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Article

Effects of Water Stress on Vegetative Growth and 'Merlot' Grapevine Yield in a Semi-Arid Mediterranean Climate

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Abstract: Water stress is considered to be the most influential type of abiotic stress to which plants may be exposed. In grapevines (*Vitis vinifera* L.), it is a common practice to keep plants under water stress at different stages of the season with the aim of reducing yield and improving the composition of the fruit. The objective of this study was to evaluate foliar development and yield of 'Merlot' grapevines grown in the field when they are subjected to different levels of water stress in a semi-arid Mediterranean climate. Four treatments with different levels of water stress were applied during two phenological intervals (flowering-veraison and veraison-maturity) to 128 grapevines for a period of two consecutive years. The levels of water stress were none-light, light-moderate, moderate-intense, and intense-intense for the flowering-veraison and veraison-maturity intervals, respectively. The results revealed that the total leaf area, the exposed leaf area, and the yield all decreased as the degree of water stress increased. The weight of the berry was a decisive factor in determining yield. The least restrictive water regime treatment gave the heaviest berries and bunches and, as a result, the highest yields.

Keywords: abiotic stress; grapevine; leaf area; *Vitis vinifera*; water stress; yield

1. Introduction

The most common types of abiotic stress to which plants may be exposed include drought (water stress), salinity, soil acidification, high temperatures, and high radiation. When plants are grown in field conditions it is difficult to discriminate the individual impacts of each environmental factor as all are interrelated [1]. Of all types of abiotic stress, the most influential is water stress, which is produced by a water deficit in plant tissues. The high importance of water stress is due to the fact that water is an essential chemical substance for the photosynthetic process given that it is the first electron donor. Water ensures a plethora of metabolic functions as it participates in biochemical reactions and transports synthesized materials and products and, through evaporation, protects against warming [2]. When tissue dehydration exceeds a critical level, a series of irreversible changes in the plant cause death. The combination of several abiotic stresses, such as water deficit, intense sunlight, and high temperatures, which are more severe during the summer season, is commonly known as summer stress [3].

The grapevine (*Vitis vinifera* L.) is one of the most abundant and important crops on the planet [4]. It adapts well to cultivation in semi-arid or arid climates and is therefore considered a water deficit-tolerant species [5,6]. This is because the grapevine has a wide and deep root system, an efficient mechanism of stomatal control and the capacity to perform osmotic adjustment [7–9]. In general, it is estimated that grapevines require between 280 to 300 L of water to create 1 kg of dry matter [10]. Lissarrague [11] (1997) estimated that these water needs are slightly higher, around 500 L. If it is considered that a vineyard reaches a total production of fresh matter between 13,000 and 45,000 kg/ha, which represents between 3000 and 9000 kg/ha of total dry matter (leaves: 15–20%, shoots: 15–20%, roots and trunk growth: 5–10%, and bunches: 35–50%), the estimated annual consumption would be between 1750 and 4500 m^3/ha. Taking into account that water needs of grapevines are different depending on the area and the production objectives, for an average condition, we estimate an annual net consumption of 2500 to 3000 m^3/ha. Gómez del Campo [12] also made measurements of water consumption throughout the season in vineyards located in the center of the Iberian Peninsula and calculated that, under water stress conditions, the consumption per plant and day was 0.53 L, while in non-stress conditions the consumption was 1.46 L.

The response of the grapevine to water stress and other environmental factors (summer stress), in order to avoid being severe affects on physiology, is to decrease the rates of transpiration and photosynthesis [13–21]. This response is characteristic of each variety and it also depends on the capacity of the grapevine to adapt to the environment [17,22–26].

Different methodologies have been employed to ascertain the water status of a vineyard. One of the most used approaches is the determination of leaf water potential (Ψ_f). The measurement of this parameter is considered by many authors [27–31] to be the most appropriate technique to evaluate the water status of the grapevine [32]. The determination of predawn leaf water potential (Ψ_{PD}), when the plant and soil are in equilibrium, provides information about the matrix potential of the soil in the root zone and it is a good reflection of the general water status of the plant.

In viticulture it is common practice to use the water limitations of the grapevine to increase the benefits since, under conditions of water restriction and by inhibiting the growth of berries, plants decrease their yields and the quality of production increases [33–38]. When situations of water deficit occur, the grapevine reacts by limiting the growth of the leaf area and yield to favor the absorption of water and minimize losses [39]. The nature and degree of these adjustments depend on the timing, duration, and intensity of the deficit.

Numerous studies have been carried out on the effects of water deficit on vegetative growth [39–43]. Some authors found that the lack of water during the development of the plant leads to a decrease in the length of the branches and thus the leaf area [39,44–49], as well as inducing an advance in the time at which growth stops [50–52]. From an agronomic point of view, the most important factor that is negatively affected by water stress is the yield. Numerous authors have verified that reductions in both the size of the grape berry and production occur with water stress [38,41,53–57]. Yield and vegetative growth are two factors that are closely related; yield depends fundamentally on the photosynthetic activity of the leaves and, as a consequence, on the amount of light intercepted by the canopy of the grapevine [58,59].

One of the most important wine growing areas in the world is La Mancha Designation of Origin (DO) (Central Spain) (Figure 1) and, as a result, it is of great interest to evaluate the possibility of adapting vineyards in this area in the future. The study reported here is important because this DO has a semi-arid climate and, as a consequence of climate change, the vineyards are increasingly suffering from the severity of summer stress. It is therefore necessary to allocate ever increasing resources for irrigation. Given the expected scarcity of water in future climate change scenarios, the need for increased irrigation poses a serious threat to the sustainability of vineyard cultivation in the coming years as the yield and the quality of the harvest, and ultimately the longevity of the grapevines, could be seriously compromised. The aim of this study was to gain a more detailed knowledge of the changes that occur in the vegetative growth and, as a consequence, in the yield of 'Merlot' grapevines when they are subjected to different levels of water stress during two key phenological intervals under semi-arid Mediterranean climate conditions and in La Mancha DO in particular.

