



# Effect of different deficit irrigation regimes on vine performance, grape composition and wine quality of the “Primitivo” variety under mediterranean conditions

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## Abstract

Climate change represents one of the current major challenges and the improper use of water resources is an impeding threat. Agricultural research can play a crucial role by developing innovative strategies and techniques to reduce water use without affecting crop productivity and quality, particularly in grapevine growing in Mediterranean areas, as both productivity and wine quality are quintessential for the economic and ecologic sustainability of this crop. The present study aimed to define a deficit irrigation strategy for the “Primitivo” grapevine cultivar, taking into account the overall pathway of the vineyard performance in terms of leaf functionality, starch reserves, vine productivity, and wine quality. The trial was carried out in Southern-Italy on a three year-old, drip irrigated vineyard, imposing four deficit irrigation regimes for two consecutive seasons, consisting of 29 (T29), 55 (T55), 85 (T85) and 100% (T100) of crop evapotranspiration ( $ET_C$ ). Mild water restriction (T85) did not affect vegetative nor reproductive vine performance. Deficit irrigation at 55%  $ET_C$  lowered leaf functionality, starch accumulation, vine vigour and yield, due to a reduction of cluster weight; however, wine acidity and phenolic compounds were increased. T29 further decreased yield, as also the number of clusters was reduced. The most water-stressed treatment revealed a low concentration of malic acid in the must and a consequent increase of the ethanol sensation in the wine. After 9 months ageing, T85 had the highest wine colour intensity suggesting this treatment as the most promising in terms of quality and quantity of wine as well as for water saving.

**Keywords** Water stress · Leaf functionality · Water use efficiency · Sensorial analysis · Starch reserves · Bud fertility malic acid

## Introduction

According to the European Environment Agency (EEA), 20% of the European territory and 30% of EU population are affected by water scarcity every year. Drought causes economic damage of up to EUR 9 billion annually and

additional unquantified damage to ecosystems and their services (EEA, 2021). Agricultural activities, which use around 70% of all human water withdrawals (Santos et al. 2020; Schultz 2017), are impacted by the water shortages and heat waves. In grapevine, water stresses coupled to heat waves negatively affect vine and leaf physiology, fruit growth and ripening, and wine quality (Gambetta et al. 2020). High temperature, for example, could enhance soluble solids content but, at the same time, can cause a decrease of anthocyanins and titratable acidity (Gutiérrez-Gamboa et al. 2021). The effects of water stress on plant physiology have been widely studied, as well as their impact on grape production and quality (Oliver-Manera et al. 2023). The expected temperature increase, of 0.3–1.7 °C in the near future (Drappier et al. 2019), the reduction of precipitation, and the occurrence of extreme events enhance the process of desertification in the Mediterranean Region (Safriel 2009),

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which is considered a hotspot of climate change (Cos et al. 2022; Lionello and Scarascia 2018). Many areas in Southern Italy are subjected to water shortages and the Apulia region has experienced a consistent decrease in annual rainfall in recent years, limiting its agricultural water resource (Gentilesco et al. 2023; Zollo et al. 2016). Modern irrigation technologies and strategies can help to improve water use efficiency (WUE) whilst benefitting grape and wine quality (Romero et al. 2022). Moderate and controlled water stress, achieved through deficit irrigation, reduced vine vigour and the competition for carbohydrates, promoting the production of secondary metabolites in the berries (Bonfante et al. 2017; Munitz et al. 2017; Romero et al. 2022), and enhancing the anthocyanin content and aroma precursors in the wine. Controlled water stress improved sensory traits, like higher astringency, better colour intensity (Intrigliolo and Castel 2011), improved aromatic profile and higher fruity and floral sensory hints, gaining persistence and balance (Gamero et al. 2014a, b). Appropriate irrigation strategies aiming at reducing water supply, matching quality and productivity, are key to make this practice economically and environmentally sustainable (Ruiz-Sanchez et al. 2010). The use of water-stress-induced strategies such as deficit irrigation, also called “sustained” deficit irrigation (DI), regulated deficit irrigation (RDI) and partial rootzone drying (PRD) (Conesa et al. 2018) represents one of the pivotal approaches. Deficit irrigation consists of a fixed reduction of water supply during the growing season so that the amount supplied is less than the water lost by the vineyard (Ruiz-Sanchez et al. 2010). The proper application of DI requires a deep knowledge of the plant response to water deficit and the economic impact of this type of management on vineyard performance (Tomás et al. 2014; Torres et al. 2021). The soil water content decreases progressively during the vegetative season due to a combination of the constant reduction of water supply and the depletion of the soil water reserve (Ferreles and Soriano 2007). The intensity of water restriction to impose may vary as a function of the cultivar and the yield target (Sadras and Schultz 2012; Romero et al. 2022). In grapevine cv. “Bobal”, a reduction of 35% of crop evapotranspiration ( $ET_C$ ) during the growing season was suggested for optimizing grape skin, seed, and volatile composition in comparison with full irrigation, allowing at the same time a yield increase in comparison to rainfed vines (Lizama et al. 2021). Water restriction, bringing the predawn leaf water potential at  $-1.0$  MPa, negatively affected “Sauvignon Blanc” aroma, which was instead enhanced under mild water deficit (Des Gachons et al. 2005). It has been reported that only severe water stress can significantly affect starch reserves. In cv. “Malbec” subjected to deficit irrigation, no significant differences were observed between fully irrigated treatment and a 60% DI;

while a water reduction to 38% and 25% of the control significantly impacted starch accumulation (Dayer et al. 2013). Another interesting strategy is the regulated deficit irrigation (RDI), which offers greater potential to save water, reduce excessive vine vigour, increase WUE and to improve berry and wine quality; however, the identification of the phenological stages in which to impose the water restriction and the intensity of the water deficit are crucial decisions (Barbagallo et al. 2021; Costa et al. 2007; García-Esparza et al. 2018; Iglesias and Garrote 2015; Intrigliolo et al. 2016). In partial root-zone drying irrigation (PRD), water is supplied alternately only to a part of the root system, while the rest is left dry. This promotes the production of chemical signals in the dry roots (e.g. abscisic acid, ABA), triggering partial stomatal closure that improves water use efficiency (Romero et al. 2022; Sadras 2009; Wang et al. 2012).

