expressed as either clusters per shoot or berries per cluster, was the yield
51 component to be last affected (in time) by water shortage. Differences between rain-fed and irrigated
52 treatments were observed in the fourth season, but no variations were found between the different
53 irrigation treatments. Sal6n et al. (2005) and Intrigliolo and Castel (2009, 2010) reported similar
54 responses, with differences between rain-fed and irrigation treatments, but not between irrigation
55 treatments. However, in the present work a significant linear correlation was found when all-season data
56 for yield and yield components were plotted against total water availability, expressed as irrigation plus
57 rainfall (Figures 1 to 4). This indicates that, regardless of the results for each year, water availability had a
58 significant long-term effect on yield. Long-term experiments are therefore needed for this type of trial as
59 annual variability may mask the true differences between treatments.

60
Although vegetative growth is the most sensitive plant response to water deficit (Jones 2004), in the
present work most treatments were started in July, when canopies were already complete at around the

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3 pea size phenological stage (7 mm berry diameter). Although there was a steady reduction in vegetative
4 growth over the years, significant differences did not appear until the third growing season (Table 5). The
5 years 2005 and 2006 were unusually dry, hence irrigation started earlier, before shoot growth had
6 stopped. Intrigliolo and Castel (2009) found differences in vegetative growth between rain-fed and
7 irrigated treatments from the first year of their trial since irrigation began around flowering when the
8 canopy was not totally complete. However, there were no differences between irrigation treatments. In the
9 second year of their study, Intrigliolo and Castel (2010) observed differences between irrigation
10 treatments involving low bud loads, but no differences were seen between irrigated treatments involving
11 high bud loads.

12
13 In the present work, water availability from budbreak to harvest explained only 32% of the differences in
14 vegetative growth, and the relationship was only of low significance ($P \leq 0.1$) (data not shown). This might
15 be explained by the fact that the rainfall prior to budbreak, which surely influences the vegetative growth
16 during early stages, was not taken into account. Further, there were some years when the plants were
17 trimmed, and the biomass removed was not taken into account when measuring the shoot weight.

18
19 The different water volumes and irrigation strategies applied during this five-year study had no
20 substantial effects on berry composition. The quantity of soluble solids increased with berry size. Sugar
21 concentration was affected only in 2006 when, due to intense dehydration, the grapes from the non-
22 irrigated vines had a higher concentration of soluble solids than those from irrigated plants. Earlier studies
23 also suggest irrigation leads to higher yields, with little effect on the concentration of soluble solids
24 (Matthews and Anderson 1988; Roby et al. 2004). However, the opposite results have also been reported
25 (Reynolds et al. 2005). In the last two years of the present trial, must acidity was affected by irrigation,
26 although, an influence of the interaction *treatment x year* was also seen. Thus, it is hard to be sure how
27 irrigation treatment alone affects must acidity. Different authors have reported different results regarding
28 the influence of irrigation on must acidity. Some indicate the water applied to have no effect on either
29 total acidity or pH (Reynolds et al. 2005), while others indicate increased must acidity in rain-fed vines
30 (Smart et al. 1985; Intrigliolo and Castel 2010). Few differences in grape phenolic composition were
31 found between the present treatments; indeed, the annual variation was greater than that associated with
32 the experimental treatments. The TPI reached approximately 30, 60 and 130 in 2004, 2005 2006
33 respectively, with no differences due to irrigation treatment observed (Table 6). In 2005, the
34 concentration of extractable anthocyanins was higher for the grapes harvested from the more irrigated
35 vines. These results are consistent with those of previous studies undertaken in warm areas, in which
36 irrigated vines, as well those with higher bud loads, reached higher phenolic contents (Esteban et al.
37 2001). However, some studies conclude that the extractability of anthocyanins is lower in grapes from
38 highly irrigated vines, due either to delayed ripening or thicker berry cell walls (Bindon et al. 2011).
39 Taking into account the years in the present work for which berry phenolic compound results were
40 available (2004-2006), the total anthocyanin content of the berries from vines under the irrigation
41 treatments was higher than that of berries from the T0 vines, with no significant differences seen between
42 irrigation strategies. Acevedo et al. (2004) reported higher anthocyanin contents for their most irrigated
43 treatment, independent of the water being applied post-setting or post-*veraison*. In the present work,
44 differences in the extractable anthocyanin concentration were observed between irrigated vines. These
45 results indicate that the effect of vine water status on anthocyanin concentration is greater than that
46 exerted on berry size. This agrees with the results of other authors (Roby et al. 2004).