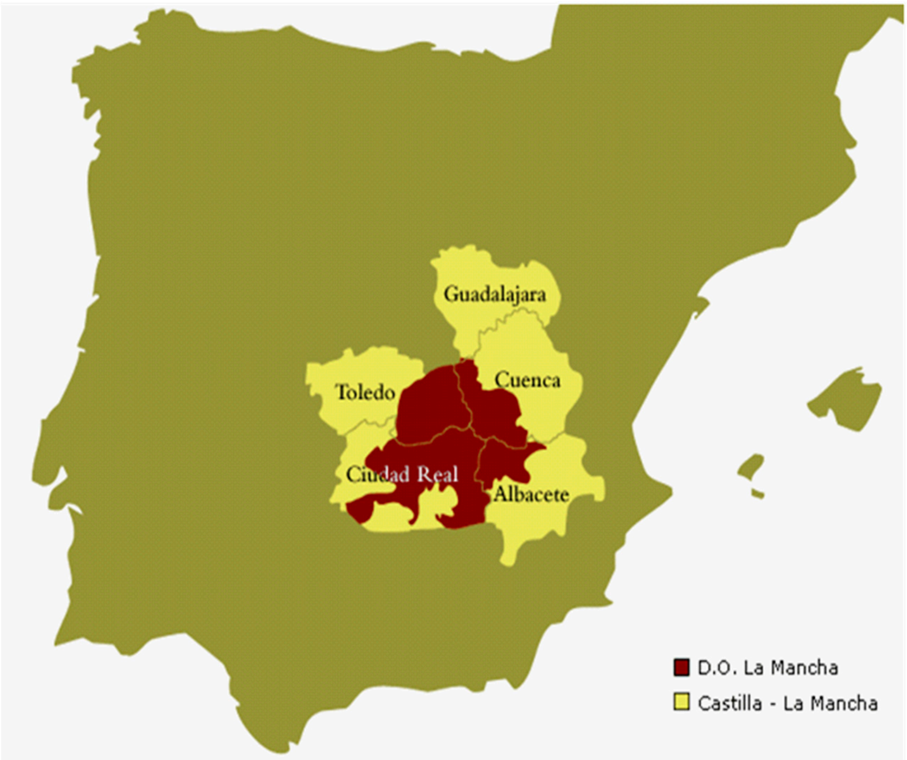

Figure 1. Area in which the study was conducted.

2. Materials and Methods

2.1. Study Area

The trial was carried out over two years in a vineyard located in the municipality of Argamasilla de Alba (La Mancha DO). The geographical coordinates of the area are 39°08′10″ North; 3°04′00″ West and the altitude is 670 m.a.s.l. According to the Winkler index, this area is classified as Region IV and it records scarce rainfall during the year (about 350 mm), with less than 50% occurring in the grapevine growing season. The reference evapotranspiration value (ET_0) is about 1300 mm/year, exceeding 1000 mm during the active vegetation period. Both years of study were exceptionally dry, with only 29.8 and 61.9 mm of rainfall in spring the first and second year, respectively, and ET_0 values of 1087 and 1056 mm the first and second year, respectively, between 1 April and 30 September. The type of soil on which the vineyard sits is one of the most representative in the region of La Mancha: Petric Calcisols [60] and Petrocalcic Calcixerept [61] showing powerful petro-calcic horizons and limited thickness, sometimes less than 40 cm. These soils, which occupy flat or almost flat areas, are the result of paleo-pedogenetic processes often linked to the presence of rubified argillic horizons. The main characteristic of the soils is the presence of a Ckm horizon that imparts the peculiarities of this soil. This horizon, which sometimes reaches a thickness of more than 1 m, is practically impenetrable to the roots of the grapevine, thus conditioning the plant cultivation. The pedoclimatic conditions of the soils correspond to a xeric moisture regime, typical of Mediterranean climates. The most superficial part of the soil is occupied by the anthropized Ap horizon, which can reach between 0 and 30 cms in depth and gives way to horizons of type Bw or Bt, with the strong development of color and structure. Other soil profile data are natural microtopography, flat or almost flat, limestone margose sediments as starting material, well drained, dry, stony class 1, no rocky outcrops, erosion is laminar hydric, salinity is nil, and human influence is due to the cultivation of the crop.

2.2. Plant Material

The study was carried out in a vineyard planted with the variety Merlot (*Vitis vinifera* L.) grafted on the Fercal rootstock (5 year old vines). The main characteristic of this rootstock is its high resistance to chlorosis [62] and its adaptation to calcareous soils. The rootstock resists up to 60% of total limestone, 40% of active limestone, and a chlorinating power index (CPI) of 120. Grapevine chlorosis is caused by a high content of active limestone in the soil, which blocks iron and therefore reduces assimilable iron content affecting chlorophyll. The study was carried out on 128 grapevines. The plants were arranged in rows that were 3 m apart and the spacing between grapevines was 1.2 m. The planting density was 2778 grapevines/ha and this was conducted in a trellis with 3 wires in double Royat cord. Each arm had 3–4 shoots and these were pruned to two buds. The average number of bunches ranged from 24 to 26 per plant. The orientation of the rows was 34° N-NW/160° S-SE.

2.3. Experimental Design

Four experimental treatments were designed. In each case the grapevines were subjected to different levels of water restriction during two phenological intervals (flowering-veraison and veraison-maturity) with their Ψ_{PD} maintained within certain ranges, which were established by Carbonneau [27] and are shown in Table 1. The treatments were applied to a total of 128 grapevines, distributed in two blocks of 64 grapevines each and distributed randomly. Each treatment involved 16 consecutive plants located in the same row. All treatments had the same frame and planting density. In order to adjust the number of shoots (12 per plant) and the number of bunches (24–26 per plant) in all grapevines, shoots were removed from the arms to give an equal number in all grapevines each year before flowering.

Table 1. Ranges of the predawn water potential (Ψ_{PD}) and levels of stress of 'Merlot' grapevines in the different water regime treatments, adapted from Carbonneau [27].

Treatment	Period		Water Status of the Vine
	Flowering-Veraison	Veraison-Maturity	Type of Stress
T1$_{(0–0.2;\ −0.2)}$	0 Mpa ≥ Ψ_{PD} ≥ −0.2 Mpa	Ψ_{PD} ≥ −0.2 Mpa	None—Light
T2$_{(−0.2–0.4;\ −0.4)}$	−0.2 Mpa > Ψ_{PD} ≥ −0.4 Mpa	Ψ_{PD} ≥ −0.4 Mpa	Light—Moderate
T3$_{(−0.4–0.6;\ −0.6)}$	−0.4 Mpa > Ψ_{PD} ≥ −0.6 Mpa	Ψ_{PD} ≥ −0.6 Mpa	Moderate—Intense
T4$_{(−0.6;\ −0.8)}$	−0.6 Mpa > Ψ_{PD}	Ψ_{PD} ≥ −0.8 Mpa	Intense

2.4. Water Regime

In order to keep the plants within the chosen ranges for each treatment, an irrigation calendar was established to provide different amounts of water. The amount of water varied depending on the treatment itself, the weather conditions, and the time of the cycle. The data are shown in Table 2. The start date of the irrigation period was determined each year, and this depended on the climate and the condition of the plants. Water meters were available for each treatment to measure the volume of water applied in each irrigation. The irrigation system used was drip and the lines were suspended from the forming wire. The drippers had a flow rate of 2.2 L/h and they were distributed 0.75 m apart. The watering was nocturnal in order to achieve maximum effectiveness and avoid losses.