Although Italy is one of the main wine exporters in the world, producing around 50.3 million hl per year (OIV 2022), and Apulia is the second national producer (ISTAT, 2021), there are only a few studies about the effects of deficit irrigation on vine performance and wine quality in this region (Storchi et al. 2005; Tarricone et al. 2017), although it could be a suitable approach to improve berry quality and WUE without affecting yields and could more easily be adopted by farmers than PRD and RDI (Buesa et al. 2017; Pérez-Álvarez et al. 2021; Shellie and King 2020; Zarrouk et al. 2016). Only a few studies have considered the effects of varying irrigation inputs in terms of vineyard performance, grape composition and wine quality, to get information about the most appropriate irrigation strategies to apply (Romero et al. 2022). The present research evaluated the impact of different deficit irrigation regimes imposed for two consecutive years on canopy functionality, vegetative performances, productivity, and wine quality of grapevine cv. “Primitivo” under semi-arid conditions.

## Materials and methods

### Experimental set up and irrigation regimes

The trial was carried out in 2020 and 2021, in a commercial vineyard of *Vitis vinifera* L. cv. “Primitivo” UBA 47/B clone, grafted on 775 Paulsen rootstock (*Vitis berlandieri* x *Vitis rupestris*) located in the Castel del Monte area, within the Denomination of Controlled and Guaranteed Origin of Corato (Apulia region, Southern Italy), 353 m above sea level (41°05'36" N 16°20'22" E) on a shallow, gravel, silty-clay soil, with sub-alkaline reaction and 1.8% organic matter. Vines (3 years old) were spaced 2.3 m between rows and 1.1 m within row (3.952 vines ha<sup>-1</sup>). The alley-row was left to a natural grass cover, which was mowed three times per

year, in Spring and early Summer; the rows were tilled in a 70 cm wide strip underneath the vines.

The area has been characterized as a Mediterranean climate (Csa) (Pinna 1970), following the Köppen-Geiger classification system (Köppen 1936). Meteorological data, collected from a weather station close to the vineyard, were utilized to classify the vineyard, using the multicriteria climatic classification system for grape-growing regions developed by Tonietto and Carbonneau (2004). The vineyard exhibited a Huglin Index (HI) (Huglin 1978) of 2631, in 2020, and 2803 in 2021, being classified as Warm ( $HI + 2; 2400 > HI \leq 3000$ ). The Cool night Index (CI) (Tonietto 1999) ranged from 16.1 °C, in 2020, to 16.4 °C in 2021, thus indicating Temperate nights ( $CI - 1; 14 > CI \leq 18$ ). Additionally, the Dryness Index (DI) (Riou et al. 1994) was 47.7 mm, in 2020, and -72.6 mm in 2021, classifying the vineyard as moderately dry ( $-100 > DI > 50$  mm). The Winkler Index (WI) (Winkler et al. 1974) was 2127, in 2020, and 2296 in 2021, placing the vineyard on the boundary between regions IV and V.

Vines were trained as vertical shoot positioned (VSP) training system and pruned as double Guyot, with 16–18 buds per vine, taking in account wood maturity. Canopy management practices included shoot positioning in the month of June, followed by mechanical shoot topping soon after.

The vineyard was outfitted with drip-irrigation, and water supply was managed through the evapotranspiration approach with  $ET_0$  (Penman-Monteith estimation), given by the microclimatic station located close to the vineyard site, and  $ET_C$  obtained estimating the crop coefficient ( $K_C$ ) according to the canopy size (Allen and Pereira 2009). The latter was estimated by NDVI derived for the vineyard by Sentinel 2 satellite data (Hornbuckle et al. 2016; Mzid et al. 2023; Trout and Johnson 2007). Water (pH: 7.8; electrical conductivity:  $0.253 \text{ dS m}^{-1}$ ) was extracted from groundwater at a depth of about 800 m and the pump was powered by photovoltaic panels. Vines were subjected to four irrigation strategies in the two years: T100 (full irrigation, no water deficit) and three deficit irrigation regimes T85, T55 and T29 with a water restitution of 85, 55 and 29% of  $ET_C$ , respectively, using self-compensating drippers with different flow rates: 3.8, 3.2, 2.1 and  $1.1 \text{ L h}^{-1}$  for the four treatments, respectively. Soil water content values at field capacity and wilting point were  $0.219$  and  $0.097 \text{ m}^3 \text{ m}^{-3}$ , respectively; the available water content was  $0.122 \text{ m}^3 \text{ m}^{-3}$ . Water was supplied when 40% of the available water in T100 (readily available water) had been lost. Soil water content was monitored on T100 treatment by one soil moisture probe per replicate (Sentek's EnviroSCAN) at various depths (5, 15, 25, 35, 45 and 55 cm); the data were used to cross check whether the water supplied using the evapotranspiration

approach was appropriate to bring the soil water content of the first 50 cm (active root zone) at field capacity.

The four treatments were arranged according to a randomized complete block design with three blocks. Within each block, each treatment had 30 vines arranged on 3 rows, with 10 plants each. Vines of the central part of the central row were used for determinations while the remaining vines served as guards.

