



# Cumulative response of Tempranillo vines to the crop forcing technique and pre-forcing and post-veraison water stress in terms of yield and grape and wine quality

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## Abstract

Elevated temperatures during berry ripening are detrimental to grape quality. The crop forcing technique (summer pruning that ‘forces’ the vine to start a new cycle) increases must acidity and malic acid concentration at harvest by delaying the date of veraison. However, little information is available on the sensitivity to water stress of forced vines. A 3-year trial was conducted to test three irrigation strategies in forced vines: a minimum threshold of mid-day stem water potential ( $\Psi_s$ ) of  $-0.75$  MPa before forcing (DI), a minimum  $\Psi_s$  threshold of  $-1.2$  MPa only after veraison (RDI), and the combination of both treatments (DI+RDI). Results were compared to a non-forced treatment with a minimum  $\Psi_s$  threshold of  $-1.2$  MPa after veraison (C-RDI). Must acidity increased, and pH decreased in the forced treatments. However, yield was reduced by 35% and irrigation requirements increased by 20% when comparing forced and unforced treatments. As a result, water use efficiency was reduced in forced treatments. Only after a dry spring did the, DI (11%) and DI+RDI (30%) treatments, save water compared to the C-RDI treatment. Moreover, although  $\Psi_s$  before forcing never fell below  $-0.75$  MPa, a significant negative correlation ( $R^2=0.76$ ) was found between the integral of water stress before the vines were forced and the number of forced bunches per vine. Post-veraison water stress in forced vines reduced the polyphenol content of the wine. Our findings suggest that forced vines are extremely sensitive to even mild water stress.

## Introduction

Under the high temperatures caused by global warming, berry ripening occurs earlier in the season and the time elapsed from veraison to harvest is reduced (Jones et al. 2005). As a result, berry quality is impaired mainly due to lower must acidity and anthocyanin content and increase sugar content (Palliotti et al. 2014). In recent years, research

in viticulture has focused on different management techniques with the aim of minimizing the effects of climate change on grape and wine quality (Gutiérrez-Gamboa et al. 2021; Palliotti et al. 2014). In climates with hot summers, some of these techniques including chemical treatments, different irrigation strategies, late winter pruning, partial leave removal, or severe trimming (Gutiérrez-Gamboa et al. 2021; Palliotti et al. 2014), have the goal of delaying the harvest date to a cooler period by altering vine carbon partitioning and availability or enhancing the competition of vegetative organs with fruit and, therefore, delaying the onset and reducing the rate of berry sugar accumulation (Gutiérrez-Gamboa et al. 2021). However, these techniques like partial leaf removal and deficit irrigation (Buesa et al. 2019) or late winter pruning (Gatti et al. 2016) are only able to delay the harvest date by around 15 days, which in hot late summer conditions may be insufficient to increase berry acidity and anthocyanin content (Buesa et al. 2019). One technique that is able to delay harvest by up to 2 months is the crop forcing technique (also known as forcing regrowth) (Dry 1987; Gu et al. 2012; Lavado et al. 2019; Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019). This technique consists

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of removing all the leaves, laterals, and fruits, leaving 2–6 buds which are “forced” to break and start a new phenological cycle with new fruits. This technique must be applied before the vines enter in endo-dormancy, before shoot maturity, which prevents the buds from breaking (Martínez De Toda et al. 2019). In forced vines, berry acidity is clearly enhanced. This can be attributed to the fact that, because berry ripening takes place under lower temperatures, malic respiration is also reduced (Lavado et al. 2019; Martínez-Moreno et al. 2019). Some phenolic enhancement, especially anthocyanin content, has also been reported (Gu et al. 2012; Lavado et al. 2019) but, in the Tempranillo cultivar, vines which were forced late (about 25 days after full bloom) had reduced anthocyanin concentration (Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019). In addition, in vines forced late, berry sugar accumulation may stop due to low temperatures after veraison (Martínez De Toda et al. 2019). However, the main limitation of the crop forcing technique for its use in commercial vineyards is the large reduction in yield (Lavado et al. 2019; Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019). This reduction has been attributed to low carbon reserve status when vines are forced and to the fact that, with a shorter cycle, forced vines gain less carbon than vines managed conventionally (Oliver-Manera et al. 2022) and to the date when forced buds unlock (Martínez De Toda et al. 2019). The carbon gained before forcing has little impact on whole vine final carbohydrate reserves of forced vines, and it has therefore been suggested that water stress before forcing may allow a better carbohydrate reserve status to start the new cycle (Oliver-Manera et al. 2022), because vegetative growth is more sensitive than photosynthesis to water stress (Hsiao 1973), benefiting forced yield and grape quality.

One of the most sensitive regions to global warming is the Iberian Peninsula (Brednar et al. 2022). In 2021, Spain was the third leading wine producing country in the world and the leading wine exporter (OIV 2022). The Tempranillo cultivar is the third most cultivated variety worldwide and is the most grown cultivar in Spain (Anderson and Nelgen 2020). It is an early–mid-ripening variety and, therefore, highly sensitive to global warming because the ripening phase may occur during the hottest part of summer (Ramos et al. 2018). In addition, elevated temperatures at harvest may increase heat risk for the farm workers who need to harvest. Therefore, due to their economic importance and sensitivity to elevated temperatures, techniques which can delay the ripening phase of Tempranillo vines have been the subject of research. Tempranillo vines are also strongly sensitive to any impairment to source: sink relationships (Intrigliolo and Castel 2011), which may be worsened under water stress conditions (Buesa et al. 2019). As global warming advances, irrigation strategies which minimize water consumption while improving yield and berry quality are necessary tools in hot and semiarid areas (Chaves et al.

2010). The effects of water stress on yield and berry quality are widely studied. However, the effects of water stress and the magnitude of these effects on yield and grape quality are quite different depending on the time and the intensity of the stress (Girona et al. 2009), the canopy management (Buesa et al. 2019), and the cultivar or even the rootstock (Palai et al. 2022). In Spain and in the Tempranillo cultivar, the most common irrigation strategy is to stop irrigation after veraison, partly because of some Spanish wine quality certificate restrictions and the belief that irrigation after veraison impairs berry quality (Santesteban et al. 2011). However, some alternative irrigation strategies based on pre-veraison water deficit have been shown to be effective in improving berry quality and limiting excessive vegetative growth (Intrigliolo and Castel 2010; Santesteban et al. 2011; Caruso et al. 2022). To our knowledge, no research studies have been published on irrigation strategies based on different phenological sensitivity when vines are pruned to force buds to regrow. Only Gu et al. (2012) reported that increasing irrigation from 65 to 80% of crop evapotranspiration enhanced yield. Forced vines shifted phenology to a completely different environment than unforced vines. It has been demonstrated that, during the berry ripening period, vines forced 23 days after anthesis keep a better vine water status than unforced vines, because cooler conditions reduce the environmental water demand and, therefore, increase the whole vine water potential (Martínez-Moreno et al. 2019). However, the first stages of growth and development occur in late spring and early summer, and so, under highly water demanding environments in forced compared to unforced vines (Martínez-Moreno et al. 2019). One of the main limitations of the crop forcing technique is the low amount of non-structural carbohydrate reserves at the time when vines are forced. Another limitation is the time required to restore a new functional leaf area which provides carbohydrates to the whole vine (Oliver-Manera et al. 2022). Therefore, it seems inappropriate to follow an early water-deficit strategy soon after forcing limiting vegetative growth.

The goal of this study is to evaluate three irrigation strategies when vines are forced and their impact on forced yield during three consecutive seasons (2018–2020). The three irrigation strategies were: water stress before forcing pruning with (DI+RDI) or without (DI) water stress after veraison and water stress only after veraison (RDI). The RDI treatment was then compared with an unforced treatment under water stress after veraison (C-RDI) commonly used by local winegrowers.

## Materials and methods

### Experimental site and design and phenology

The trial was conducted in 2018, 2019, and 2020 in a commercial vineyard in Lleida (Catalonia, Spain) (41.65°N, 0.52°E; 320 m above sea level) on Tempranillo vines grafted on R110 rootstock and planted in 2013. The rows were north–south (31.6°N-E) oriented with a slope of 2% and with 1.65 m between vines and 2.5 m between rows. Vines were trained with double cordons and had a vertically positioned canopy. The criterion for winter pruning was to leave about 12 spurs on each vine and two buds per spur. The soil was poor in organic matter (< 1.5%), non-saline (EC=0.27 dS/m), and stony (≈35%), with silty loam texture (22.7% clay, 49.3% silt, 28% sand) and maximum deepness explorable by the roots of 1 m. Vines were drip irrigated with 2.3 L/h pressure compensating emitters spaced at 0.6 m intervals. The climate is characterized by cold winters with fog and hot summers with the possibility of heat waves with temperatures above 40 °C exceptionally. The regional Winkler index (Amerine and Winkler 1944) is 1905 growing degree days (GDD), calculated with 10 °C as the baseline temperature, and classified as III but close to IV. Mean annual precipitation and reference evapotranspiration are 340 mm and 1064 mm, respectively (data from 2007 to 2017). Rainfall events occur mainly in spring and autumn, mostly outside the growing season. Weather data were collected from a weather station located 6.8 km from the plot property of the meteorological network of the Generalitat de Catalunya (Servei Meteorològic de Catalunya).