2.5. Water Potential and Leaf Development

The water status of the grapevines was evaluated by measuring Ψ_{PD} [63]. Measurements were made between the phenological stages of flowering and maturity with a Scholander pressure chamber (SKPM-1400, Skye Instruments Ltd., Llandrindod Wells, Wales, UK). Measures were taken during 33 days in the first year and 34 days in the second year. These were done on days 1, 3, and 5 of each week. In addition, each year, two series of 3 and 5 consecutive days were carried out with the aim of tracking more accurately the behavior of the Ψ_{PD}. Each daily Ψ_{PD} data assigned to each treatment corresponded to the average of 8 measurements made on 8 leaves, from 8 different grapevines.

Table 2. Irrigation period and volumes of water contributed to the different water regime treatments during the two-year trial.

Treatment	Year	Repetition	Irrigation Period		Volume (mm)	
			Start	Final	Total	Average
T1$_{(0–0.2;–0.2)}$	1	T1-1	17-June	17-August	131.01	132.27
		T1-2			133.54	
	2	T1-1	25-May	11-August	132.69	131.71
		T1-2			130.72	
T2$_{(−0.2–0.4;\ −0.4)}$	1	T2-1	17-June	16-August	114.95	117.04
		T2-2			119.14	
	2	T2-1	5-June	11-August	110.37	110.12
		T2-2			109.86	
T3$_{(−0.4–0.6;\ −0.6)}$	1	T3-1	17-June	12-August	93.82	93.07
		T3-2			92.33	
	2	T3-1	27-June	11-August	66.12	67.36
		T3-2			68.61	
T4$_{(−0.6;\ −0.8)}$	1	T4-1	17-June	12-August	70.95	70.63
		T4-2			70.31	
	2	T4-1	28-June	11-August	55.22	56.46
		T4-2			57.70	

The leaf development of the grapevines was characterized by analysis of the total leaf area (TLA), the external leaf area (ELA), and the leaf index (LI). Eighty grapevines were analyzed each year. The total leaf area was calculated by measuring the leaf area index (LAI), which is an indirect method based on the percentage of light extinction, using a diffuse radiation detector LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE, USA) [64]. This index shows the relationship between the leaf area of the vineyard and the soil surface and it is expressed as m^2 foliar area/m^2 soil area. To make the measurement, first, a reference measurement was made with the sensor placed above the canopy in each grapevine of the series in such a way that the part of the sensor not covered by the vision cap was oriented perpendicularly to the trellis and the sun was positioned frontally. Then, with the sensor at ground level and always maintaining the same orientation, five measurements were made at equidistant points along the trellis section occupied by two grapevines. These points were positioned in a diagonal line, drawn from the trunk base of the first grapevine to the third part of the width of the street, on the vertical of the next grapevine. This procedure was repeated for all grapevines in each series (10 consecutive plants).

The ELA measurements were carried out on the same plants that were used to determine the LAI using an adaptation of the digital image analysis method [65]. A template of 1 cm^2 grids, which was equivalent to 100 cm^2, was applied to the digital images. Once gridded, the cells that were occupied were counted deducting the gaps and obtaining the approximate area. The average width of each grapevine was also measured, which was obtained from the average of 5 measures in each grapevine. It is expressed as m^2 foliar area/m^2 soil area.

The leaf index was obtained by dividing the external leaf area by the total leaf area of the grapevine. This parameter is used to estimate the porosity and overlap of vegetation. It has no units; it is a dimensionless parameter.

2.6. Yield and Its Components

The yield, expressed as kg of grape/m^2 of surface, was calculated at the time of commercial maturity (harvest) as the average of the production of 10 grapevines selected at random for each treatment and for each repetition. Once the production was weighed, the number of bunches on each grapevine was counted. The mean bunch weight was obtained indirectly by dividing the production of each grapevine by the number of bunches and calculating the average. To determine the mean berry weight, a random sample of 100 berries was taken from each of the 10 grapevines and the mean weight was calculated.

2.7. Statistical Analysis

Statistical analysis of the results was performed with version 23.0 of the SPSS statistical package. The possible effect of the treatments on the different variables considered was evaluated by performing an analysis of variance and the means were compared using Tukey's honestly significant difference test, with a probability level of $\alpha \leq 0.05$. The contrast between the means of the years was assessed by a Student *t*-test for independent samples, with a probability level of $p \leq 0.05$.

3. Results

3.1. Effects on Leaf Development

In each year, the TLA generally decreased as the level of water stress experienced by the grapevines in the different treatments increased, with both years showing significant differences (Table 3). In the first year, T2 barely varied with respect to T1, while the decrease of T3 and T4 compared to T1 was 6.2% and 15.4%, respectively. In contrast, during the second year, the differences were greater, with the decreases in T2, T3, and T4 with respect to T1 were 10%, 13.9%, and 23.2%, respectively. Comparing years, the value of TLA decreased in the second year by 13.6% compared to the first year. The trend in the ELA was similar to that of the TLA, with decreases observed in the two years as the level of water stress increased in the different treatments. In contrast, significant differences in ELA were only observed between treatments during the second year and differences were not observed between years, with similar mean values obtained. A comparison of the two years shows that the values in the second year were lower than in the first year in the most restrictive treatments (T3 and T4), although in a much smaller proportion than in the TLA (approximately 5%). In the second year, the average leaf index was slightly higher than the first (13%), and significant differences were observed between the different treatments in the second year. Furthermore, differences between seasons were significant.

Table 3. Means and standard deviations (SD) of leaf development parameters: total leaf area (TLA m^2 foliar area/m^2 soil area), external leaf area (ELA m^2 foliar area/m^2 soil area), and leaf index (LI) for each of the four water regime treatments in the two years.

Year	Treatment	Samples	TLA	ELA	LI
	(Ψ_{PD})	**(*n*)**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
1	**T1$_{(0–0.2; −0.2)}$**	20	1.62ab ^{z} ± 0.08	0.83b ± 0.02	0.56 ± 0.07
	T2$_{(−0.2–0.4; −0.4)}$	20	1.64b ± 0.18	0.83ab ± 0.00	0.51 ± 0.06
	T3$_{(−0.4–0.6; −0.6)}$	20	1.52ab ± 0.18	0.81ab ± 0.06	0.52 ± 0.06
	T4$_{(−0.6; −0.8)}$	20	1.37a ± 0.08	0.75a ± 0.03	0.55 ± 0.08
Sig ^{y}			**	*	ns
2	**T1$_{(0–0.2; −0.2)}$**	20	1.51c ± 0.03	0.85b ± 0.03	0.57a ± 0.05
	T2$_{(−0.2–0.4; −0.4)}$	20	1.36b ± 0.17	0.86b ± 0.02	0.64b ± 0.10
	T3$_{(−0.4–0.6; −0.6)}$	20	1.30b ± 0.08	0.76a ± 0.04	0.60ab ± 0.06
	T4$_{(−0.6; −0.8)}$	20	1.16a ± 0.00	0.72a ± 0.04	0.62ab ± 0.04
Sig			***	***	*
	Year 1	80	1.54b ± 0.12	0.81 ± 0.04	0.54a ± 0.02
	Year 2	80	1.33a ± 0.15	0.80 ± 0.07	0.61b ± 0.03
Sig			***	ns	***