47
48 The yield and vegetative growth of the vines subjected to water deficits higher than that produced in the
49 T45 treatment, fell over the years. Their physiological activity was probably reduced in the same way.
50 The lack of consistent differences in grape composition between the irrigation treatments could be due to
51 the buffering effect of the environmental conditions and cropping load, as suggested by other authors
52 (Intrigliolo and Castel 2010), or to different physiological activities. Even if the concentration of solids
53 were the same in grapes from different treatments, the total amount of solids per hectare might be very
54 different due to crop yield differences.

55 CONCLUSIONS

56
57 In this five-year study of different irrigation strategies for grapevines, the differences seen in yield, yield
58 components and vigour due to plant water availability increased over the years. A linear correlation
59 between yield and water supplied during the growing season (irrigation plus rainfall) was observed. Berry
60 weight was found to be the yield component most sensitive to water availability.

The maintenance of water deficit over the years led to a gradual reduction in yield and vegetative growth. A water supply (rainfall plus irrigation) of at least 300 mm was required to achieve a sustainable yield of 7-8 t/ha. In the last two years of the study, when environmental conditions were the most critical, and water deficit had been accumulating over the years, berry weight was more strongly affected by a lack of irrigation in pre-*veraison* than in post-*veraison*.

After consecutive years of low rainfall (less than 100 mm during each cycle), the non-irrigated vines had fewer clusters per shoot and berries per cluster than did the irrigated plants. When considering the whole period of study, the irrigated treatment plants had higher total anthocyanin and extractable anthocyanin contents due to differences in berry weight. In terms of concentration, grape composition was scarcely affected by the irrigation volume. The effect of berry weight, metabolic activity and solute concentration partly compensated for one another, which resulted in few differences in phenol concentrations.

Taking into account yield, water use efficiency, and berry composition, the T45-20 treatment was the most efficient irrigation strategy.

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Table 1 Dates and growing degree days for main phenological stages

Year		Budbreak	Bloom	Veraison	Harvest	Days from budbreak to harvest
2002	Date	24 April	15 June	20 August	8 October	168
	GDD ^a (°C)	31	353	1261	1642	
2003	Date	22 April	7 June	4 August	6 October	168
	GDD (°C)	22	333	1182	1887	
2004	Date	25 April	17 June	18 August	30 September	160
	GDD (°C)	22	329	1146	1603	
2005	Date	29 April	7 June	12 August	8 September	134
	GDD (°C)	70	494	1326	1684	
2006	Date	17 April	7 June	5 August	29 August	135
	GDD (°C)	37	404	1238	1518	

^a GDD: Cumulative growing degree days, base 10 °C

Table 2 Seasonal and annual meteorological data and total amount of irrigation water supplied in each treatment

Year	Seasonal ^a GDD ^b		Seasonal ETo ^c		Rainfall (mm)		Irrigation (mm)			
	(°C)	(°C/day)	(mm)	(mm/day)	Seasonal	Annual	T20	T20-45	T45-20	T45
2002	1611	9.6	800	4.8	141	480	70	116	110	156
2003	1865	11.1	847	5.0	105	524	134	189	126	218
2004	1581	9.9	749	4.7	180	512	89	146	143	200
2005	1614	12.0	753	5.6	2	162	155	195	299	339
2006	1481	11.0	716	5.3	78	373	106	166	179	239
Total 2002-2006							554	812	857	1152

^a Seasonal values are from budbreak to harvest

^b GDD: Cumulative growing degree days, base 10 °C

^c ETo: Reference evapotranspiration

Table 3 Effect of water supply on midday leaf water potential during phase III of berry development