Four treatments were studied: unforced vines grown conventionally according to the winery criteria with a regulated deficit irrigation strategy from veraison to harvest (C-RDI), forced vines under regulated deficit irrigation before forcing (DI), forced vines under regulated deficit irrigation before forcing and from veraison to harvest (DI + RDI), and forced vines under regulated deficit irrigation only from veraison to harvest (RDI). For the periods without water stress, vines were irrigated at 100% of the estimated evapotranspiration (ETc). It should be noted that the forced treatments had been previously forced in 2017 but without any differentiated irrigation strategy (vines were irrigated only until the end of July). Crop forcing pruning was performed between 80 and 85 days after budburst (DABB) which usually occurs at mid-June in the study site. The exact forcing dates were June 28, June 17, and June 22 for 2018, 2019, and 2020, respectively. Crop forcing pruning was performed mechanically with a pre-pruner (Pellenc DISCO, Pellenc SAS, Pertuis, France) attached to a tractor, leaving 6–8 buds per shoot, and manually removing the remaining leaves and bunches. In C-RDI vines, a standard removal of excessive/basal lateral shoots

was undertaken in early summer, together with the removal of the bunch zone leaves (below nodes 3–6) to allow exposure of the bunch to the sun, and a mechanical hedging and lateral trimming was executed. Additionally, fruit thinning was performed 1 month before veraison to establish a fruit load of about 20 bunches per vine. In forced treatments, no other management operation was performed apart from the crop forcing. Each treatment was replicated three times with 20 vines per replication. The experimental design was a completely randomized block design.

Phenological stages were monitored weekly according to the modified E–L system (Coombe 1995) in two vines per repetition (six vines per treatment). A certain phenological stage was considered to have been reached when 50% of the population was at the same phenological stage.

### Water status, irrigation control and irrigation scheduling

To monitor vine water status, stem water potential ( $\Psi_s$ ) was measured every 15 days from May to October following the Shackel et al. (1997) methodology. On two vines per replicate, a shaded leaf located near the trunk was bagged in an aluminium bag 30 min before the measurement. Measurements were carried out between 11:30 and 12:30 (GMT), using a pressure chamber (Model 3005, Soil Moisture Equipment Co. Sta. Barbara, CA, USA).

To consider both the intensity and the duration of water stress,  $\Psi_s$  was integrated as water stress integral (Int $\Psi$ ). However, the first measurement of  $\Psi_s$  differed between years. Therefore, the first measurement was normalized to 48 days after budburst (DABB), which was the earliest measurement taken in the whole trial (in 2020). Int $\Psi$  was calculated following the equation defined by Myers (1988) (1):

$$\text{Int}\Psi = \left| \sum_{i=0}^{i=t} (\bar{\Psi}_{i,i+1} - c)n \right| \quad (1)$$

where  $\bar{\Psi}_{i,i+1}$  is the average of  $\Psi_s$  between two measurements,  $n$  is the number of days elapsed between the two measurement dates, and  $c$  is the maximum  $\Psi_s$  measured in the study period ( $c = -0.3$  MPa).

To monitor the amount of water applied, each plot was equipped with a water meter (CZ 3000, Contazara S.A., Zaragoza, Spain). Irrigation dose was calculated each week through Eq. 2

$$\text{Irrigation Dose} = \text{ETc}/0.9 - P_{\text{eff}} \quad (2)$$

where ETc is the crop evapotranspiration for non-stressed vines calculated from the ETo Penman–Monteith formula

of  $ET_c = ET_o K_c$  (Allen et al. 1998) with crop coefficients  $K_{c1} = 0.2$  (from budburst),  $K_{c2} = 0.7$  (mid-season, from veraison until harvest), and  $K_{c3} = 0.3$  (at leaf senescence) derived from previous trials located at the same orchard (Girona et al. 2006; Marsal et al. 2008).  $P_{eff}$  is the effective rainfall, estimated as 60% of the rainfall for a week with more than 10 mm of precipitation and otherwise considered to be zero. The factor 0.9 is the efficiency of the irrigation system.

Irrigation dose was, however, modulated based on  $\Psi_s$  thresholds proposed by Girona et al. (2006): prior to full bloom  $-0.6$  MPa (no stress) and  $-0.75$  MPa (deficit irrigation) from full bloom to veraison  $-0.75$  MPa (no stress) and  $-1.0$  MPa (deficit irrigation), and in the post-veraison period  $-0.85$  MPa (full irrigation) and  $-1.2$  MPa (deficit irrigation). Irrigation was cancelled for 3 days if  $\Psi_s$  was higher than  $-0.4$  MPa to avoid overirrigation based on the mid-point between the leaf turgor loss point and the leaf full turgor pressure point determined from pressure volume curves. A total of eight pressure volume curves were generated in May of 2018 following the procedure described in Marsal and Girona (1997).

Water use efficiency (WUE) was calculated as the ratio between yield and the water provided by irrigation and effective rainfall during the growing season (from non-forced budburst to leaf fall).

### Fraction of intercepted radiation

The fraction of photosynthetically active radiation intercepted by the vines (FIPAR) was measured before forcing on DOY 144, 141, and 148 for 2018, 2019, and 2020, respectively, just before forcing only in 2018 and 2019 on DOY 177 and 167, respectively, and after forcing on DOY 243, 206, and 215 for 2018, 2019, and 2020, respectively. In 2019 and 2020, an extra measurement was added at the end of the season on DOY 275 and 286, respectively, to indicate maximum growth for all treatments. All the measurements were carried out between 11:00 and 12:00 (GMT) using a Li-191R line quantum sensor (Li-191R, Li-Cor, Inc., Lincoln, NE, USA). For each plot, one measure was taken above the canopy ( $I_{above}$ ) and two rows of 50 measurements were taken below the canopy of 10 vines per plot ( $I_{below}$ ). Measurements below the canopy were taken perpendicular to the row and 0.5 m apart to cover the entire ground allocated per vine. The FIPAR was calculated as follows (Eq. 3):

$$FIPAR = 1 - \frac{\sum I_{below}/100}{I_{above}} \quad (3)$$

### Yield, yield components, must and wine quality

The optimal harvest date was set when total soluble solids (TSS) content was between 22.5 and 23.5°Brix for all treatments. Thus, to ensure the optimal harvest date, from 1 month after veraison until harvest, a sample of berries was collected approximately every 3 days to extract the juice and measure the TSS with a refractometer (Palette, PR-32 $\alpha$ , ATAGO Co., LTD., Tokyo, Japan). At harvest, yield, number of bunches per vine and bunch weight were determined. A sub-sample of 50 berries per replication was also weighed (berry weight). The number of berries per bunch and per vine was then estimated as the ratio between bunch weight and yield by berry fresh weight. All samples were weighed shortly after collection and dried at 65 °C to constant weight to determine the dry weight. Berry juice was extracted from one sample of each replication and TSS content was determined. The same juice was used to measure pH using a pH meter (Crison PLG-22, HACH LANGE, SLU, Barcelona, Spain) and titratable acidity (TA). To measure must TA (g/L tartaric acid), 10 mL of filtered juice was diluted with 10 mL of distilled water and titrated with a 0.1 N NaOH solution to a final pH of 8.2. The concentration of malic acid in must was determined enzymatically. After harvest, clusters from each plot were moved to an experimental winery. Grapes were destemmed and crushed and fermented at 22 °C in stainless steel containers. Subsequently, SO<sub>2</sub> (40 mg/L) and 0.2 g/kg of *Saccharomyces cerevisiae* yeast were added, and the skin and grape juice was left for 7 days. After alcoholic fermentation, grapes and juice were pressed and decanted to glass bottles for 6 months under carbon dioxide atmosphere until analysis. Total polyphenol index (TPI) and colour properties were determined by measuring absorbances at a determined wavelength ( $A_{nm}$ ): total polyphenol index ( $A_{280}$ ), colour intensity ( $A_{420} + A_{520} + A_{620}$ ), and hue ( $A_{420}/A_{520}$ ). Absorbances were measured with a spectrophotometer (T60, PG Instruments Ltd., Wibtoft, Leicestershire, UK). However, due to technical problems with the absorbance measurements, these were delayed by 1 year in 2020. Because of the low yield in forced treatments in 2018, must and wine quality analysis were not performed.

### Statistical analysis

A univariate analysis of variance (ANOVA) was performed to reveal differences between treatments ( $P < 0.05$ ). The normal distribution of experimental errors was assessed with the Shapiro–Wilk test (Shapiro and Wilk 1965). Homogeneity of error variances was assessed with Levene’s test (Levene 1960) ( $P < 0.05$ ). Differences between means were determined using the Tukey test. All statistical analyses were

performed with JMP14 software (SAS Institute Inc., Cary, NC, 1989–2021).

## Results

### Phenology, forcing pruning and harvest dates

Because budburst for the C-RDI treatment differed between years (Table 1) and the forcing date was set at about 80 days after forcing budburst, the date of the forcing pruning differed between years. Harvest was postponed by 47 days on average in forced treatments (Table 1). On average, forced budburst (the time when forced buds unlocked) was detected 9 days (131 GDD) after the forcing pruning. Veraison date was observed 119 days after budburst (C-RDI) and 81 days after the time when forced buds unlocked (forced treatments) and, on average, was delayed by 53 days in forced treatments. Minimal differences (4%) were observed in GDD from budburst to veraison between the C-RDI (1089) and forced treatments (1134) (in this case from budburst of forced buds to veraison). Harvest date differed between treatments and years as this was a technical decision based on TSS.