^{z} The letters indicate statistically significant differences between treatments, according to the Tukey's honestly significant difference test (α = 0.05). The contrast between the means of the years was performed by the Student *t*-test for independent samples (α = 0.05). ^{y} Significance (Sig): *, **, ***, ns: significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

3.2. Effects on Yield

The mean values of the performance components were lower in the second year than the first year (Table 4). The average weight of the berry decreased by 28.3% with respect to the first year for T3 and by 29.2% for T4. These decreases had a detrimental effect on production, which decreased by 42.2% and 37.1% for these treatments, respectively. In terms of treatments, in the two years there were marked differences in yield and bunch weight, but for the weight of the berry, the only differences were observed in the second year. During the first year, the decreases in the weight of a berry in T2, T3, and T4 compared to T1 were 4.6%, 9.2%, and 12%, respectively, while in the second year these decreases were considerably greater (7.8%, 21.1%, and 24.4% for T2, T3, and T4, respectively). Between years, the weight of the berry decreased by 23.5% in year 2 compared to year 1. In terms of yield, in the first year, the decreases in T2, T3, and T4 compared to T1 were 3.2%, 13.5%, and 29.4%, respectively, while in the second year these decreases were much greater (3.3%, 30%, and 37.8% for T2, T3, and T4, respectively). Between years, yield decreased by 33.9% in year 2 compared to year 1. All of the yield parameters, except for the number of bunches, decreased as the level of water stress increased with the different treatments in both years of the trial.

Table 4. Means and standard deviations (SD) of the yield components: yield (kg/m^2), number of bunches per grapevine (bunches), bunch weight (g), and berry weight (g) for each of the four water regime treatments in the two years.

Year	Treatment (Ψ_{PD})	Samples (*n*)	Yield Mean ± SD	Bunches Mean ± SD	Bunch Weight Mean ± SD	Berry Weight [z] Mean ± SD
1	**T1**$_{(0–0.2; -0.2)}$	20	1.26b [y] ± 0.03	25.63 ± 4.72	177.01b ± 28.71	1.09 ± 0.02
	T2$_{(-0.2–0.4; -0.4)}$	20	1.22b ± 0.01	25.05 ± 4.49	176.21b ± 25.23	1.04 ± 0.07
	T3$_{(-0.4–0.6; -0.6)}$	20	1.09ab ± 0.07	25.60 ± 3.97	155.25ab ± 23.61	0.99 ± 0.08
	T4$_{(-0.6; -0.8)}$	20	0.89a ± 0.03	24.90 ± 4.16	131.79a ± 20.11	0.96 ± 0.07
Sig [x]			***	ns	*	ns
2	**T1**$_{(0–0.2; -0.2)}$	20	0.90b ± 0.02	24.10 ± 1.54	136.07b ± 18.68	0.90d ± 0.03
	T2$_{(-0.2–0.4; -0.4)}$	20	0.87b ± 0.11	24.80 ± 2.36	126.59b ± 15.92	0.83c ± 0.04
	T3$_{(-0.4–0.6; -0.6)}$	20	0.63ab ± 0.02	24.65 ± 1.64	93.08a ± 12.99	0.71b ± 0.03
	T4$_{(-0.6; -0.8)}$	20	0.56a ± 0.14	24.65 ± 3.47	83.00a ± 14.65	0.68a ± 0.03
Sig			*	ns	**	***
	Year 1	80	1.12 ± 0.17	25.30 ± 0.37	160.07 ± 21.37	1.02 ± 0.06
	Year 2	80	0.74 ± 0.17	24.55 ± 0.31	109.69 ± 25.62	0.78 ± 0.10
Sig			***	ns	***	***

[z] Number of samples (*n*) for berry weight is $n = 8$ in year 1 and $n = 16$ in year 2. [y] The letters indicate statistically significant differences between treatments, according to the Tukey's honestly significant difference test ($\alpha = 0.05$). The contrast between the means of the years was performed by the Student *t*-test for independent samples ($\alpha = 0.05$). [x] Significance (Sig): *, **, ***, ns: significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

4. Discussion

4.1. Effects on Leaf Development

When plants perceive signals of abiotic stress, they express complex defense responses which can be reversible or irreversible depending on the duration and intensity of stress and the organ or tissue involved [3]. One of the first responses of plants to water stress is the reduction of vegetative growth, which allows grapevines to restrict water losses [66]. The measurements of the different leaf development parameters were carried out in part during the phenological stage of veraison as this is the moment in which the maximum leaf area is reached. In trials carried out with the variety 'Tempranillo', Cuevas [42] found that the maximum values of both TLA and ELA depended on the year but were always reached around veraison. From that time until the harvest date there was a loss of leaves from the main branches and under dry conditions this represented up to 29% of the leaf area of the branch. Sommer and Clingeleffer [67] also found that the greatest foliar surface was obtained around the time of veraison.

In this study both the planting density and the number of branches per grapevine were the same in all treatments. Therefore, the differences between the plants in terms of vegetative growth can be attributed to the different levels of water stress. The decrease in the total leaf area, from 1.62 to 1.37 m^2 foliar area/m^2 soil area in the first year and from 1.51 to 1.16 m^2 foliar area/m^2 soil area the second year, as the degree of water stress increased, was produced by the limitation that the water stress caused on the elongation of the branches [47], thus giving rise to shoots with a lower foliar surface (fewer leaves and smaller area). These results are consistent with those obtained by other authors [42,56,68].

The effects of water stress on leaf area differ depending on the period in which the stress occurs, and these have been studied by numerous authors. Some authors [43,69] found that grapevines that were supplied with water until the time of veraison showed greater leaf development than those where the water supply was suppressed during this period. Sommer and Clingeleffer [67] arrived at similar conclusions on comparing the leaf development of grapevines subjected to different water availability before and after veraison. These authors argued that differences in leaf area were significant as variations in water supply took place before veraison. However, any leaf area differences produced after full growth were practically negligible. Buesa et al. [56] carried out a similar study over three years on the variety 'Moscatel of Alejandría', and they also observed that the grapevines subjected to water deficit before veraison had a smaller leaf area than those in which the deficit occurred after veraison, although significant differences were only found during the first year. In contrast, well-irrigated plants, in addition to a larger leaf surface, had a more compact foliar mass.