## Leaf functionality and water relations

Ecophysiology measures were performed only in 2021. On June 21st, July 27th and August 27th, corresponding to berry cell division, cell expansion and close to harvest, midday leaf gas exchanges, temperature and chlorophyll fluorescence were measured with an integrated fluorometer and gas exchange system fitted with an artificial and adjustable light source (iFL, ADC BioScientific Ltd., Global House, Geddings Road, Hoddesdon, United Kingdom). Light intensity (PPFD) was maintained constant across the treatments by setting the LED light source to the natural irradiance experienced by the leaves immediately before the measurements (PPFD of 1000, 1900 and  $1700 \mu\text{mol m}^{-2} \text{ s}^{-1}$  on June 21st, July 27th and August 27th, respectively); the reference  $\text{CO}_2$  was set at 400 ppm. The following measures were taken on 3 vines of the central row for each block and treatment, at solar noon: leaf net photosynthesis ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ); stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{ s}^{-1}$ ); transpiration ( $E$ ,  $\text{mmol m}^{-2} \text{ s}^{-1}$ ); effective efficiency of PSII ( $\Phi_{\text{PSII}} = [F_m' - F_s] / F_m'$ ); electron transport rate exiting PSII ( $J_{\text{PSII}}$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ); leaf and air temperature ( $T_{\text{leaf}}$ ,  $T_{\text{air}}$ , °C). Midday stem water potential ( $\Psi_s$ , MPa) was measured on the same vines using a Scholander chamber (3005F01, Soil Moisture Equipment Corp., Santa Barbara CA, USA), following Naor et al. (1995).

## Starch reserves and bud fertility

During the 2020 Winter, one-year pruning wood was collected from the same vines. Five woody sections were obtained in 3 internodes (3rd, 5th, 7th) from each stem. A total of 180 woody sections were analysed following the method described by Rustioni et al. (2017). Reflectance spectra of the tissues were collected by a Jaz System spectrometer (Ocean Optics, B.V., Dunedin, USA) before and after on-solid starch iodine complexation obtained by Lugol stain, according to Rustioni et al. (2016). The starch index was calculated as:

$$\text{Starch Index} = \frac{R_{Re(900)}}{R_{Re(555)}} - \frac{R_{t0(900)}}{R_{t0(555)}}$$

$R_{x(w)}$  = Reflectance (% calibrated on the reference blank).  
 $x$  = spectrum type:  $t_0$  = before reaction;  $Re$  = after Lugol reaction.

$w$  = wavelength of interest (nm): 900 = normalization reference; 555 = starch-iodine complex absorption maximum.

To investigate the effect of water deficit on the next vegetative season, during the bud break period of 2021 and 2022, the number of buds, clusters and shoots, as well as the bud fertility (number of clusters per bud) were calculated for the four treatments on the same vines.

## Vegetative and productive performances

At the end of each vegetative season, the number and weight of clusters and the pruning weight were measured for each vine. In addition, the average cluster weight (g), yield ( $\text{t ha}^{-1}$ ) and Water Productivity (WP), expressed as kg of clusters per  $\text{m}^3$  of supplied water irrigation (Fernández et al. 2020), were calculated. The Ravaz index (Ravaz 1903) was calculated as kg of grape per kg of pruning weight for each vine under investigation.

## Quality and wine sensorial analysis

The quality of must was evaluated by measuring: soluble sugar residues (SSR, °Brix), pH, titratable acidity ( $\text{g l}^{-1}$ ) and malic acid ( $\text{g l}^{-1}$ ) according to standard procedures (EEC2676). In 2021, micro-vinifications were carried out from the grapes of all treatments: about 100 kg of grapes were manually harvested and vinified at the experimental winery of CREA-VE according to the procedure described by Gambacorta et al. (2022). The grapes were crushed and de-stemmed with a stainless-steel crusher-destemmer and placed in 100 L vertical stainless-steel vats. Potassium metabisulphite (6 g/100 kg), yeast (*Saccharomyces cerevisiae* var. Bayanus, Mycoferm CRU05, 20 g/100 kg, Ever, Pramaggiore, Italy) and yeast activator (Enovit, AEB, Venice, Italy) were added. Maceration was performed for 9 days with 2 punch-downs per day. Then, free-run wine was recovered by draining, and the grape pomace was gently pressed to recover press-run wine using a 80 L stainless-steel hydropress. The free-run and press-run wines were blended and raked after 2 weeks to eliminate gross lees. The wines were bottled after 6 months, without any additional treatment, and analyzed for ethanol concentration (%v/v), pH, titratable acidity as tartaric acid (TA,  $\text{g l}^{-1}$ ), malic acid ( $\text{g l}^{-1}$ ), and dry reduced extract (DRE,  $\text{g l}^{-1}$ ). Total polyphenols as gallic acid (TP,  $\text{mg l}^{-1}$ ) and anthocyanins, as malvidin-3-glucoside (A,  $\text{mg l}^{-1}$ ), were assayed according to Di Stefano et al. (1989).

A first round of sensorial analysis was conducted after 6 months of wine aging and a second round, aimed at

evaluating the evolution of wines in time, was replicated three months later. Thirteen expert panellists performed the sensorial analysis; 30 ml of each wine, labelled with four random numbers and covered with plastic film were served at 20 °C in 250 ml ISO goblets. The evaluation entailed two sections: the first consisted of a list of descriptors related to visual, olfactory and taste aspects to be scored by using a structured 10-point scale; the second one reported a list of specific sensorial notes to be flagged by the panellists.

## Statistical analysis

The four irrigation regimes were compared for leaf functionality, starch reserves, bud fertility, productivity, must and wine quality by means of ANOVA considering the randomized complete block design; mean separation was performed with post-hoc SNK test. For those variables tested for the two consecutive years a 2-way ANOVA was performed, considering the year and the water regime as factors. A non-parametric approach was used for sensorial analysis data. Kruskal-Wallis test was performed to compare medians of each descriptor, Mann Whitney test was used to verify differences in the perception of each descriptor between the first and the second round of sensorial analysis. Furthermore, a chi-square test was conducted on each individual note perceived by the 13 panellists for each wine to assess its statistical significance, as follows:

$$x^2 = \frac{(\text{observed} - \text{expected})^2}{(\text{expected})}$$

$$= \frac{(\text{Nr panelists perceiving hint} - \text{Total Nr panelists})^2}{(\text{Total Nr panelists})}$$