### Weather data, irrigation, and plant water status

Weather conditions were different between years (Fig. 1A, C and E). On average, mean temperature during berry ripening (from veraison to harvest) was reduced from 23.5 °C for unforced vines to 15.4 °C for forced vines. The most evaporative demand period (more than 5 mm/day) and hotter days (average temperature above 20 °C) were concentrated between DOY 140 and DOY 240 (Fig. 1A, C and E). Annual rainfall was higher in 2018 (485 mm) (Fig. 1A) and 2020 (501 mm) (Fig. 1E) than the typical values of the region whereas 2019 was a typical year of this semiarid region (331 mm) (Fig. 1C). The period of the year with fewest rainfall events was between DOY 160 and DOY 280 for the 3 different years (Fig. 1A, C and E). After DOY 285,

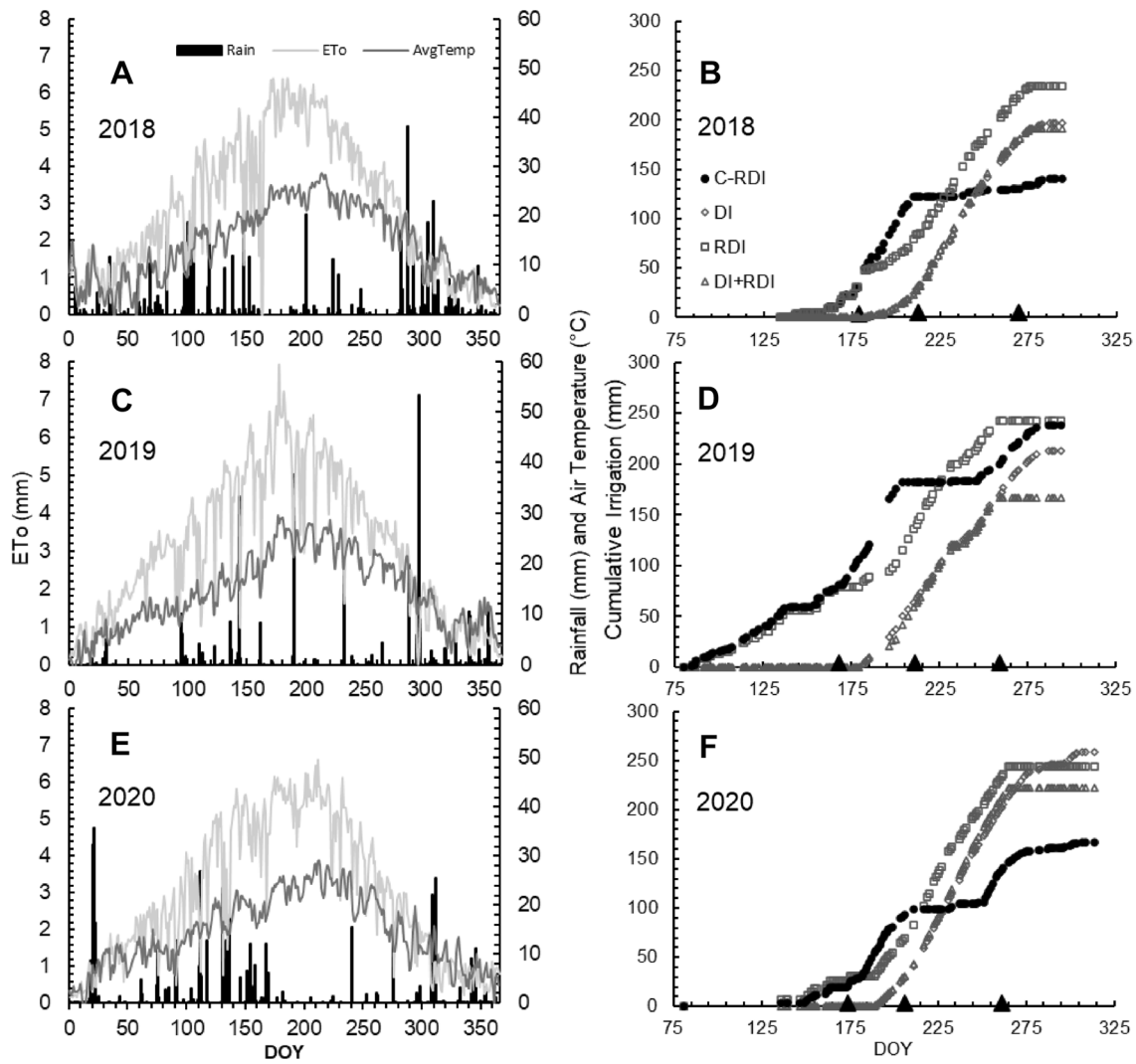
rainfall events appeared in years 2018 and 2019 (Fig. 1A and C), with some of them providing high rainfall amounts (more than 20 mm), but in 2020 significant autumn rainfall appeared later (after DOY 309).

The potential ET<sub>c</sub> (without deficit irrigation strategy) estimated for the C-RDI treatment (475.5 mm) was higher than for the forced treatments (394.1 mm) (Table 2). For the whole season, rainfall provided 29% and 41% of the ET<sub>c</sub> for the C-RDI and forced treatments, respectively. However, the average amount of water applied in the C-RDI treatment for the 3 trial years was lower than in the DI (18%), RDI (25%), and DI + RDI (7%) treatments (Table 2). Differences came from the amount of water provided by the rainfall and the ET<sub>c</sub> for each period in which different irrigation treatments were applied. Before forcing, the period in which deficit irrigation was applied in the DI and DI + RDI treatments, rainfall provided 55% of the ET<sub>c</sub> (Table 2). However, from budburst to veraison, the period in which water deficit was avoided for all treatments, 32% of the ET<sub>c</sub> was provided by rainfall for the C-RDI treatment, whereas for forced treatments, rainfall accounted for only 12% of the ET<sub>c</sub>. From veraison to harvest, the period in which deficit irrigation was applied in the C-RDI, DI + RDI and RDI treatments, rainfall was almost zero for the C-RDI treatment, whereas in forced treatments, 83% of the ET<sub>c</sub> was provided by rainfall. Only in season 2019, after a dry spring (Fig. 1C) which obliged the supply of irrigation water to the C-RDI and RDI treatments early in the season, the amount of water applied in the C-RDI treatment was the same as in the RDI and higher than in the DI (11%) and DI + RDI (30%) treatments (Fig. 1D). Among forced treatments, irrigation was higher in the RDI than in the DI (7%) and DI + RDI (19%) treatments (Table 2). Only in 2020 was the amount of irrigation higher (by 6%) in the DI than in the RDI treatment (Fig. 1F), because from veraison to harvest (DOY 261 to 307), the amount of water provided by rain (23.4 mm) occurred late (Fig. 1E). Only C-RDI vines required post-harvest irrigation, because in forced treatments, leaves were senescent soon

**Table 1** Main phenological stages, forcing dates, and harvest dates for each year of the trial in Julian days and GDD (in brackets)

Treatment	Year	Forcing	Budburst	Full bloom	Veraison	Harvest	Leaf fall
C-RDI	2018	–	94	152 (322)	213 (1145)	264 (1850)	319 (2139)
	2019	–	85	149 (245)	211 (1067)	259 (1681)	315 (2026)
	2020	–	93	142 (286)	206 (1056)	244 (1639)	305 (2042)
Forced	2018	179 (634)	189 (149)	215 (399)	270 (1130)	303 (1333)	333 (1362)
	2019	168 (412)	177 (122)	200 (360)	259 (1145)	302 (1464)	324 (1490)
	2020	174 (612)	181 (102)	207 (369)	261 (1128)	307 (1342)	322 (1368)

For the C-RDI treatment, GDD are computed from budburst to each phenological stage. For forcing dates, GDD indicated are computed from C-RDI budburst to forcing date. For forced budburst dates, GDD indicated are computed from forcing date. For phenological stages after forcing, GDD indicated are computed from the time when buds unlock (forced buds budburst) to each phenological stage



**Fig. 1** In the left-hand column: daily rainfall, ETo, and average temperature for 2018 **A**, 2019 **C** and 2020 **E**. In the right-hand column: cumulative irrigation for each treatment for 2018 **B**, 2019 **D**, and

2020 **F**. Triangles indicate (from left to right) forcing date, veraison for C-RDI, and veraison for forced treatments

**Table 2** Average of 2018, 2019, and 2020 seasons of estimated cumulative potential ETC, effective rainfall ( $P_{eff}$ ), and cumulative irrigation (Irr) in mm, before forcing, between two phenological stages, and for the total season (from non-forced budburst to leaf fall)

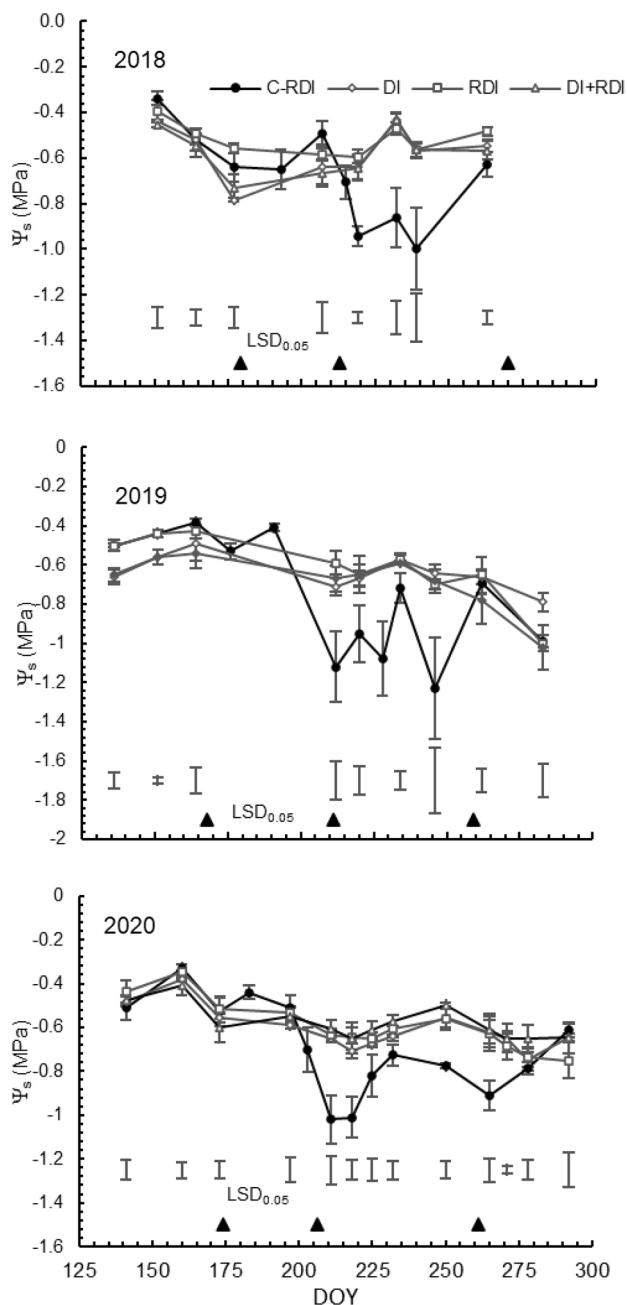
Phenology	Pre-forcing			Budburst to veraison			Veraison to harvest			Post-harvest			Total season		
	ETc	$P_{eff}$	Irr	ETc	$P_{eff}$	Irr	ETc	$P_{eff}$	Irr	ETc	$P_{eff}$	Irr	ETc	$P_{eff}$	Irr
C-RDI	137.0	74.9	60.6	270.4	87.2	132.5	143.7	11.1	12.0	62.1	40.7	37.4	475.5	139.0	181.9
DI			0.8	196.9	23.5	181.7	52.3	43.3	40.6	7.9	20.8	0.0	394.1	162.5	223.1
RDI			53.7			178.6			7.5			0.0			240.1
DI+RDI			1.0			185.0			7.9			0.0			193.9