More recently, other authors, such as Munitz et al. [49], concluded that the amount of water supplied to grapevines during the season has a positive effect on vegetative growth, when the stage from flowering to closure of the bunch shows the fastest vegetative growth, which coincides with the period of maximum activity of the cambium. In contrast to these results, in a trial conducted in Chile by Acevedo-Opazo et al. [70] in which plants of the variety 'Cabernet Sauvignon' were kept at different levels of water stress from fruit set to maturity, significant treatment differences were not found in most of the vegetative growth parameters (branch length, number of nodes, internode length, and pruning wood weight) in any year, although there was a tendency for all of the aforementioned parameters to decrease with increasing levels of stress.

The ELA provides an estimate of the proportion of the total leaf area of the grapevine that receives direct solar radiation. Smart [71] considered that a high proportion of the total CO_2 that is assimilated by grapevines is due to the amount of sunlight that directly reaches the leaves. Therefore, the productivity of a grapevine will mainly depend on the number of external (exposed) leaves and their area. Escalona et al. [13] showed that the leaves located on the inside of the canopy represent 35–50% of the total leaf area surface. However, their contribution to the total net carbon gain is less than 5% of the total grapevine. Other authors [72] found that in cases of severe water deficits, the leaves of the grapevines closed their stomata and photosynthesis was reduced to a minimum, thus decreasing the photosynthetically-active foliar area and, consequently, reducing the impact of the solar radiation intercepted by the plants. These alterations caused a decrease in the production of photoassimilate and, as a result, a reduction in the quantity and quality of the harvest. Fernández [43] also found similar effects in stressed grapevines and cited the lack of availability of water before veraison as a trigger for this response.

Comparison of the ELA data shows that the values in the second year were lower with respect to the first year only in the most restrictive treatments (T3 and T4). These values decreased from 0.81 to 0.76 m^2 foliar area/m^2 soil area and from 0.75 to 0.72 m^2 foliar area/m^2 soil area, for T3 and T4, respectively, but to a much lesser extent than the TLA (approximately 5%). The smaller differences are probably due to the fact that the external leaf area represented about half of the value of the total leaf area (52.59% to 60.15% on average in the first and second years, respectively) and therefore the differences with respect to the LAI were reduced.

The higher average leaf index values obtained in the second year were mainly due to the fact that, as a consequence of the higher levels of water stress maintained during the season, the TLA values in the first year were higher, since ELA values were practically constant during the two years (from 0.51 to 0.64). These results are consistent with those obtained by other authors, e.g., Rubio [68] who studied the 'Tempranillo' variety and compared treatments with and without irrigation. There were higher values for both TLA and ELA in irrigated grapevines, although the highest LI values (0.4) were obtained for grapevines without irrigation, with statistically significant differences occurring in three of the four years of study. Likewise, Fernandez [43] worked with the 'Cabernet Sauvignon', which were subjected to different levels of water deficits during the pre-veraison and post-veraison stages. In that study, the maximum LI values (0.8) were also obtained with the treatment involving continuous severe deficit, which was the most stressed case of all.

According to our data, it is evident that water stress produced a decrease in the density of vegetation, and this favors a higher percentage of leaves being exposed, which in turn results in fully productive plants. In addition, the leaf index values in all treatments were between 0.50 and 0.65, i.e., within the range considered as acceptable [73], and this indicates that the plants were productive.

4.2. Effects on Yield

Some authors have indicated that, in the grapevine, water availability seems to have a greater effect at the vegetative level than on productivity [40,74], but this factor is considered to be one of the most important for the proper development of the plant as an excess of water stress results in a decrease in yield [55,75]. Other researchers [33,48,56,57,76–78] have established relationships between the level of stress, the weight of the berry, and the production of the grapevines.

In addition to the water deficit stress, the high temperatures that occurred during the seasons also may have influenced [74,79] our study. The lowest yields obtained, in the second year, were likely due to the fact that the volumes of water applied were lower (0.4% in T1, 5.9% in T2, 27.6% in T3, and 20.1% in T4) and, as a result, water stress levels increased slightly. The justification can be found by comparing the meteorological data from both years (Figure 2). The mean of the monthly mean temperatures (Tm) and maximum daily temperatures (Tmax) in the period that elapsed from the phenological stage of flowering (May) to maturity (August) were lower in the second year than the first year (0.3 and 0.4 °C, respectively) with equal minimum daily temperatures (Tmin). Regarding the monthly mean of daily solar radiation (RS), the mean values during the same period were equal (27.2 MJ/m^2), while the monthly mean of accumulated rainfall (R) was 19.8 mm higher the second year. These differences increased as the intensity of the stress increased and were greater for the more restrictive treatments. Therefore, yield showed a close relationship with the level of water stress experienced by the grapevines during the year, which in turn depended on the water provided by the irrigation. These results are in line with those obtained by other authors [35,80], who concluded that the effect of the water regime on the production and weight of the berry differed depends on the year. Martinez et al. [78] also observed significant differences in the production parameters of the 'Albariño' variety due to yearly differences. This effect has also been observed in other varieties such as 'Bobal' [33], 'Cabernet Sauvignon' [76], and 'Merlot' [77].

The average number of bunches in the two years remained practically constant for all treatments (25.30 and 24.45 for the first and second years, respectively). This finding is due to the fact that, in both years, the number of branches in the grapevines (12) was adjusted in order to make grapevines comparable. These results show that the decrease in yield (from 1.26 to 0.89 kg/m^2 and from 0.90 to 0.56 kg/m^2 for the first and second year, respectively) was due to the fact that, as the level of water stress increased, the weight of the berry decreased (from 1.09 to 0.96 g and from 0.90 to 0.68 g for the first and second years, respectively), and thus the weight of the bunch decreased (from 177.01 to 131.79 g and from 136.07 to 83 g for the first and second years, respectively). Our observations are consistent with those reported [33]. These authors also adjusted the number of buds by pruning and they concluded that the increase in productivity on decreasing the level of water stress was mainly due to the increase in the weight of the berry, since both the number of bunches and the number of berries per bunch remained similar in all treatments. Therefore, the fertility of the buds and the setting rate were not affected by the level of water stress [81]. In contrast, other authors, such as Shellie [75] with the variety 'Merlot', Girona et al. [82] with 'Pinot Noir', Santesteban et al. [83] with 'Tempranillo', and Romero et al. [36] with 'Monastrell', observed that water stress, in addition to decreasing the mean weight of a berry and the weight of a bunch, also affected the number of bunches per grapevine.