Treatment	Midday leaf water potential (MPa)					Average	Year	Treatment x Year
	2002	2003	2004	2005	2006			
T0	-1.46 c ^b	-1.59 c	-1.46 c	-1.59 c	-1.44 b	-1.50 c		
T20	-1.18 ab	-1.41 bc	-1.18 ab	-1.36 b	-1.38 b	-1.30 b		
T20-45	-1.25 bc	-1.44 bc	-1.25 bc	nc ^c	-1.15 b	-1.27 b	***	***
T45-20	-1.01 a	-1.32 ab	-1.01 a	nc	-1.36 a	-1.17 a		
T45	-1.11 ab	-1.22 a	-1.11 ab	-1.14 a	-1.07 a	-1.13 a		
Treatment	** ^a	**	**	***	***	***		

^a *, **, ***, ns: significant at $P \leq 0.05$, 0.01, 0.001 and not significant respectively

^b Means within columns followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

^c nc: data not collected

Table 4 Effect of water supply on crop yield and yield components

Crop yield (t/ha)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	3.95	4.60	3.16 c ^b	1.35 d	1.58 c	2.93 c		
T20	5.46	7.25	5.51 b	4.87 c	5.65 ab	5.75 b		
T20-45	4.42	8.85	9.09 a	6.94 b	3.97 bc	6.65 ab	***	***
T45-20	3.85	7.73	6.69 ab	8.91 a	7.30 a	6.91 a		
T45	4.35	8.34	7.68 ab	8.19 ab	7.90 a	7.29 a		
Treatment	ns ^a	ns	**	***	**	***		
Berry weight (g)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	0.89	0.70 b	0.70 d	0.43 c	0.56 c	0.66 c		
T20	0.90	0.97 a	0.88 b	0.65 b	0.70 b	0.82 b		
T20-45	0.95	1.08 a	0.95 b	0.66 b	0.61 bc	0.85 b	***	**
T45-20	0.89	1.02 a	0.94 b	0.78 a	0.70 b	0.87 b		
T45	0.96	1.10 a	1.00 a	0.79 a	0.89 a	0.95 a		
Treatment	ns	**	***	***	**	***		
Clusters/shoot								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	1.8	1.8	1.6	1.2 b ^b	1.2 b	1.5 b		
T20	1.7	2.0	1.8	1.8 a	1.9 a	1.8 a		
T20-45	1.5	2.0	1.8	2.0 a	1.8 a	1.8 a	ns	ns
T45-20	1.8	1.8	1.9	1.9 a	1.8 a	1.8 a		
T45	1.8	2.0	1.7	1.8 a	1.9 a	1.8 a		
Treatment	ns ^a	ns	ns	*	**	**		
Cluster weight (g)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	42	55 b	46 b	23 c	25 c	38 c		
T20	62	80 ab	76 ab	62 b	59 b	68 b		
T20-45	57	101 a	103 a	77 ab	46 bc	77 ab	***	ns
T45-20	46	83 ab	80 ab	93 a	67 ab	74 ab		
T45	54	89 a	94 a	93 a	79 a	82 a		
Treatment	ns	*	*	***	**	***		
Berries/cluster								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	48	79	65	54 b	44 b	58 b		
T20	67	83	87	95 a	81 a	83 a		
T20-45	60	93	107	117 a	72 ab	90 a	***	ns
T45-20	53	81	84	119 a	97 a	87 a		
T45	58	81	95	117 a	88 a	88 a		
Treatment	ns	ns	ns	**	*	***		

^a *, **, ***, ns: significant at $P \leq 0.05$, 0.01, 0.001 and not significant respectively

^b Means within columns followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

Table 5 Effect of water supply on shoot weight

Treatment	Shoot weight (g)					Average	Year	Treatment x Year
	2002	2003	2004	2005	2006			
T0	52	37	32 b	10 c	9 c	28 d		
T20	50	41	32 b	23 b	15 b	32 cd		
T20-45	52	51	46 a	29 b	14 bc	38 ab	***	**
T45-20	33	37	42 ab	45 a	20 b	35 bc		
T45	44	49	47 a	43 a	29 a	42 a		
Treatment	ns	ns	*	***	***	***		