Note that phenological stages did not coincide in dates for C-RDI and forced treatments

after harvest and ETc was vastly compensated by rainfall (Table 2).

During the periods in which differentiated irrigation treatments were not triggered,  $\Psi_s$  was almost always between

the previously established thresholds ( $-0.4$  to  $-0.8$  MPa) (Fig. 2). Only on DOY 151 (2018) and DOY 160 (2020) did we observe  $\Psi_s$  higher than  $-0.4$  MPa in the C-RDI and RDI treatments, and in 2019  $\Psi_s$  dropped below  $-1.0$  MPa



**Fig. 2** Seasonal pattern of the mid-day stem water potential ( $\Psi_s$ ) for the 3 years of the trial for each treatment. Black triangles indicate, from left to right, forcing date, veraison of the C-RDI treatment and veraison of the forced treatments. The vertical bars represent the least significant difference (LSD) ( $P \leq 0.05$ ) between irrigation treatments for those dates in which  $\Psi_s$  was measured in all the treatments

for the C-RDI treatment in the post-harvest period. Prior to application of forcing pruning, we observed  $\Psi_s$  lower than  $-0.6$  MPa only on DOY 177 (2018), DOY 136 (2019), and DOY 173 (2020) for the DI and DI+RDI treatments. Although  $\Psi_s$  prior to forcing for the C-RDI and RDI

treatments tended to be higher than in the DI and DI+RDI treatments (14% higher on average for the 3 years), only on DOY 136 and 151 (2019) and 177 (2018) did we observe statistical differences in  $\Psi_s$  for the DI and DI+RDI compared to the C-RDI and RDI treatments. Regarding the post-veraison deficit strategies, the  $\Psi_s$  in the C-RDI treatment was close to  $-1.1$  MPa for the three seasons and never exceeded  $-1.25$  MPa. However, only in 2019, did  $\Psi_s$  reach the minimum value of  $-1.0$  MPa for the RDI and DI+RDI treatments, and in 2020, the minimum  $\Psi_s$  observed for the DI+RDI treatment was  $-0.65$  MPa whereas for the RDI was  $-0.8$  MPa. No statistical differences were observed in post-veraison  $\Psi_s$  between the forced treatments. In 2018, post-veraison  $\Psi_s$  was not measured, because 45% of the days were rainy days.

Only in 2019 was pre-forcing Int $\Psi$  significantly reduced in the DI and DI+RDI treatments (Table 3) compared to the RDI treatment, although there was a tendency to observe lower (more negative) pre-forcing Int $\Psi$  in 2018 and 2020 in the DI and DI+RDI treatments than in the RDI treatment. As a result, when pre-forcing Int $\Psi$  was averaged for all the years of the trial, Int $\Psi$  was significantly lower in the DI and DI+RDI treatments and there was no statistical significance of the interaction YxT. In 2018, the DI and DI+RDI treatments were significantly more stressed than the RDI treatment (lower Int $\Psi$ ) in the period between forcing and veraison in which no differentiated treatments were applied (Table 3). As in the case of pre-forcing Int $\Psi$ , the tendency of the DI and DI+RDI treatments to be more stressed from forcing to veraison is confirmed by the appearance of significant differences when averaging the same period for the 3 years of the trial. In addition, the interaction YxT was not significant. Differences in the water stress integral were observed between years but not treatments from veraison to harvest and for the entire year.

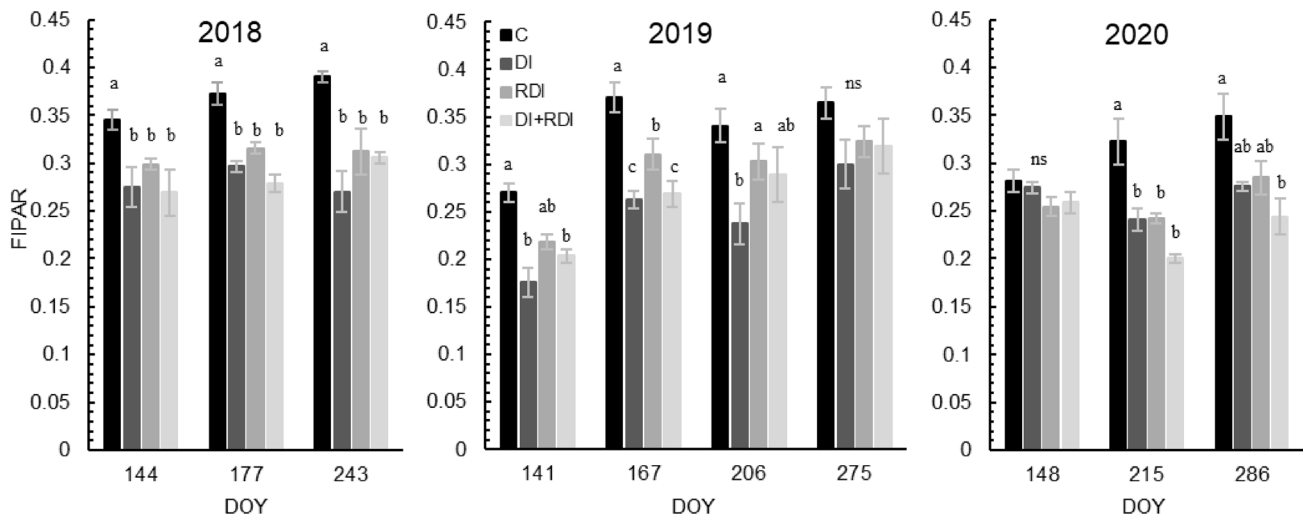
### Fraction of intercepted photosynthetically active radiation

In year 2018, when comparing forced treatments we did not observe differences in FIPAR due to the irrigation strategy before forcing (DOY 144 and 177). However, we observed lower FIPAR in forced treatments compared to the C-RDI treatment. Note that in 2017, the same vines were also forced, and therefore, the differences observed in Fig. 3 for the pre-forcing period in 2018, must be attributed to the forcing operation carried out in 2017 and the subsequent management by the winery which did not include differentiated irrigation management for forced treatments. After forcing (DOY 243), forced treatments did not achieve FIPAR comparable to the C-RDI treatment, although this measurement was taken 27 days before veraison in forced vines.

**Table 3** Water stress integral (IntΨ) for forced treatments for years 2018, 2019, and 2020 and during the whole trial (average) split by periods from the first measurement to application of forcing pruning (Pre-forcing), from the first measurement after application of forcing pruning to veraison (Forcing-Veraison), from veraison to harvest (Veraison-Harvest) and the sum of the entire year (Total)

Year	Treatment	Pre-forcing (MPa)	Forcing-veraison (MPa)	Veraison-harvest (MPa)	Total (MPa)
2018	DI	-8.87 ± 0.33	-19.87 ± 2.52 <sup>b</sup>	-7.32 ± 1.50	-36.06 ± 4.29
	RDI	-6.35 ± 0.70	-15.43 ± 1.69 <sup>a</sup>	-6.37 ± 0.82	-28.15 ± 1.80
	DI+RDI	-9.08 ± 1.10	-19.96 ± 2.97 <sup>b</sup>	-8.52 ± 1.69	-37.56 ± 5.58
	Significance	ns	*	ns	ns
2019	DI	-7.66 ± 1.55 <sup>ab</sup>	-21.61 ± 2.99	-17.98 ± 2.23	-47.25 ± 6.71
	RDI	-3.98 ± 0.21 <sup>a</sup>	-19.46 ± 3.46	-24.23 ± 2.52	-47.67 ± 6.10
	DI+RDI	-7.93 ± 1.46 <sup>b</sup>	-21.86 ± 4.47	-26.38 ± 5.08	-56.17 ± 10.25
	Significance	*	ns	ns	ns
2020	DI	-4.34 ± 0.64	-20.06 ± 1.52	-16.37 ± 3.21	-40.77 ± 5.17
	RDI	-3.11 ± 1.40	-18.67 ± 3.93	-18.79 ± 4.53	-40.57 ± 8.45
	DI+RDI	-4.98 ± 1.56	-16.67 ± 1.95	-15.37 ± 2.57	-37.02 ± 5.91
	Significance	ns	ns	ns	ns
Average	DI	-6.96 ± 0.84 <sup>b</sup>	-20.51 ± 1.24 <sup>b</sup>	-13.89 ± 2.05	-41.36 ± 3.18
	RDI	-4.48 ± 0.66 <sup>a</sup>	-17.85 ± 1.57 <sup>a</sup>	-16.45 ± 3.05	-38.78 ± 4.18
	DI+RDI	-7.33 ± 0.92 <sup>b</sup>	-19.50 ± 1.81 <sup>ab</sup>	-16.76 ± 3.11	-43.59 ± 4.92
	Significance	**	*	ns	ns
Year	2018	-8.10 ± 0.59 <sup>b</sup>	-18.43 ± 1.43 <sup>a</sup>	-7.40 ± 0.76 <sup>a</sup>	-33.93 ± 2.55 <sup>a</sup>
	2019	-6.53 ± 0.89 <sup>b</sup>	-20.98 ± 1.88 <sup>b</sup>	-22.86 ± 2.16 <sup>c</sup>	-50.37 ± 4.21 <sup>b</sup>
	2020	-4.14 ± 0.69 <sup>a</sup>	-18.46 ± 1.26 <sup>a</sup>	-16.85 ± 1.84 <sup>b</sup>	-39.45 ± 3.38 <sup>a</sup>
	Significance	**	*	**	**
YxT	Significance	ns	ns	ns	ns