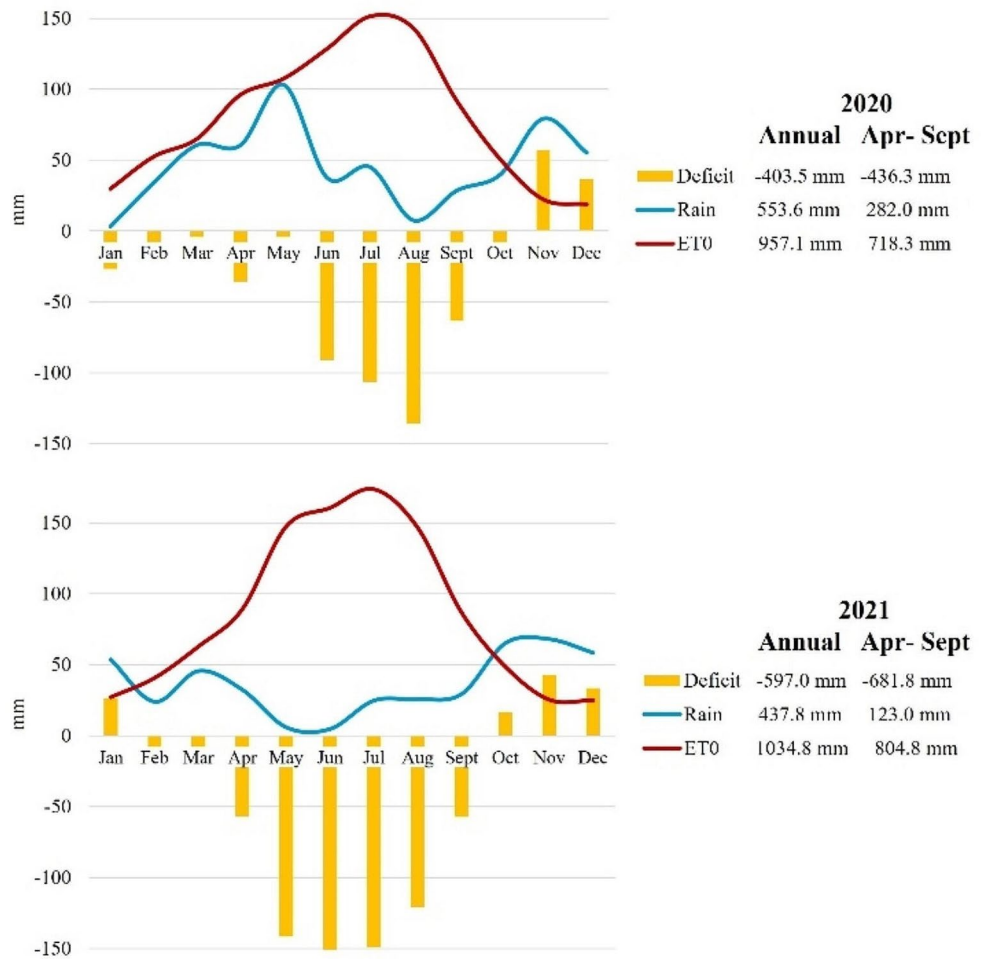
Specifically, the threshold value for 12 degrees of freedom (GL) and  $\alpha < 0.05$  was 5.23. Therefore, a note was considered significantly perceived by the panel when indicated by at least 5 tasters on a total of 13.

## Results

### Microclimate and irrigation regimes

Climate patterns in 2020 and 2021 were in line with average Mediterranean climate (Koppen 1936). The Winter and the initial part of the Spring 2021 were characterized by rains; at the beginning of the Spring air temperature increased and rainfall frequency and intensity diminished. The highest air temperatures were recorded from June to August with maximum values often exceeding 33 °C. In the same period rainfalls amounted to 90 and 52.2 mm in 2020 and 2021, respectively. The vapor pressure deficit (VPD) increased

**Fig. 1** Seasonal water deficit in 2020 and 2021, calculated as the difference between the reference Evapotranspiration (ET<sub>0</sub>) and rainfall, during the entire year and from April to September



**Table 1** Water supplied to the four irrigation regimes in 2020 and 2021 seasons

Year	Treatment	Water supply (m <sup>3</sup> ha <sup>-1</sup> )
2020	T29	174.2
	T55	330.4
	T85	510.6
	T100	600.7
2021	T29	222.1
	T55	421.2
	T85	651
	T100	765.9

during the year reaching the maximum values during Summer, with average daily VPDs of 1.5 and 2.17 kPa in 2020 and 2021, respectively. The seasonal water deficit, calculated as the difference between the reference Evapotranspiration (ET<sub>0</sub>) and rainfall, was higher in 2021 than 2020; it increased approaching Summer and from June to August it was 333 and 425 mm in 2020 and 2021, respectively (Fig. 1). Despite this, the vineyard area was classified in both years as warm, with temperate nights and a moderate dryness, in line with previous studies (Alba et al. 2021; Gentileco et

al. 2023) and compliant with an area in the Mediterranean basin. Water was supplied from July 24th to September 2nd in 2020 and from June 21st to August 2nd in 2021, with seasonal volumes in T100 of 600 and 765 m<sup>3</sup>ha<sup>-1</sup>, respectively. Seasonal water supply in T29 was 174 and 222 m<sup>3</sup>ha<sup>-1</sup> for 2020 and 2021, respectively (Table 1).