^a *, **, ***, ns: significant at $P \leq 0.05$, 0.01, 0.001 and not significant respectively

^b Means within columns followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

Table 6 Effect of water supply on grape composition

Soluble solids (°Brix)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	23.2	24.0	26.8	25.7	26.3 a ^b	25.2 a		
T20	21.2	24.0	25.3	24.3	23.1 bc	23.6 b		
T20-45	23.4	25.4	25.5	25.9	24.6 b	24.9 a	***	ns
T45-20	23.6	25.9	25.3	25.7	24.2 bc	24.9 a		
T45	23.2	25.7	26.2	25.3	23.0 c	24.7 a		
Treatment	ns ^a	ns	ns	ns	**	***		
Titratable acidity (g/L)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	6.0	5.2	5.6	7.5 a	6.6 c	6.2 b		
T20	7.0	5.9	6.0	8.1 a	7.0 c	6.8 a		
T20-45	5.7	5.2	5.8	6.1 b	7.6 b	6.1 b	***	***
T45-20	6.6	5.5	5.7	5.8 b	7.6 b	6.2 b		
T45	6.8	5.0	6.1	7.9 a	8.1 a	6.8 a		
Treatment	ns	ns	ns	*	**	**		
pH								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	3.35	3.51	3.27	3.53	3.31 ab	3.39		
T20	3.22	3.42	3.21	3.41	3.27 bc	3.31		
T20-45	3.27	3.44	3.27	3.35	3.35 a	3.34	ns	ns
T45-20	3.23	3.54	3.29	3.40	3.24 bc	3.34		
T45	3.23	3.48	3.30	3.57	3.23 c	3.36		
Treatment	ns	ns	ns	ns	*	ns		
Total phenolic index								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	nc ^c	nc	29	60	118	69		
T20	nc	nc	30	64	131	75		
T20-45	nc	nc	29	54	134	72	***	ns
T45-20	nc	nc	30	54	136	73		
T45	nc	nc	28	58	135	74		
Treatment	-	-	ns	ns	ns	ns		
Total anthocyanins (mg malvidin/L)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	nc	nc	444	1401	1406	1084 b		
T20	nc	nc	748	1472	2062	1427 a		
T20-45	nc	nc	714	1458	1768	1313 a	***	ns
T45-20	nc	nc	497	1605	2104	1402 a		
T45	nc	nc	609	1473	1769	1284 a		
Treatment	-	-	ns	ns	ns	*		
Extractable anthocyanins (mg malvidin/L)								
Treatment	2002	2003	2004	2005	2006	Average	Year	Treatment x Year
T0	nc	nc	476	775 bc	1071	626 c		
T20	nc	nc	540	816 bc	990	782 ab		
T20-45	nc	nc	473	674 c	868	672 bc	***	ns
T45-20	nc	nc	450	995 a	1194	880 a		
T45	nc	nc	434	954 ab	989	792 ab		
Treatment	-	-	ns	*	ns	**		

^a *, **, ***, ns: significant at P ≤ 0.05, 0.01, 0.001 and not significant respectively

^b Means within columns followed by different letters differ significantly at P ≤ 0.05 (Duncan's multiple range test)

^c nc: data not collected

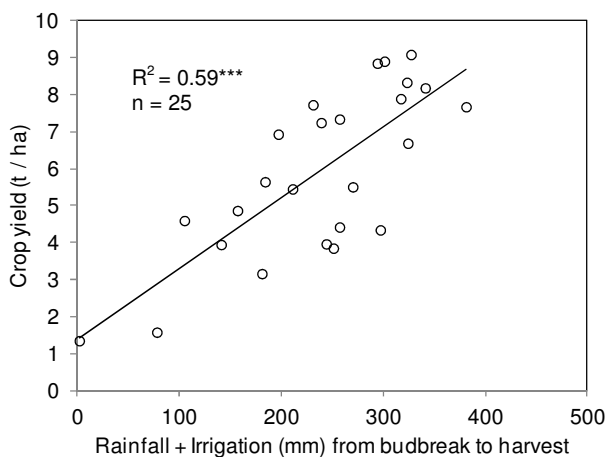


Fig. 1 Relationship between water supply (rainfall + irrigation) from budbreak to harvest and crop yield. Values are means per treatment replicate, pooling data across seasons. *** indicates significant linear trend at $P \leq 0.001$