Treatment effects were analysed using ANOVA and the means were separated with the Tukey test. Means followed by different letters are different at  $P < 0.05$ . \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; ns not significant. Data are means of three replications ± standard error



**Fig. 3** Effects of the forcing and irrigation treatments on the fraction of intercepted photosynthetically active radiation (FIPAR) in 2018, 2019, and 2020 measured between 11:00 and 12:00 (GMT). Bars are

standard deviation of the three replicates. Letters on the top of each column represent means significant differences with the Tukey test at  $P < 0.05$ . ns means no significant difference

In 2019, prior to forcing, the C-RDI treatment had higher FIPAR than forced treatments and differences attributed to the irrigation treatment were observed on DOY 167 in which the irrigated RDI treatment had higher FIPAR than both

non-irrigated treatments (DI and DI + RDI) (Fig. 3). After the forcing action, FIPAR was statistically lower in the DI than the C-RDI and RDI treatments on DOY 206, but at the end of the season (DOY 275), all forced treatments were able



to achieve FIPAR values like those of the C-RDI treatment. In 2020, reduced FIPAR in forced treatments before forcing was not present. However, after forcing, the DI + RDI treatment did not achieve FIPAR values comparable to the C-RDI treatment, but the RDI and DI treatments did although 20% lower. Excepting DOY 148 in 2020, generally the RDI treatment had higher FIPAR than the other forced treatments.

### Yield, yield components, must and wine quality

To assess the effect of forcing treatment, only the RDI treatment was included to avoid any interaction with the irrigation strategies (deficit prior to forcing) which cannot be applied to unforced vines. On average, yield was reduced by 34% in the RDI compared to the C-RDI treatment (Table 4). The number of bunches per vine at harvest was higher in the RDI (26) than in the C-RDI (17) treatment. However, it should be noted that the number of bunches per vine was fixed to about 20 in the unforced treatment, following winery criteria. Bunch weight and fresh berry weight in the RDI treatment were, respectively, 59% and 38% lower than in the C-RDI treatment. The number of berries per bunch was also reduced by 56.5% in the RDI compared to the C-RDI treatment. However, because of the higher number of bunches per vine in the RDI treatment, the total berries per vine were not significantly reduced compared to the C-RDI treatment. In 2018, and more severely in 2020, the C-RDI treatment was affected by spring mildew damaging part of the commercial yield. Thanks to the forcing operation, forced bunches were unaffected by the illness. Therefore, the interaction YxT was statistically significant, since in 2020, yield was higher in the

RDI than C-RDI treatment mainly due to the higher number of bunches per vine (Table 4).

Comparing irrigation treatments in forced vines, we observed that yield in the RDI treatment was 34% higher than in the DI treatment (although not with statistical significance) and 51% higher than in the DI + RDI treatment and with significant differences (Table 5). The origin of the differences in yield was the higher number of bunches per vine in the RDI (26) treatment than in the DI (21) and the DI + RDI (19) treatments. As a result, the number of berries per vine was higher in the RDI (1497) than in the DI + RDI (947) treatment, with the DI (1135) treatment in the middle. No differences were observed in bunch weight, berry fresh and dry weight, and berries per bunch between treatments (Table 5).

Water use efficiency (WUE) was reduced by the forcing operation (Table 4) compared to the C-RDI treatment although in 2020, due to mildew illness, WUE was higher in the RDI than the C-RDI treatment. However, no differences in WUE were observed between forced treatments, although WUE was 32% higher in the RDI treatment than the DI and DI + RDI treatments (Table 5).

A significant correlation was found between yield and the integral of  $\Psi_s$  (Int $\Psi$ ) for forced treatments ( $R^2=0.70$ ) prior to the date when vines were pruned for forcing (Fig. 4a). The correlation was even stronger ( $R^2=0.76$ ) when plotting the number of bunches per vine against Int $\Psi$  (Fig. 4b). In forced vines, the higher the water stress before forcing (Int $\Psi$  more negative) the lower the number of bunches per vine.

Grape quality was affected by the forcing operation (Table 6). TSS was lower in the DI and DI + RDI treatments

**Table 4** Effects of dormant bud forcing (RDI treatment) on yield and yield components and water use efficiency (WUE) compared to unforced vines (C-RDI treatment)

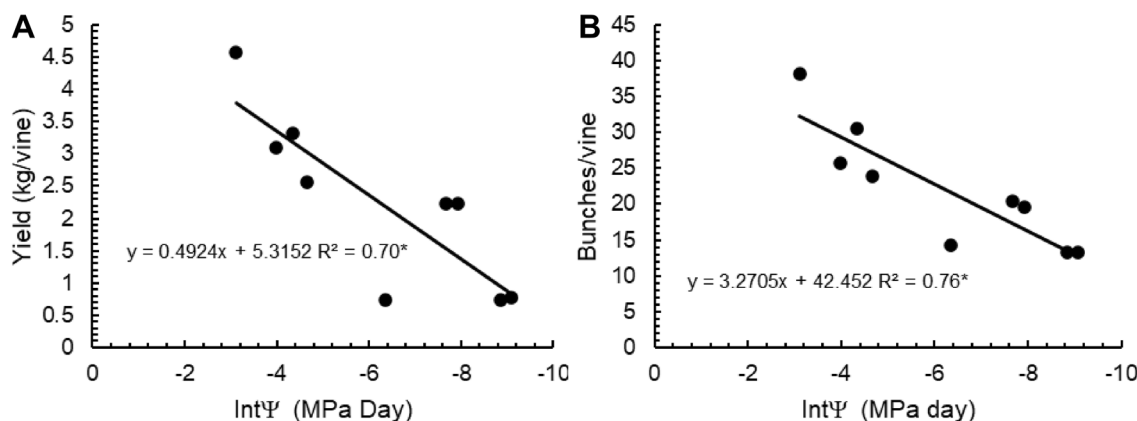
Parameter	T	2018	2019	2020	Average	T	Year	TxYear
Yield (kg/vine)	C-RDI	3.25 ± 0.60	6.87 ± 1.09	2.68 ± 0.54	4.27 ± 0.76	*	**	**
	RDI	0.73 ± 0.12	3.11 ± 0.14	4.58 ± 0.29	2.81 ± 0.57			
Bunches/vine	C-RDI	16 ± 2	21 ± 1	15 ± 2	17 ± 1	**	**	**
	RDI	14 ± 1	26 ± 2	38 ± 3	26 ± 4			
Bunch weight (g)	C-RDI	198.7 ± 13	318.7 ± 39	177.5 ± 19	231.6 ± 25.6	**	**	**
	RDI	50.8 ± 5	119.2 ± 4	115.3 ± 4	95.2 ± 11.4			
BerryFW (g)	C-RDI	2.25 ± 0.14	2.52 ± 0.08	2.73 ± 0.14	2.50 ± 0.09	**	**	ns
	RDI	1.52 ± 0.06	2.08 ± 0.16	1.83 ± 0.05	1.81 ± 0.10			
BerryDW (g)	C-RDI	0.56 ± 0.04	0.62 ± 0.01	0.70 ± 0.03	0.63 ± 0.03	**	**	*
	RDI	0.39 ± 0.02	0.54 ± 0.02	0.45 ± 0.00	0.46 ± 0.02			
Berries/bunch	C-RDI	89 ± 3	126 ± 12	65 ± 7	93 ± 10	**	**	**
	RDI	34 ± 4	58 ± 7	63 ± 4	52 ± 5			
Berries/vine	C-RDI	1430 ± 175	2711 ± 381	983 ± 207	1708 ± 292	ns	**	**
	RDI	488 ± 92	1502 ± 64	2500 ± 148	1497 ± 295			
WUE (kg/m <sup>3</sup> )	C-RDI	2.60 ± 0.41	4.77 ± 0.59	2.34 ± 0.34	3.24 ± 0.45	**	**	**
	RDI	0.41 ± 0.06	2.10 ± 0.00	2.85 ± 0.17	1.79 ± 0.36			

Treatment effects were analysed using ANOVA. \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; ns not significant. Data are means of three replications ± standard error

**Table 5** Effects of the irrigation treatment (T) on yield and yield components and water use efficiency (WUE) between the forced treatments