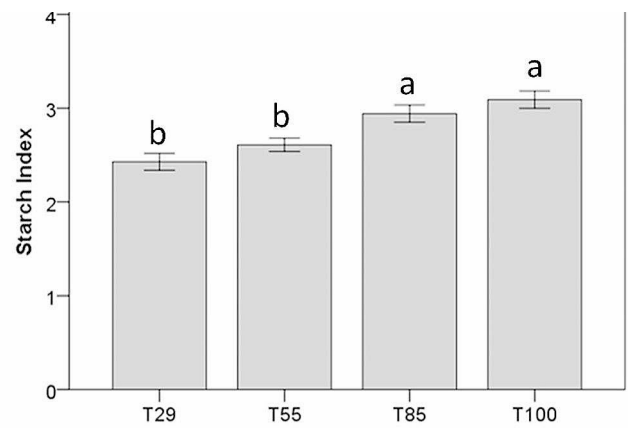
### Leaf functionality and water relations

Leaf functionality was first measured on June 21st, 2021, two days after the onset of the treatments. Air temperature and VPD were 38.6 °C and 3.64 kPa, respectively; net photosynthesis (P<sub>n</sub>) and stomatal conductance (g<sub>s</sub>) were generally low and T100 and T85 showed higher values than the remaining treatments (Table 2). Leaf temperature exceeded 40 °C with the highest values recorded on T55 and T29. The electron transport rate exiting from PSII (J<sub>PSII</sub>) was higher in T100 and T85 than T55 and T29 (Table 2). The second measurement was performed on July 27th during cell expansion. Midday air temperature and VPD were 37.4 °C and 4.98 kPa. No differences were observed for all the eco-physiology variables even if T29 showed a slightly lower



**Table 2** Net photosynthesis (pn), stomatal conductance (gs), leaf transpiration (E), leaf temperature ( $T_{leaf}$ ), electron transport rate exiting PSII ( $J_{PSII}$ ) and stem water potential ( $\Psi_s$ ), recorded on the four treatments during fruit cell division (21/6), fruit cell expansion (27/7) and close to the harvest (27/8) in 2021. Within each date and for each variable, different letters indicate a statistical difference at  $p=0.05$

Date	Treatment	Pn ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	gs ( $\text{mol m}^{-2} \text{s}^{-1}$ )	E ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$T_{leaf}$ ( $^{\circ}\text{C}$ )	$J_{PSII}$ ( $\mu\text{mol e m}^{-2} \text{s}^{-1}$ )	$\Psi_s$ (MPa)
21/6	T29	1.26	0.013	0.67	41.15	98.52	-
	T55	1.14	0.013	0.83	43.15	80.61	-
	T85	3.61	0.027	1.48	40.59	148.41	-
	T100	2.83	0.023	1.33	40.60	140.70	-
27/7	T29	3.61	0.051	3.33	44.21	119.03	-1.28
	T55	5.42	0.087	5.17	45.18	119.01	-1.17
	T85	3.28	0.043	2.71	44.14	96.06	-1.04
	T100	6.40	0.093	5.73	44.34	137.25	-1.05
27/8	T29	7.59	0.108	2.89	34.39	158.86	-1.20
	T55	7.62	0.127	3.31	34.82	174.72	-0.97
	T85	9.55	0.141	3.32	33.85	180.22	-0.86
	T100	10.62	0.176	3.93	34.24	232.33	-0.83



**Fig. 2** Starch content quantified by means of the Starch Index recorded on canes of the four treatments under investigation in 2020. Different letters indicate a statistical difference at  $p=0.05$

midday stem water potential, close to -1.3 MPa; the average Pn, gs and  $\Psi_s$  were  $4.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $0.07 \text{mol m}^{-2} \text{s}^{-1}$  and  $-1.18 \text{MPa}$ , respectively (Table 2). Close to harvest, air and leaf temperature recorded at midday were 27.8 and 34.2  $^{\circ}\text{C}$ , respectively. Pn values in T100 and T85 were higher than in T55 and T29; the highest gs was recorded in T100 followed by T85, T55 and T29. Leaf temperature was similar among the treatments and the highest and the lowest values of  $J_{PSII}$  were recorded on T100 and T29, respectively. The minimum midday stem water potential was observed in T29 with  $\Psi_s$  of -1.20 MPa (Table 2).

### Starch reserves and bud fertility

DI significantly impacted starch reserve accumulation in woody canes, with similar values of starch index in T100 and T85, decreasing in the treatments receiving less water (Fig. 2).

No year by treatment interaction was recorded for number of buds, clusters, shoots, and bud fertility. The former three variables showed lower values in 2021 than in 2022 (Table 3). The number of buds and shoots was lower in T29 and T55 in comparison with T85 and T100. Bud fertility, expressed as the number of clusters per bud, was slightly lower in T29 than in the remaining treatments (Table 3); the lowest number of clusters was recorded in T29. No difference for the percentage of budbreak was observed, suggesting that the lower number of shoots of T29 was dependent by the lower number of buds rather than a higher level of closed buds (Table 3).

### Vegetative and productive performances

The interaction between year and irrigation regime was not significant. Excluding the average cluster weight, the

**Table 3** Number of buds left with Winter pruning, and the derived number of clusters, shoots and bud fertility detected on the four irrigation treatments in the two years of trial. Within each experimental factor, (\*) or different letters indicate a statistical difference at  $p=0.05$ 

Experimental factor	Buds		Clusters		Shoots		Bud fertility
	(number vine <sup>-1</sup> )						(cluster bud <sup>-1</sup> )
<b>Year</b>							
2021	10.18	*	12.06	*	8.98	*	1.32
2022	17.11		25.17		15.89		1.44
<b>TRT</b>							
T29	11.89	b	14.56	b	10.56	b	1.19
T55	13.22	ab	20.22	a	12.17	ab	1.54
T85	15.31	a	20.33	a	13.56	a	1.42
T100	14.72	a	20.28	a	13.94	a	1.39

second year of investigation revealed a general reduction of productivity, attributable to the lower number of buds left with the Winter pruning and the related number of clusters (Table 4). The heaviest clusters were observed in T100 and T85, followed by T55 and T29 (Table 4); T29 showed the lowest number of clusters per vine (Table 4). Yield was not different between T100 and T85 ( $\sim 7 \text{ t ha}^{-1}$ ), while it was reduced in T55 and T29 showing values of 4.21 and 2.86  $\text{t ha}^{-1}$ , respectively (Table 4). Water productivity did not differ among the treatments while the pruning weight was different between T100, T85 and T55, T29. The Ravaz index decreased progressively passing from T100 to T29 (Table 4).