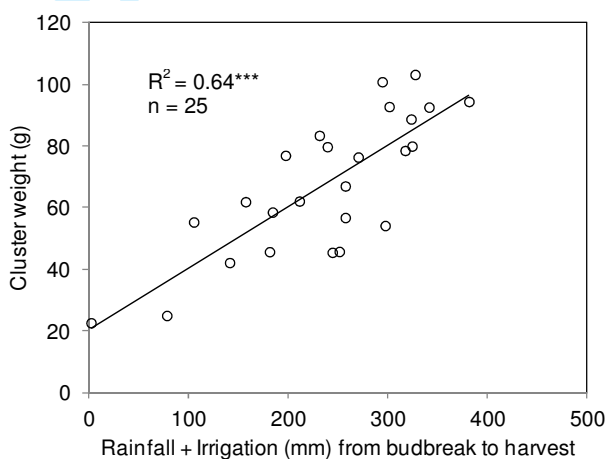


Fig. 2 Relationship between water supply (rainfall + irrigation) from budbreak to harvest and cluster weight. Values are means per treatment replicate, pooling data across seasons. *** indicates significant linear trend at $P \leq 0.001$

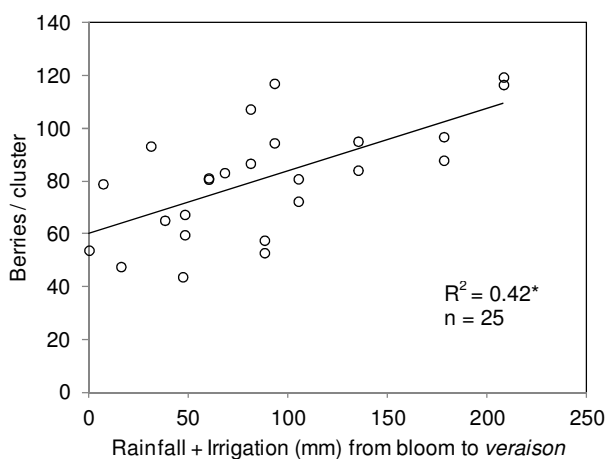


Fig. 3 Relationship between water supply (rainfall + irrigation) from bloom to *veraison* and the number of berries per cluster. Values are means per treatment replicate, pooling data across seasons. * indicates significant linear trend at $P \leq 0.05$

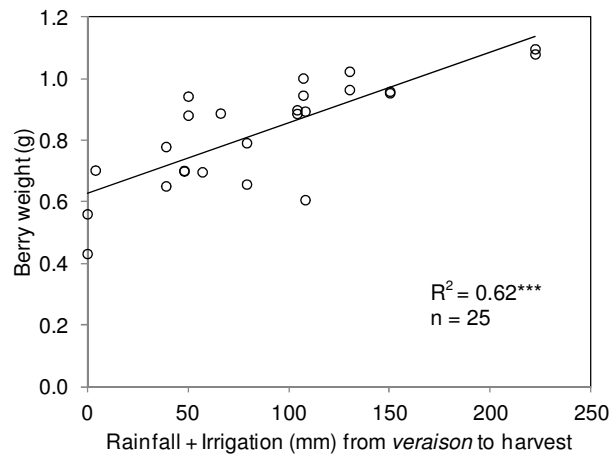


Fig. 4 Relationship between water supply (rainfall + irrigation) from *veraison* to harvest and berry weight. Values are means per treatment replicate, pooling data across seasons. *** indicates significant linear trend at $P \leq 0.001$