	Yield (kg/vine)	Bunches/vine	Bunch weight (g)	BerryFW (g)	BerryDW (g)	Berries/bunch	Berries/vine	WUE (kg/m <sup>3</sup> )
T								
DI	2.09 ± 0.51 <sup>ab</sup>	21 ± 3 <sup>ab</sup>	86.5 ± 10.8	1.73 ± 0.11	0.44 ± 0.03	48 ± 5	1135 ± 271 <sup>ab</sup>	1.36 ± 0.32
RDI	2.81 ± 0.57 <sup>a</sup>	26 ± 4 <sup>a</sup>	95.2 ± 11.4	1.81 ± 0.10	0.46 ± 0.02	52 ± 5	1497 ± 295 <sup>a</sup>	1.79 ± 0.36
DI+RDI	1.86 ± 0.39 <sup>b</sup>	19 ± 2 <sup>b</sup>	87.9 ± 10.2	1.88 ± 0.10	0.49 ± 0.03	46 ± 4	957 ± 181 <sup>b</sup>	1.36 ± 0.30
Significance	*	*	ns	ns	ns	ns	*	ns
Year (Y)								
2018	0.75 ± 0.12 <sup>c</sup>	14 ± 1 <sup>c</sup>	51.9 ± 4.2 <sup>b</sup>	1.48 ± 0.05 <sup>c</sup>	0.38 ± 0.01 <sup>c</sup>	35 ± 3 <sup>b</sup>	503 ± 79 <sup>c</sup>	0.45 ± 0.07
2019	2.52 ± 0.28 <sup>b</sup>	22 ± 2 <sup>b</sup>	112.8 ± 4.9 <sup>a</sup>	2.10 ± 0.07 <sup>a</sup>	0.55 ± 0.013 <sup>a</sup>	54 ± 4 <sup>a</sup>	1216 ± 138 <sup>b</sup>	1.87 ± 0.19
2020	3.48 ± 0.47 <sup>a</sup>	31 ± 3 <sup>a</sup>	104.9 ± 6.4 <sup>a</sup>	1.89 ± 0.03 <sup>b</sup>	0.48 ± 0.01 <sup>b</sup>	56 ± 4 <sup>a</sup>	1870 ± 265 <sup>a</sup>	2.18 ± 0.29
Significance	**	**	**	**	**	**	**	**
T x Y	ns	ns	ns	ns	ns	ns	ns	ns

Treatment effects were analysed using ANOVA and the means were separated with the Tukey test. Means followed by different letters are different at  $P < 0.05$ . \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; ns not significant. Data are means of three replications ± standard error



**Fig. 4** Linear regression between yield **a** and the number of bunches per vine; **b** for the three seasons only including CF treatments with the integral of  $\psi_s$  calculated prior to forcing. Data are for the RDI, DI, and DI+RDI treatments for the 3 years of the study.  $P < 0.01$  for both regressions

than in the C-RDI treatment, but higher than the lower threshold set of 22.5°Brix. Must TA from forced berries was higher (187% on average) and pH lower (9% on average) than the must from the C-RDI vines. On average, the must from forced vines had a malic acid concentration 269% higher than the must from unforced vines. The higher TA and the same or slightly lower TSS resulted in doubling of the TSS:TA ratio. No differences attributable to the irrigation regime between forced treatments were observed in TA, pH, malic acid, or the TSS:TA ratio. It is notable, however, that TA, pH, malic acid concentration, and the TSS:TA ratio were different between 2019 and 2020, with the latter having higher TA and malic acid concentration values and lower pH and TSS:TA ratio values for all treatments.

After vinification, the lactic acid concentration was increased in wines from forced treatments (Table 7), since

malic acid was higher in forced musts (Table 6). Alcohol content was reduced in wines from forced treatments and the DI + RDI treatment had lower alcohol content than the other forced treatments (Table 7). TA and pH were enhanced in all wines from forced treatments, especially in those from the DI + RDI treatment. Colour intensity was not altered by the forcing treatments in 2019, but it was drastically reduced in 2020. Hue was generally not altered by forcing and contradictory results were obtained in 2019 compared with 2020. On average, TPI was higher in the C-RDI treatment. However, in 2019, TPI was higher in the DI treatment compared to the C-RDI treatment and both forced treatments with post-veraison (RDI and DI + RDI) water restriction reduced TPI, although this was only statistically significant in the DI + RDI treatment when compared to the DI treatment. Note that the colour traits and TPI analyses were performed 6 months (2019) and one

**Table 6** Effects of forcing and irrigation treatments (T) on grape quality in seasons 2019 and 2020

Parameter	T	2019	2020	Average	T	Year	TxYear
TSS °Brix	C-RDI	24.3 ± 0.2	25.1 ± 0.4	24.7 ± 0.3 <sup>a</sup>	**	ns	**
	DI	23.4 ± 0.2	23.4 ± 0.3	23.4 ± 0.2 <sup>b</sup>			
	RDI	24.7 ± 0.0	23.1 ± 0.4	23.9 ± 0.4 <sup>ab</sup>			
	DI + RDI	23.2 ± 0.2	23.7 ± 0.2	23.4 ± 0.2 <sup>b</sup>			
TA (g/L)	C-RDI	3.97 ± 0.11	5.57 ± 0.37	4.77 ± 0.40 <sup>b</sup>	**	**	ns
	DI	8.75 ± 0.12	9.59 ± 0.13	9.17 ± 0.15 <sup>a</sup>			
	RDI	7.90 ± 0.06	9.33 ± 0.32	8.61 ± 0.35 <sup>a</sup>			
	DI + RDI	8.67 ± 0.25	9.24 ± 0.32	8.96 ± 0.16 <sup>a</sup>			
pH	C-RDI	3.46 ± 0.03	3.58 ± 0.04	3.52 ± 0.04 <sup>a</sup>	**	**	**
	DI	3.24 ± 0.02	3.16 ± 0.01	3.20 ± 0.02			
	RDI	3.30 ± 0.01	3.12 ± 0.02	3.21 ± 0.04 <sup>b</sup>			
	DI + RDI	3.23 ± 0.01	3.16 ± 0.01	3.20 ± 0.01 <sup>b</sup>			
Malic acid (g/L)	C-RDI	1.58 ± 0.21	2.10 ± 0.10	1.83 ± 0.16 <sup>b</sup>	**	**	ns
	DI	4.4 ± 0.09	5.87 ± 0.13	5.13 ± 0.23 <sup>a</sup>			
	RDI	4.13 ± 0.03	5.43 ± 0.34	4.78 ± 0.33 <sup>a</sup>			
	DI + RDI	4.23 ± 0.17	5.47 ± 0.07	4.85 ± 0.21 <sup>a</sup>			
TSS: TA	C-RDI	61.1 ± 1.2	45.4 ± 2.7	53.3 ± 3.8 <sup>a</sup>	**	**	*
	DI	26.8 ± 0.6	24.4 ± 0.6	25.6 ± 0.5 <sup>b</sup>			
	RDI	31.3 ± 0.3	24.9 ± 1.2	28.1 ± 1.5 <sup>b</sup>			
	DI + RDI	26.8 ± 1.0	25.6 ± 0.6	26.2 ± 0.6 <sup>b</sup>			

Treatment effects were analysed using ANOVA and the means were separated with the Tukey test. Means followed by different letters are different at  $P < 0.05$ . \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; ns not significant. Data are means of three replications ± standard error

and a half years (2020) after vinification due to technical problems.

## Discussion

Crop forcing technique succeeded in delaying the berry ripening period to a cooler environment as has been reported in previous works (Gu et al. 2012; Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019; Oliver-Manera et al. 2022). The cumulative GDD from budburst to veraison was a robust tool to predict veraison date for forced treatments, an observation reported previously in fully irrigated vines (Prats-Llinàs et al. 2020; Oliver-Manera et al. 2022) but contrary to Martínez-Moreno et al. (2019) who reported significantly more GDD required to reach veraison in forced treatments. This extra GDD requirement was attributed to competition for resources between the attempt to refill carbohydrate reserves after forced budburst and vine development. These contradictory results may be due to the vine water status. In Martínez-Moreno et al. (2019), pre-veraison water potentials were lower than  $-1$  MPa in forced vines. Therefore, a good vine water status soon after forcing (lower than  $-0.65$  MPa) may benefit both, vine development and carbohydrate reserves refilling, minimizing the effect of forcing on the required GDD for vine development.

The crop coefficients ( $K_c$ ) used in this study are quite similar to those reported using weighing lysimeters with Tempranillo cultivar ( $K_c = 0.7$ ) for a FIPAR of 0.35 (Picón-Toro et al. 2012). Therefore, we consider that the estimated  $ET_c$  is a reliable tool to analyse the amount of water demanded by vines. Although for the whole growing season (from budburst to leaf fall), the potential  $ET_c$  was higher and rainfall lower for unforced vines than for forced vines, after the 3 years of the trial the irrigation strategies we applied in forced treatments did not save water compared to the unforced treatment. In the C-RDI treatment, the water-deficit strategy was applied from veraison (DOY 213 on average) to harvest (DOY 256 on average) and was based on the criterion of maintaining  $\Psi_s$  above the threshold of  $-1.2$  MPa proposed by Girona et al. (2006). Although the period in which water was restricted for the C-RDI treatment coincided with the driest and hottest period of the year (the estimated  $ET_c$  of this period represented 30% of the total estimated  $ET_c$  and rainfall was practically nil) meeting the  $\Psi_s$  threshold criteria ( $\Psi_s$  above  $-1.2$  MPa) barely required irrigation water. In addition, before veraison, 32% of the  $ET_c$  was compensated by rainfall in the C-RDI treatment, which, together with the water available in the soil from previous autumn and winter rainfall, allowed the C-RDI treatment to compensate for only 49% of the  $ET_c$  by irrigation without  $\Psi_s$  dropping below the  $-0.6$  MPa threshold assigned for non-stressed vines (Girona