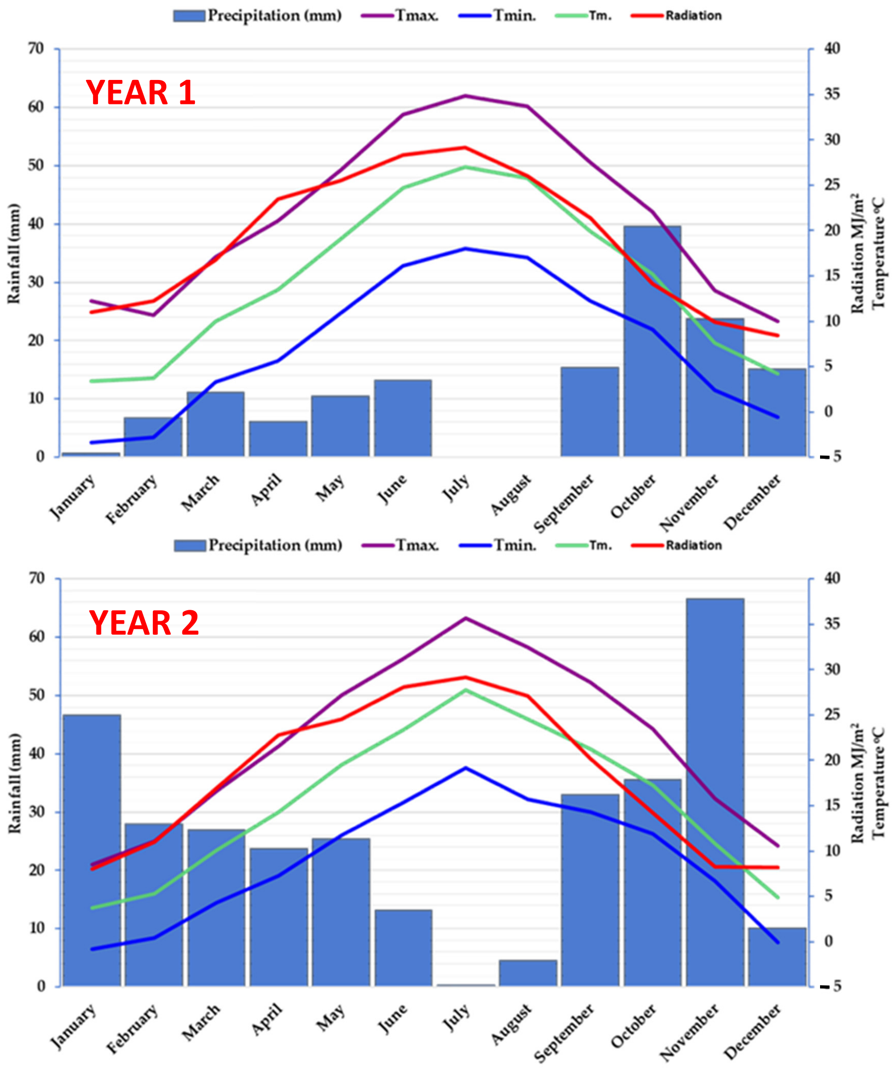

Figure 2. Meteorological data of the two years (Tmax: monthly mean of maximum daily temperatures, Tmin: monthly mean of minimum daily temperatures, Tm: monthly mean temperature).

The impact of water stress on the yield of the grapevines depends, in addition to the intensity, on when the stress occurs. Buesa et al. [56] studied the variety 'Moscatel de Alejandría' in the eastern part of Spain and observed that when water restriction took place before veraison, the yield decreased by 10% more than when it occurred after veraison. These authors attributed this finding to the fact that the early water deficit caused a greater reduction in the growth of the berry than when it was late. This led to a decrease in the productivity of the branches and the weight of the bunches because the berries were smaller. Similar results were previously obtained by Ferreyra et al. [84], for the 'Chardonnay' variety. In our study, a positive relationship between berry weight and yield was observed (Figure 3).

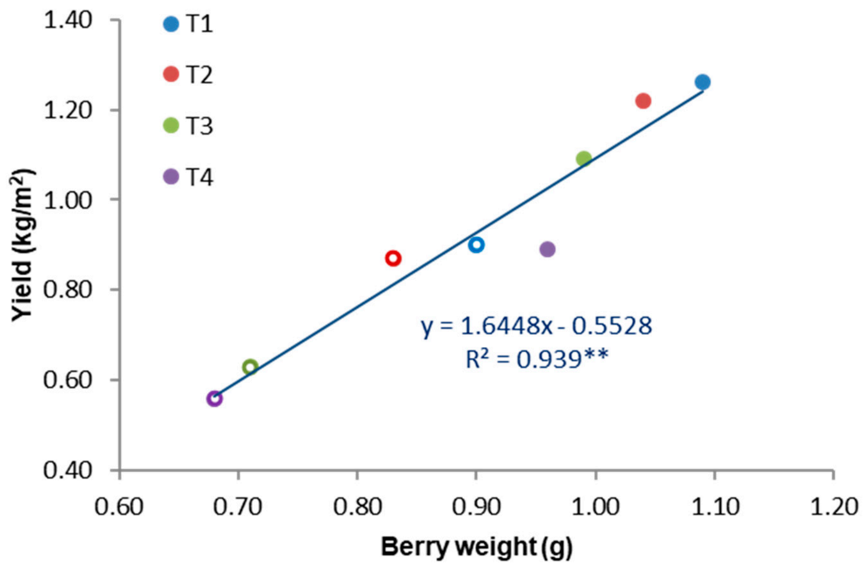

Figure 3. Ratio of berry weight (g) to yield (kg/m^2), for each treatment. The solid symbols correspond to the data of year 1 and the outlined symbols year 2. Each spot corresponds to the average of two repetitions for each treatment. Significance of the coefficient of determination R^2 by correlation analysis: **, significant at $p \leq 0.01$.

Therefore, it can be stated that while water stress restricted production and all the components of yield, the weight of the grape was the most decisive. These results are consistent with those obtained by other authors [35,49,85–91], who worked with grapevines with different levels of irrigation and therefore different levels of water stress. They concluded that the increase in yields with the availability of water was mainly due to differences in the weight of the berry. In contrast, Cuevas [39] did not find significant differences in the weight of the berry between treatments with different levels of water stress but differences were observed in the number of branches and number of bunches per branch.

Other authors [92] carried out a greenhouse trial in which well-irrigated grapevines were compared with others subjected to water deficit and they found, as we did, that plants with stress had a lower leaf area and smaller berry size. Ferlito et al. [93] carried out a study in Sicily with four varieties and concluded that the reduction of the foliar surface in the grapevines resulted in production of bunches with a lower weight and smaller berries.

The present study was carried out under field conditions and, as a result, the amount of water added during the two years differed depending on the environmental conditions that prevailed in each year, and this, in turn, conditioned the degree of water stress during the different stages of the season. Future research should be aimed at monitoring the effects of water stress under controlled temperature, humidity, and radiation conditions in order to be able to repeat the test over several years and to obtain more reliable results. These tests should also be repeated with other grapevine varieties to ascertain whether the results are similar.