### Wine quality and sensorial analysis

Harvest was performed at the same time for all the treatments according to the “Primitivo” appellation guidelines: when the Total Soluble Solids (TSS) were at least 19.5–20.0 °Brix. Harvest occurred on October 3rd and on September 2nd in 2020 and 2021, respectively. There was no interaction between year and irrigation regime for must quality (Table 5). TSS and malic acid (mal) values were lower in 2020 than in 2021, probably due to the higher cropload recorded in 2020; pH and titratable acidity were quite similar in the two years. T100 and T85 had the lowest TSS ( $\sim 21.5$  °Brix) and T55 the highest (23.1 °Brix); T29 showed an intermediate level of total soluble solids (Table 5). No difference for pH was observed, while the concentration of TA was reduced in T29 in comparison with T100 and T85. A decrease of malic acid concentration was observed in T29 (Table 5).

Chemical analysis on the 2021 wines was performed after 6 months ageing. The lowest ethanol content was observed in T29 and T85 in comparison with the remaining water regimes. Total acidity decreased in the severe water stress treatment, while T55 revealed a malic acid concentration higher than the other treatments as well as the dry reduced extract (DRE). T55 had the highest phenolic compounds

content with T85 recording an anthocyanins content lower than the remaining treatments (Table 6).

The first sensory analysis was conducted after 6 months ageing on wines derived from the four treatments. No difference was found for all the descriptors (Fig. 3a). The notes perceived by at least 5 panellists on a total of 13 showed a prevalence of hints of berries, fresh and sour cherry in spirit, and spicy notes of black pepper, cloves, cinnamon. The ethanol sensation was particularly high in T29 (Fig. 3b). The second sensory analysis was performed after 9 months ageing on the same wines. A difference was found on the descriptor “Color Intensity”, highest in T85 (Fig. 4a). The notes perceived by panellists resulted similar to the previous sensory analysis, with red fruits hints, alcoholic sensation. In addition, notes of black pepper and liquorice emerged clearly after 9 months ageing. During this analysis the ethanol sensation in T29 was reduced in comparison with the previous tasting (Fig. 4b). No differences emerged for descriptors between the first and second tasting both in T29 and in T100. T55 showed a decrease of “sweetness” with the ageing of the wine. The same was observed for T85 and in addition the perception of viscosity increased in the second tasting (Fig. 5).

### Discussion

Water restriction below 45%  $ET_C$  (T55, T29) progressively decreased yield, due to the reduction of cluster weight, probably caused by water shortage, decreased sugar synthesis and accumulation (Chaves et al. 2010; Lovisolo et al. 2010). The lower number of clusters in T29, further decreased productivity (Table 4). Water restriction in T55 and T29 in 2020 affected annual wood starch accumulation (Fig. 2); consequently, the already short Winter pruning performed for the four treatments was made even more severe for the less irrigated treatments, leaving less buds (Table 3). However, T55 compensated this limitation with more clusters per bud, 1.54 (Tables 3 and 4). Severe DI may have modified vine source/sink balance by reducing the gas exchanges

**Table 4** Vegetative-reproductive performance and Water Productivity (WP) recorded on the four irrigation treatments in the two years of trial. Within each experimental factor, (\*) or different letters indicate a statistical difference at  $p=0.05$

Experimental factor	Clusters		Cluster weight (g)	Yield (t ha <sup>-1</sup> )	WP (kg m <sup>-3</sup> )	Pruning weight (g vine <sup>-1</sup> )	Ravaz Index kg grapes/kg pruning weight
	(number vine <sup>-1</sup> )	(number vine <sup>-1</sup> )					
<b>Year</b>							
2020	25.23	*	89.67	8.94	19.29	373.35	6.06
2021	12.06		79.33	3.80	7.77	237.10	4.03
<b>TRT</b>							
T29	12.97	b	56.05	2.86	11.61	228.29	3.18
T55	15.77	ab	67.85	4.21	10.27	277.82	3.85
T85	15.66	ab	112.75	6.83	11.50	361.81	4.88
T100	18.21	a	96.07	7.10	10.56	352.98	4.96

through the closure of stomata (Müller et al. 2011), which was reflected in a reduced allocation of non-structural carbon reserves to perennial organs (Dayer et al. 2013; Herrera et al. 2015; Rustioni et al. 2019). On the other hand, mild drought conditions did not impact significantly the starch accumulation in canes. Water restriction also reduced vegetative activity in T55 and, above all, T29 showing the lowest pruning weight (Chaves et al. 2010; Matthews et al. 1990; Smart and Coombe 1983). WP was maintained stable among the treatments with a parallel reduction of reproductive and vegetative activity in the most stressed treatments (Table 4), suggesting a good plasticity of this variety as other grapevine cultivars (Intrigliolo et al. 2008).

Excluding T29, all the treatments showed a midday stem water potential between  $-0.8$  to  $-1.1$  MPa, considered as mild stress for grapevine.  $\Psi_s$  in T29 was  $\leq -1.20$  MPa, within the range of moderate-severe water stress for this species (Sadras and Shultz 2012). The generally low values of net photosynthesis recorded during the first and the second measurements could be attributable to the high VPD and air temperature encountered in this period. VPD was 3.64 and 4.98 kPa during the first and the second date, respectively, and the vines coped with the high evapotranspirative demand of the environment closing their stomata. In addition, air temperature was excessive (38.6 and 37.4 °C on June 21st and July 27th, respectively) for optimum RuBisCO carboxylative activity, and led to more photorespiration (von Caemmerer 2000; Foyer et al. 2009). Water restriction can affect carbon assimilation (Oliver-Manera et al. 2023) due to stomatal and non-stomatal limitations (Cifre et al. 2005; Flexas et al. 2002; Osmond and Grace 1995; Seaton and Walker 1990). Stomatal closure reduced CO<sub>2</sub> intake lowering carboxylation in T29 and T55 (Table 2). At the same time the reduction of leaf transpiration would have affected leaf thermoregulation increasing leaf temperature and the photoprotective (but dry matter consuming) pathways, i.e. photorespiration and the alternative electron transports (Escalona et al. 1999). At the very low  $g_s$  levels, recorded on June 21st, for example, the rate of electrons exiting PSII ( $J_{PSII}$ ) was proportional to the rate of net photosynthesis, indicating that the plant funnelled more energy to non-photochemical quenching processes instead of moving electrons no longer usable by RuBisCO (Table 2). On August 28th  $g_s$  levels were higher; stomatal conductance and Pn were reduced passing from T85 to T55, however  $J_{PSII}$  remained quite stable as the electrons that could not be used for carboxylation were used by photorespiration and the alternative electron processes. These mechanisms are quite common in C3 plants, including fruit crops such as apple, pear, peach and apricot (Losciale et al. 2008, 2011, 2023), as well as in grapevine where Pn decreased with  $g_s$  while  $J_{PSII}$  remained stable till a threshold of around 0.1 mol