**Table 7** Effects of forcing and irrigation treatment (T) on wine quality in seasons 2019 and 2020

Parameter	T	2019	2020	Average	T	Year	TxYear
Lactic acid (g/L)	C-RDI	0.99 ± 0.04 <sup>b</sup>	1.13 ± 0.11 <sup>b</sup>	1.06 ± 0.06 <sup>b</sup>	**	**	ns
	DI	2.18 ± 0.09 <sup>a</sup>	2.52 ± 0.05 <sup>a</sup>	2.35 ± 0.07 <sup>a</sup>			
	RDI	2.14 ± 0.02 <sup>a</sup>	2.40 ± 0.08 <sup>a</sup>	2.27 ± 0.07 <sup>a</sup>			
	DI+RDI	2.15 ± 0.10 <sup>a</sup>	2.35 ± 0.05 <sup>a</sup>	2.25 ± 0.06 <sup>a</sup>			
Alcohol content (%Vol)	C-RDI	14.8 ± 0.1 <sup>a</sup>	15.2 ± 0.2 <sup>a</sup>	15.0 ± 0.2 <sup>a</sup>	**	ns	**
	DI	14.1 ± 0.1 <sup>a</sup>	13.8 ± 0.1 <sup>b</sup>	14.0 ± 0.1 <sup>b</sup>			
	RDI	14.2 ± 0.1 <sup>a</sup>	13.5 ± 0.3 <sup>b</sup>	13.8 ± 0.2 <sup>bc</sup>			
	DI+RDI	13.2 ± 0.1 <sup>b</sup>	13.9 ± 0.2 <sup>b</sup>	13.6 ± 0.2 <sup>c</sup>			
TA (g/L)	C-RDI	4.47 ± 0.03 <sup>c</sup>	4.16 ± 0.10	4.31 ± 0.08 <sup>c</sup>	**	**	**
	DI	4.60 ± 0.03 <sup>bc</sup>	4.33 ± 0.04	4.47 ± 0.05 <sup>b</sup>			
	RDI	4.75 ± 0.03 <sup>b</sup>	4.32 ± 0.07	4.54 ± 0.10 <sup>ab</sup>			
	DI+RDI	5.02 ± 0.03 <sup>a</sup>	4.23 ± 0.06	4.63 ± 0.12 <sup>a</sup>			
pH	C-RDI	4.06 ± 0.03 <sup>a</sup>	4.07 ± 0.04	4.07 ± 0.02 <sup>a</sup>	**	**	**
	DI	3.99 ± 0.02 <sup>a</sup>	4.00 ± 0.02	4.00 ± 0.02 <sup>ab</sup>			
	RDI	3.98 ± 0.02 <sup>a</sup>	3.98 ± 0.01	3.98 ± 0.02 <sup>bc</sup>			
	DI+RDI	3.83 ± 0.01 <sup>b</sup>	4.01 ± 0.07	3.92 ± 0.03 <sup>c</sup>			
Colour intensity	C-RDI	12.3 ± 0.3	12.2 ± 0.4 <sup>a</sup>	12.2 ± 0.2 <sup>a</sup>	**	**	**
	DI	11.8 ± 0.3	8.9 ± 0.2 <sup>b</sup>	10.4 ± 0.5 <sup>b</sup>			
	RDI	11.9 ± 0.0	9.3 ± 0.0 <sup>b</sup>	10.6 ± 0.4 <sup>b</sup>			
	DI+RDI	11.7 ± 0.3	9.4 ± 0.5 <sup>b</sup>	10.6 ± 0.6 <sup>b</sup>			
Hue	C-RDI	0.53 ± 0.01 <sup>a</sup>	0.68 ± 0.01 <sup>ab</sup>	0.61 ± 0.03	ns	**	**
	DI	0.56 ± 0.01 <sup>a</sup>	0.67 ± 0.01 <sup>ab</sup>	0.61 ± 0.02			
	RDI	0.54 ± 0.01 <sup>a</sup>	0.65 ± 0.01 <sup>b</sup>	0.59 ± 0.03			
	DI+RDI	0.49 ± 0.00 <sup>b</sup>	0.69 ± 0.01 <sup>a</sup>	0.59 ± 0.03			
TPI	C-RDI	62.6 ± 2.2 <sup>b</sup>	79.2 ± 1.1 <sup>a</sup>	70.9 ± 3.9 <sup>a</sup>	**	**	**
	DI	73.5 ± 1.5 <sup>a</sup>	54.0 ± 0.7 <sup>b</sup>	63.8 ± 3.3 <sup>b</sup>			
	RDI	69.4 ± 0.7 <sup>ab</sup>	52.3 ± 0.2 <sup>b</sup>	60.9 ± 3.9 <sup>b</sup>			
	DI+RDI	66.8 ± 1.2 <sup>b</sup>	57.6 ± 2.5 <sup>b</sup>	62.3 ± 1.9 <sup>b</sup>			

Treatment effects were analysed using ANOVA and the means were separated with the Tukey test. Means followed by different letters are different at  $P < 0.05$ . \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; ns not significant Data are means of three replications ± standard error

et al. 2006). As a result, although some post-harvest irrigation was required to recover vine water status to  $\Psi_s$  above the threshold of  $-0.85$  MPa for non-stressed vines (Girona et al. 2006), the C-RDI treatment supplied only 38.2% of the seasonal potential ET<sub>c</sub> by irrigation.

On the other hand, for forced vines, the period from forced buds budburst (DOY 182 on average) to veraison (DOY 263 on average) in which no deficit irrigation was applied, coincided with the most water demanding period (representing 50% of the whole year ET<sub>c</sub>), with low compensation by rainfall (only 10% of the ET<sub>c</sub>). Therefore, in forced treatments, irrigation water was required to compensate almost 90% of the ET<sub>c</sub> from forced budburst to veraison which represented 50% of the total ET<sub>c</sub>. In addition, in forced treatments, the pre-forcing water stress strategy could have saved a maximum of 35% of the total ET<sub>c</sub>, but spring rainfall compensated 55% of the ET<sub>c</sub>, reducing the required water provided by irrigation.

Only in 2019, after a dry spring (only 30% of ET<sub>c</sub> was compensated by rainfall), did the DI and DI+RDI treatments save water compared to the C-RDI treatment at the end of the season. In fact, 2019 was the only season in which  $\Psi_s$ , which is a very robust indicator to discriminate different vine water status independently of the environment (Santesteban et al. 2019), was clearly affected by the irrigation regime in the DI and DI+RDI treatments compared to the C-RDI and RDI treatments. However, when analysing pre-forcing Int $\Psi$ , it was evident that the DI and DI+RDI treatments were more stressed than RDI.

On the other hand, the post-veraison period in forced treatments represented only 13% of the total ET<sub>c</sub> and is likely to be compensated by rainfall in autumn. Delayed veraison by an average of 53 days (Table 1) moved the ripening period to environmental conditions and daylength that drive low daily evaporative demand. As a result, excepting 2019, we did not observe any sign of water stress after

veraison in any forced treatment either analysing the  $\Psi_s$  or the  $\text{Int}\Psi$ . The difficulty of applying post-veraison water stress in forced vines was previously reported by Martínez-Moreno et al. (2019), but neither the amount of water nor the exact irrigation strategy were reported. It should be considered that annual rainfall in 2019 (331 mm) was close to the regional mean (340 mm) but not in 2018 (485 mm) and 2020 (501 mm). Therefore, in terms of saving water, the DI and DI + RDI strategies showed promising results but not the RDI strategy.

To study plant responses to irrigation treatments, multi-year studies are recommended (Intrigliolo and Castel 2010). As a perennial crop, grapevine responses to water stress may be extended to the subsequent year. The same idea is valid for defoliation experiments. Reducing photosynthetic capacity one year may affect the next season in the form of reduced carbohydrate reserves (Bennett et al. 2005). When using the crop forcing technique, the physiological response to irrigation is even more complex than in classical irrigation or defoliation experiments. The effects on forced vine performance may come from the period before forcing or even from the previous year (Oliver-Manera et al. 2022). Vine response to the crop forcing technique is a reduction of yield due to lower bunch weight, number of berries per bunch and individual berry weight (Gu et al. 2012; Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019; Oliver-Manera et al. 2022), observations which are in agreement with our results. Yield reduction has been attributed to low carbon availability when vines are forced, the date of forcing, an impaired seasonal carbon balance and to the temperature distribution of forced vines (very elevated at first stages of development and low after veraison), altering the duration of the developmental stages (Gu et al. 2012; Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019; Oliver-Manera et al. 2022). Our results of reduced FIPAR, which is positively correlated to whole vine photosynthesis (Poni et al. 2003), in forced treatments compared to the C-RDI treatment confirm the limitation on photosynthetic capacity in forced vines. However, it is notable that in our trial, yield in forced vines increased year after year, suggesting vine acclimation to forcing. This observation is consistent with the capacity to restore carbohydrate reserves after vines are forced, attributable to a high source:sink ratio since forced vines give priority to vegetative growth over yield (Oliver-Manera et al. 2022) and to the tendency of the reduction in yield to remain constant after 2 years of forcing (Martínez-Moreno et al. 2019).

When comparing the different irrigation strategies of forced treatments, we observed a reduction in yield, but only caused by a reduction in the number of bunches per vine in those treatments in which irrigation was withheld before forcing. We did not observe a reduction in berry fresh weight, which is highly sensitive to post-veraison water

stress (Girona et al. 2009) in any treatment, which is consistent with the lack of post-veraison water stress. However, the main limitation for berry post-veraison growth may be low temperature (Martínez De Toda et al. 2019) rather than water stress.