5. Conclusions

Water deficit stress, in addition to acting as a limiting factor on leaf development, had a marked influence on the mean size of a berry and consequently on yield. The vegetative growth of the grapevines was greatly affected by water stress. The stress reduced the density of the vegetation, which favored a greater percentage of the leaves being exposed and resulted in the plants being fully productive. The yield decreased as the stress level increased. The component that had most influence on the yield was berry weight.

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References

1. Tester, M.; Bacic, A. Abiotic Stress Tolerance in Grasses. From Model Plants to Crop Plants. *Plant Physiol.* **2005**, *137*, 791–793. [CrossRef] [PubMed]
2. Huglin, P.; Schneider, C. Cap. 6.2. Relations entre les facteurs du milieu naturel et la vigne. In *Biologie et Écologie de la Vigne*, 2nd ed.; Lavoisier-Technique & Documentation: París, France, 1998; pp. 283–286.
3. Cramer, G.R.; Urano, K.; Delrot, S.; Pezzotti, M.; Shinozaki, K. Effects of abiotic stress on plants: A systems biology perspective. *BMC Plant Biol.* **2011**, *11*, 163. [CrossRef]
4. Bernardo, S.; Dinis, L.-T.; Machado, N.; Moutinho-Pereira, J.M. Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. A review. *Agron. Sustain. Dev.* **2018**, *38*, 66. [CrossRef]
5. Chaves, M.; Zarrouk, O.; Francisco, R.; Costa, J.M.; Santos, T.; Regalado, A.P.; Rodrigues, M.L.; Lopes, C.M. Grapevine under deficit irrigation: Hints from physiological and molecular data. *Ann. Bot.* **2010**, *105*, 661–676. [CrossRef]
6. Zarrouk, O.; Costa, J.; Francisco, R.D.B.; Lopes, C.; Chaves, M.M. Drought and water management in Mediterranean vineyards. In *Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective*; GeróS, H., Chaves, M.M., Medrano, H., Delrot, S., Eds.; Wiley: Hoboken, NJ, USA, 2016; pp. 38–67.
7. Rodrigues, M.L.; Chaves, M.M.; Wendler, R.; David, M.M.; Quick, W.P.; Leegood, R.C.; Stitt, M.; Pereira, J.S. Osmotic Adjustment in Water Stressed Grapevine Leaves in Relation to Carbon Assimilation. *Funct. Plant Biol.* **1993**, *20*, 309–321. [CrossRef]
8. Chaves, M.; Santos, T.; Souza, C.; Ortuño, M.; Rodrigues, M.; Lopes, C.; Maroco, J.; Pereira, J. Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Ann. Appl. Biol.* **2007**, *150*, 237–252. [CrossRef]
9. Blum, A. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* **2009**, *112*, 119–123. [CrossRef]
10. Hidalgo, L. *Tratado de Viticultura General*, 2nd ed.; Mundi-Prensa, S.A., Ed.; Artes Gráficas Cuesta: Madrid, Spain, 1999; p. 743.
11. Lissarrague, J.R. Necesidades de agua de la vid. Consecuencias del estrés hídrico y del riego en el viñedo. *Agricultura* **1997**, *785*, 943–950.
12. Gómez del Campo, M.V. Mecanismos de Adaptación a la Sequía en la Vid. Evaluación del Consumo de Agua, Crecimiento y Desarrollo Vegetativo, Productividad y Eficiencia en el Uso del Agua de Cuatro Genotipos de Vid (*Vitis vinifera* L.). Ph.D. Thesis, Universidad Politécnica de Madrid, Madrid, Spain, 1998.
13. Escalona, J.M.; Flexas, J.; Medrano, H. Stomatal y non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Aust. J. Plant Physiol.* **1999**, *26*, 421–433. [CrossRef]
14. FlexasA, J.; Bota, J.; Escalona, J.M.; Sampol, B.; Medrano, H. Effects of drought on photosynthesis in grapevines under field conditions: An evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol.* **2002**, *29*, 461–471. [CrossRef]
15. Cuevas, E.; Baeza, P.; Lissarrague, J.R. Variation in stomatal behaviour and gas exchange between mid-morning and mid-afternoon of north–south oriented grapevines. (*Vitis vinifera* L. cv. Tempranillo) at different levels of soil water availability. *Sci. Hortic.* **2006**, *108*, 173–180. [CrossRef]
16. Moutinho-Pereira, J.; Magalhaes, N.; Gonçalves, B.; Bacelar, E.A.; Brito, M.E.B.; Correia, C. Gas exchange and water relations of three *Vitis vinifera* L. cultivars growing under Mediterranean climate. *Photosynthetica* **2007**, *45*, 202–207. [CrossRef]