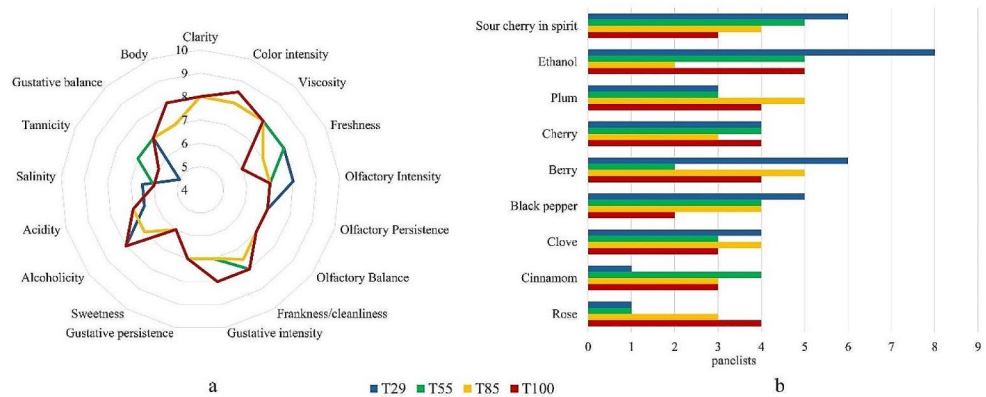
**Table 5** Total soluble solids (TSS), pH, Titratable Acidity (TA) and malic acid (mal), recorded on the must obtained from the four irrigation treatments in the two years of trial. Within each experimental factor, (\*) or different letters indicate a statistical difference at  $p=0.05$

Experimental factor	TSS (°Brix)		pH	TA (g L <sup>-1</sup> )		mal (g L <sup>-1</sup> )	
<b>Year</b>							
2020	19.83	*	3.71	4.75		1.26	*
2021	24.27		3.75	4.59		3.09	
<b>TRT</b>							
T29	22.14	ab	3.74	3.90	b	1.72	b
T55	23.13	a	3.80	4.63	ab	2.40	a
T85	21.71	b	3.75	4.99	a	2.40	a
T100	21.23	b	3.63	5.17	a	2.19	a

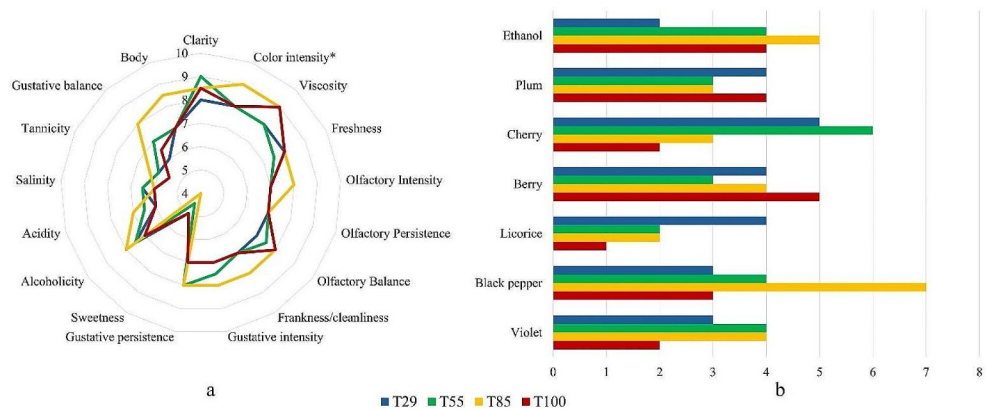
**Table 6** Chemical characteristics of Primitivo wines as a function of four irrigation regimes. E ethanol; TA-titratable acidity as tartaric acid; Mal-malic acid; DRE-dry reduced extract; TP-total polyphenols as gallic acid; A-anthocyanins as malvidin-3-glucoside. For each variable, different letters indicate a statistical difference at  $p=0.05$

YEAR	TREATMENT	E (% v/v)	pH	TA (g L <sup>-1</sup> )	Mal (g L <sup>-1</sup> )	DRE (g L <sup>-1</sup> )	A (mg L <sup>-1</sup> )	TP (mg L <sup>-1</sup> )
2021	T29	13.58 b	3.94	4.94 b	0.19 b	33.10 c	404.77 a	1762.67 b
	T55	14.90 a	3.91	5.91 a	0.67 a	36.56 a	438.67 a	2001.33 a
	T85	13.21 b	3.66	5.93 a	0.25 b	31.79 d	398.71 b	1569.33 d
	T100	14.77 a	3.73	5.73 a	0.27 b	34.07 b	410.33 a	1664.10 c