Despite both irrigation management and defoliation being cited as interesting techniques to be studied in grapevine management in the face of global warming (Gutiérrez-Gamboa et al. 2021; Palliotti et al. 2014), there remains a huge scope for research when combining both techniques. No reduction in bunches per vine was observed when combining water deficit with leaf removal with Tempranillo (Buesa et al. 2019) or Merlot cultivar (Yu et al. 2016). On the other hand, after 5 trial years, Williams (2012) observed lower bunches per vine in both vines under water stress and defoliated vines, although no interaction was observed. Ferlito et al. (2014) observed a reduction in yield when combining defoliation and water stress, but the reduction was cultivar-dependant. However, early water stress applied about full bloom (Guilpart et al. 2014; Matthews and Anderson 1989; Santesteban et al. 2011), as well as early reduction of the whole canopy functional leaf area (about full bloom and flowering) (Gatti et al. 2016; Risco et al. 2014), can result in a reduction in the number of bunches per vine the subsequent year or even the abortion of inflorescences to the same year in late pruning techniques (Gatti et al. 2016). In a crop forcing study, the number of bunches per vine increased when applied irrigation was increased from 60% of the pre-veraison ETC to 80% of seasonal ETC the following year, but only 1 year of each irrigation regime was reported (Gu et al. 2012). It is widely accepted that inflorescence induction in grapevines occurs from budburst until before the bud enters dormancy the year before berry formation (Boss et al. 2003; Mullins et al. 1992). Therefore, in forced vines, inflorescence primordia, which would become the forced crop, are formed from unforced budburst (between DOY 85 and 94 in this study) to a few days after forcing pruning (at least before the time when forced inflorescences appear). This observation is supported by the fact that, with the Tempranillo cultivar, the later the vines are forced (always before the bud enters dormancy), the higher the number of forced bunches (Martínez-Moreno et al. 2019; Martínez De Toda et al. 2019; Oliver-Manera et al. 2022). In our trial, the irrigation treatment in DI and DI + RDI coincides with the induction of the inflorescence primordia that will become the forced crop after the forcing operation. The good negative correlation observed between the water stress integral prior to forcing and the number of bunches per vine suggests high sensitivity of inflorescence formation to water stress which rises as the soil becomes drier (Guilpart et al. 2014). This is the case of the DI + RDI treatment, since a lower proportion of ETC was compensated through irrigation the previous year. In fact, the first  $\Psi_s$  measured after forced buds were

unlocked (DOY 207 and 212 for 2018 and 2019) tended to be lower (12% averaging 2018 and 2019) and the  $\text{Int}\Psi$  more negative between forcing and veraison in treatments with pre-forcing water stress except when winter and spring rainfall compensate the pre-forcing vine water demand as was the case of 2020. Since  $\Psi_s$  is a good indicator of plant water status and is well correlated with soil water status (Intrigliolo and Castel 2006), this suggests some recovery time required to refill the soil in the DI and DI + RDI treatments. Moreover, the probable impact of forcing pruning on the hydraulic system of the vine should not be ruled out. It is accepted that cutting shoots when under stress causes xylem embolism (Wheeler et al. 2013). Because the forcing pruning was performed under high evaporative demand and low soil water content for the DI and DI + RDI treatments, it is likely that the hydraulic system for both treatments needed more time to recover from the pruning. Dysfunctionality of the hydraulic system can reduce vine growth and photosynthesis (Lovisololo et al. 2010), which is consistent with our observation of slightly higher FIPAR in the RDI treatment.

It is also well known that the number of bunches per vine is correlated to carbohydrate reserves at budburst (Bennett et al. 2005; Lebon et al. 2008), which are in turn dependent on canopy photosynthetic capacity and carbon utilization the year before (Holzapfel et al. 2010; Williams 1996). Before the time vines were forced, we only observed a significant reduction in FIPAR in the DI and DI-RDI treatments, which is consistent with the lower  $\Psi_s$  for the same treatments compared to the RDI treatment in 2019. In addition, the averaged  $\Psi_s$  we observed in the DI and DI + RDI treatments before forcing was about  $-0.54$  MPa, whereas the averaged minimum  $\Psi_s$  was  $-0.66$  MPa for both treatments, which is not considered to drastically reduce leaf net photosynthesis in Tempranillo (Intrigliolo and Castel 2011). Furthermore, although soil water content is one of the main factors affecting the use of carbohydrate reserves (Smith and Holzapfel 2009), vegetative growth is more sensitive to water stress than photosynthesis (Hsiao 1973) and, therefore, we expected that mild water stress before forcing might have resulted in an increase of carbohydrate reserves when forced buds unlock which is contrary to the yield reduction observed in the DI and DI + RDI treatments. Therefore, our results suggests that inflorescence formation is more sensitive to mild water stress than photosynthesis as previously reported (Guilpart et al. 2014). However, our results do not allow to rule out reduced carbon availability in the reduction of bunches per vine we observed in the DI + RDI treatment.

Must acidity and malic acid concentration increased and pH reduced by the forcing operation, with TSS levels within the previously determined range ( $22.5$ – $23.5^\circ$ Brix) although lower than in the C-RDI. Therefore, the TSS: TA ratio was enhanced due to the forcing. The above were all goals of the crop forcing technique, and are in accordance with results

previously reported with Tempranillo (Lavado et al. 2019; Martínez-Moreno et al. 2019; Martínez De Toda 2021). The increased must acidity and lower pH in forced treatments has been attributed to the lower temperature at which berries ripen, because malic acid degradation as a respiratory substrate is dependent on temperature (Martínez De Toda et al. 2019). The irrigation treatments did not alter must quality, suggesting the need for more research on new irrigation strategies based on our results. However, although in wine made using forced grapes acidity was increased and alcohol content decreased, the effect on colour attributes and TPI differed depending on the year, and in 2020, wine colour and TPI clearly were impaired in forced treatments which could be related to the mildew affection in the C-RDI treatment possibly affecting the source:sink relationship and increasing anthocyanin biosynthesis (Mirás-Avalos et al. 2017), which is closely related to wine colour in red varieties. However, the DI treatment succeeded in increasing TPI in 2019 compared to forced vines under post-veraison water stress and unforced vines. The latter observation is consistent with previous works which observed higher polyphenol content in well-irrigated forced vines than unforced vines attributed to a better coupling of sugar and anthocyanin accumulation due to a more suitable temperature regime after veraison in forced vines (Gu et al. 2012; Lavado et al. 2019). Regarding the effect of water stress, Buesa et al. (2019) observed that rain-fed vines defoliated soon before veraison reduced TPI compared to undefoliated and irrigated vines, which was attributed to reduced carbohydrate availability after veraison. In forced vines, post-veraison carbohydrate uptake at whole vine level is dramatically reduced even for fully irrigated vines (Oliver-Manera et al. 2022). Therefore, since 2019 was the only year in which post-veraison  $\Psi_s$  for the RDI and DI + RDI treatments dropped below  $\Psi_s -1.0$  MPa which can limit leaf photosynthesis (Intrigliolo and Castel 2011), reduced carbohydrate availability may have affected phenolic biosynthesis.

In a climate change scenario in which the water demand of plants and aridity are expected to increase, techniques which increase yield while reducing water consumption have become a priority (Flexas et al. 2010). This is not the case of the crop forcing technique and the irrigation strategies studied in our trial. Due to the greater water consumption and reduced yield in forced than unforced vines, WUE was dramatically reduced in forced vines regardless of the irrigation strategy. There are two main ways to increase WUE in forced vines: (i) yield must be increased by maintaining or reducing the amount of water supplied, or (ii) water consumption should be reduced by maintaining or increasing yield. Early deficit irrigation (from full bloom to veraison) reduces berry size and vine vigour (Intrigliolo and Castel 2010), which are also vine responses to the crop forcing technique. Therefore, in our opinion, early water-deficit strategies should be

avoided in forced vines. However, based on our results and the apparent positive yield response to the increase in water supply suggested by Gu et al. (2012), we suggest that sustained deficit irrigation (a controlled reduction of the ETC) would be a good irrigation strategy to increase WUE. In addition, in this study, we used previously defined  $\Psi_s$  thresholds determined under environmental conditions in which vines are adapted to grow. However, it is well known that  $\Psi_s$  thresholds are affected not only by phenology but also by the vapour deficit pressure (Olivo et al. 2009). Therefore, sustained deficit irrigation strategies would be an interesting object of research to define physiological irrigation management thresholds in forced vines. Interestingly, a recent variation of crop forcing called double cropping, in which the primary crop and the leaves from the first six nodes were not removed, overcame the yield reduction associated with forced vines (Poni et al. 2021). Therefore, research in irrigation strategies and water stress responses using this new technique would be of interest to enhance WUE.

## Conclusions

The crop forcing technique indisputably enhanced must acidity, pH, and malic acid content when compared to unforced vines. However, our study failed to maintain or increase water use efficiency in forced vines. Forced vines have been proved to be highly sensitive to water stress before the forcing pruning (resulting in reduced yield) and after veraison (which may reduce the polyphenol content of wine). Therefore, as an alternative to the irrigation strategies proposed in this study, it is suggested to focus research on sustained deficit irrigation strategies, based on irrigating with a controlled percentage of crop evapotranspiration, which would allow the establishment of water stress indicators adapted to the crop forcing technique. On the other hand, double cropping may overcome yield reduction. Further research is needed combining irrigation strategies with double cropping with the aim of increasing the water use efficiency of forced vines and making the crop forcing technique more sustainable.

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**Author contributions** JG and OG-T conceived, planned, and supervised this study. JO-M contributed to the planning of the experiment. JO-M and MM performed most of the field measurements and tasks. JO-M did the processing and analysis of all the data and drafted and finalized the manuscript. JG and OG-T reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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**Data availability** The data that support the findings of this study are available upon reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

**Conflict of interest** The authors declare no competing interests.

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