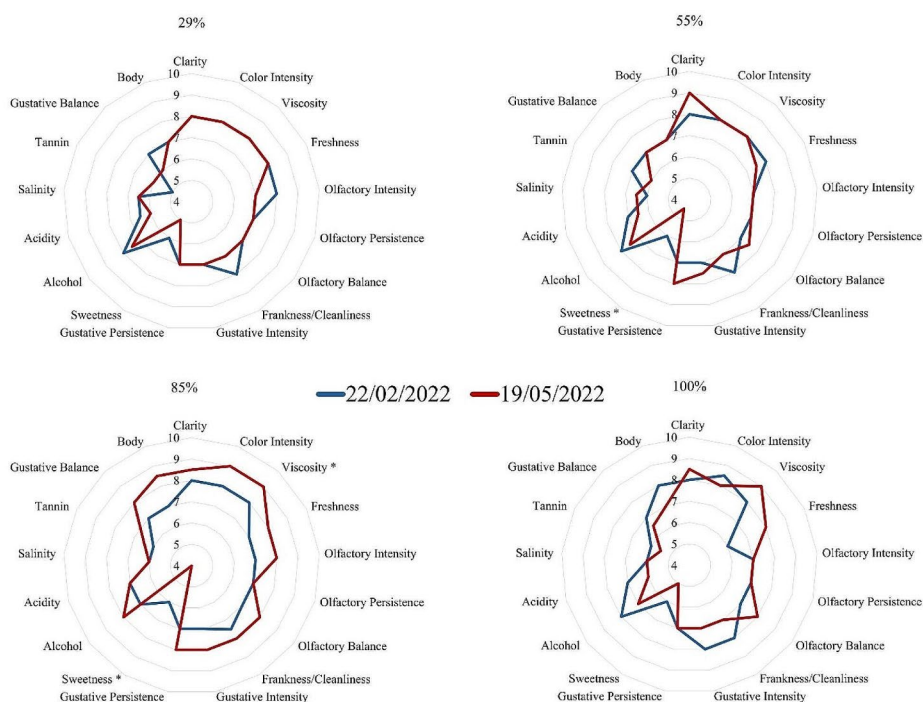
**Fig. 3** Sensory evaluation in wines obtained from full irrigation (T100) and deficit irrigation (T25, T50, T85) of Primitivo grapevines after 6 months ageing (a) and sensorial perceived hints (b). Hints indicated by at least 5 tasters are significantly perceived at  $\alpha < 0.05$



**Fig. 4** Sensory evaluation in wines obtained from full irrigation (T100) and deficit irrigation (T25, T50, T85) of Primitivo grapevines after 9 months ageing (a) and sensorial perceived hints (b). Hints indicated by at least 5 tasters are significantly perceived at  $\alpha < 0.05$



**Fig. 5** Comparison of the sensory profiles of wines obtained from full irrigation (T100) and deficit irrigation (T25, T50, T 85) of Primitivo grapevines and tasted at two different aging stages: after 6 months (22/02/2022) and 9 months (19/05/2022)



$m^{-2}s^{-1}$ . Below this value also the electron transport rate ( $J_{PSII}$ ) dropped with a consequent increase of the photoprotective non-photochemical quenching (Flexas et al. 2002).

After seed hardening the water flux into the berries mainly occurs through the phloem (Düring et al. 1987; Findlay et al. 1987; Greenspan et al. 1996), which in turn depends on the concentration of sugars downloaded by leaves after the carboxylation process. The reduction in photosynthesis in T29 and T55 could have limited the phloem flux, contributing to the reduction of cluster weight (Table 4). Moderate DI (T85) did not affect leaf functionality as well as the productive and vegetative performances of vines, in accordance with previous studies on other grapevine cultivars, which report yield decreases when water restitution was lower than 80%  $ET_C$  (Du et al. 2006; Grimes and Williams 1990; Intrigliolo et al. 2008; Messaoudi and El-Fellah 2004).

A general reduction of the must quality was observed in T29 (Table 5). Sugar content slightly increased while the concentration of TA and malic acid declined. The low vigour of these vines probably exposed berries at a high level of direct light with a consequent increase of their temperature, leading to malic acid degradation (Buttrose et al. 1971; Ruffner et al. 1976). Wine quality did not always follow the same trend as the must, revealing the importance of the wine making process on the quality of the final product (Iorio et al. 2022). In general, a good wine quality, according to the standard of the variety, was achieved for all the treatments (Suriano et al. 2016). High water deficit (T29) decreased alcohol content, titratable acidity, malic acid

and the dry reduced extract. Water supply of about half of evapotranspiration (T55) induced a higher alcohol content, titratable acidity, dry reduced extract, total polyphenols and anthocyanins of wines, associated with low yields per hectare. This water shortage could provide positive qualitative effects in terms of acidity and phenolic compounds (Greven et al. 2009). Sensorial analysis suggested that the paradigm “less quantity more quality” is confirmed within a certain range. After 6 months, wines were quite similar even if the ethanol sensation was higher in T29 (Fig. 3), maybe caused by the low concentration of malic acid, which could not compensate this sensation (Table 6). After 9 months, the colour intensity increased in T85, together with viscosity (Fig. 5). Considering the sensorial analysis, T85 can be considered a favourable compromise between high productivity, wine quality and water saving.

## Conclusions

The Deficit Irrigation strategy, supplying 85% of  $ET_C$  along the season, led to a satisfactory result from a quantitative and qualitative point of view, on grapevine cv. “Primitivo”, while allowing to save water resources. Water shortages higher than 45%  $ET_C$  affected whole vine behaviour in terms of reproductive and vegetative activity, limiting carbon fixation and plant water status. Water supply of 55%  $ET_C$  had a positive effect on wine quality with a considerably negative impact on yield, cluster weight, as well as on the vegetative

activity, lowering the starch reserve accumulation, the quality of wood, the number of shoots and the general vigour of the plant. Severe water stress further reduced the plant vigour and productivity also lowering the number of clusters in addition to their weight. Particularly interesting are the results of T55 where the wine quality, assessed by chemical analysis after 6 months, was increased but to the cost of a severe loss of productivity. Further studies aiming at investigating the effect of an intermediate DI, between 85 and 55 ET<sub>C</sub>, on the general behaviour of Primitivo grapevine, as well as the long-term effects on the quantitative and qualitative performances of the vines are needed.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

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

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