17. Martínez, J.; Chacón, J.L. Variability of stomatal conductance with leaf water potential in 5 grapevine cultivars: Consequences for water efficiency. In Proceedings of the XVI International GiESCO Symposium, Davis, CA, USA, 14–16 July 2009; pp. 333–336.
18. Pinheiro, C.; Chaves, M. Photosynthesis and drought: Can we make metabolic connections from available data? *J. Exp. Bot.* **2010**, *62*, 869–882. [CrossRef] [PubMed]
19. Dinis, L.-T.; Ferreira, H.; Pinto, G.; Bernardo, S.; Correia, C.M.; Moutinho-Pereira, J.M. Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. *Photosynthetica* **2016**, *54*, 47–55. [CrossRef]
20. Conesa, M.R.; De La Rosa, J.; Domingo, R.; Banon, S.; Perez-Pastor, A. Changes induced by water stress on water relations, stomatal behaviour and morphology of table grapes (cv. Crimson Seedless) grown in pots. *Sci. Hortic.* **2016**, *202*, 9–16. [CrossRef]
21. Van Leeuwen, C.; Destrac-Irvine, A. Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One* **2017**, *51*, 147–154. [CrossRef]
22. Bota, B.J.; Flexas, J.; Medrano, H. Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Ann. Appl. Biol.* **2001**, *138*, 353–361. [CrossRef]
23. Ojeda, H.; Lebon, E.; Deis, L.; Vita, F.; Carbonneau, A. Régulation stomatique de cépages méditerranéens en situation de déficit hydrique dans le sud de La France. Resultats preliminaires. In Proceedings of the XIV International Symposium of GESCO, Geisenheim, Germany, 23–27 August 2005; pp. 581–587.
24. Deluc, L.G.; Quilici, D.R.; Decendit, A.; Grimplet, J.; Wheatley, M.D.; Schlauch, K.A.; Mérillon, J.M.; Cushman, J.C.; Cramer, G.R. Water deficit alters differentially metabolic pathways affecting important flavour and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genom.* **2009**, *10*, 212. [CrossRef]
25. Tomás, M.; Medrano, H.; Brugnoli, E.; Escalona, J.; Martorell, S.; Pou, A.; Ribas-Carbó, M.; Flexas, J. Variability of mesophyll conductance in grapevine cultivars under water stress conditions in relation to leaf anatomy and water use efficiency. *Aust. J. Grape Wine Res.* **2014**, *20*, 272–280. [CrossRef]
26. Martorell, S.; Díaz-Espejo, A.; Tomàs, M.; Pou, A.; El Aou-Ouad, H.; Escalona, J.M.; Vadell, J.; Ribas-Carbó, M.; Flexas, J.; Medrano, H. Differences in water-use-efficiency between two Vitis vinifera cultivars (Grenache and Tempranillo) explained by the combined response of stomata to hydraulic and chemical signals during water stress. *Agric. Water Manag.* **2015**, *156*, 1–9. [CrossRef]
27. Carbonneau, A. Aspects qualitatifs. In *Traité D'irrigation*; Tiercelin, J.R., Ed.; Tec & Doc: Paris, France, 1998; pp. 257–276.
28. Choné, X.; Van Leeuwen, C.; Dubordieu, D.; Gaudillère, J.P. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* **2001**, *87*, 477–483. [CrossRef]
29. Williams, L.; Araujo, F. Correlations among Predawn Leaf, Midday Leaf, and Midday Stem Water Potential and their Correlations with other Measures of Soil and Plant Water Status in Vitis vinifera. *J. Am. Soc. Hortic. Sci.* **2002**, *127*, 448–454. [CrossRef]
30. Deloire, A.; Carbonneau, A.; Wang, Z.; Ojeda, H. Vine and water: A short review. *OENO One* **2004**, *38*, 1–13. [CrossRef]
31. Ojeda, H. Irrigation qualitative de précisión de la vigne. *Progrès Agric. Vitic.* **2007**, *124*, 133–141.
32. Medrano, H.; Escalona, J.M.; Flexas, J. Indicadores integradores del estado hídrico de la planta. In *Fundamentos, Aplicación y Consecuencias del Riego en la Vid*; Editorial Agrícola Española; Baeza, P., Lisarrague, J.R., de Miguel, P.S., Eds.; AMV Ediciones: Madrid, Spain, 2007; 264p.
33. Salón, J.L.; Chirivella, C.; Castel, J.R. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: Water relations, yield and wine quality. *Am. J. Enol. Vitic.* **2005**, *56*, 1–8.
34. Reynolds, A.G.; Lowrey, V.D.; Tomec, L.; Hakimi, J.; de Savigny, C. Influence of irrigation on vine performance, fruit composition and wine quality of Chardonnay in a cool, humid climate. *Am. J. Enol. Vitic.* **2007**, *58*, 217–228.
35. Intrigliolo, D.; Castel, J.R. Response of grapevine cv. "Tempranillo" to timing and amount of irrigation: Water relations, vine growth, yield and berry and wine composition. *Irrig. Sci.* **2010**, *28*, 113–125. [CrossRef]
36. Romero, P.; Gil-Muñoz, R.; Amor, F.M.; Valdés, E.; Fernández, J.I.; Martínez-Cutillas, A. Regulated deficit irrigation based upon optimun water status improves phenolic composition in Monastrell grapes and wines. *Agric. Water Manag.* **2013**, *121*, 85–101. [CrossRef]
37. Herrera, J.C.; Bucchetti, B.; Sabbatini, P.; Comuzzo, P.; Zulini, L.; Vecchione, A.; Peterlunger, E.; Castellarin, S.D. Effect of water deficit and severe shoot trimming on the composition ofVitis vinifera L. Merlot grapes and wines. *Aust. J. Grape Wine Res.* **2015**, *21*, 254–265. [CrossRef]

38. Casassa, L.F.; Larsen, R.C.; Harbertson, J.F. Effects of Vineyard and Winemaking Practices Impacting Berry Size on Evolution of Phenolics during Winemaking. *Am. J. Enol. Vitic.* **2016**, *67*, 257–268. [CrossRef]
39. Keller, M.; Romero, P.; Gohil, H.; Smithyman, R.P.; Riley, W.R.; Casassa, L.F.; Harbertson, J.F. Deficit Irrigation Alters Grapevine Growth, Physiology, and Fruit Microclimate. *Am. J. Enol. Vitic.* **2016**, *67*, 426–435. [CrossRef]
40. Williams, L.E.; Dokoozlian, N.K.; Wample, R. Grape. In *Handbook of Environmental Physiology of Fruit Crops. Vol 1. Temperate Crops*; Schaffer, B., Anderson, P.C., Eds.; CRC Press: Boca Ratón, FL, USA, 1994; pp. 85–133.
41. Ginestar, C.; Eastham, J.; Gray, S.; Lland, P. Use of sap flow sensors to schedule vineyard irrigation. I. Effects of post-veraison water deficit on water relations, vine growth and yield of Shiraz grapevine. *Am. J. Enol. Vitic.* **1998**, *49*, 413–420.
42. Cuevas, E. Estudio de Mecanismos de Adaptación Ecofísiológica de la Vid (*Vitis vinifera* L., cv. Tempranillo) al Déficit Hídrico. Evaluación del Consumo de Agua y de las Respuestas Agronómicas en Diferentes Regímenes Hídricos. Ph.D. Thesis, Departamento de Producción Vegetal: Fitotecnia, Universidad Politécnica de Madrid, Madrid, Spain, 2001; 219p.
43. Fernández, O. Análisis de las Diferencias en el Crecimiento Vegetativo, en los Componentes del Rendimiento y en la Composición de las Uvas y Vinos de cv. Cabernet Sauvignon (*Vitis vinífera* L.) Producidos por el Déficit Hídrico Durante Pre-Envero y Post-Envero. Ph.D. Thesis, Departamento de Producción Vegetal: Fitotecnia, Universidad Politécnica de Madrid, Madrid, Spain, 2013; 381p.
44. García-Escudero, E.; López, R.; Santamaría, P.; Zaballa, O. Control del rendimiento en viñedos conducidos en régimen de riego localizado. *Vitic. Enol. Prof.* **2000**, *69*, 12–24.
45. Hardie, W.D.; Martin, S.R. Shoot growth on de-fruited grapevines: A physiological indicator for irrigation scheduling. *Aust. J. Grape Wine Res.* **2000**, *6*, 52–58. [CrossRef]
46. Paranychianakis, N.V.; Aggelides, S.; Angelakis, A.N. Influence of rootstock, irrigation level and recycled water on growth and yield of Soultanina grapevines. *Agric. Water Manag.* **2004**, *69*, 13–27. [CrossRef]
47. Shellie, K.C. Vine and berry response of Merlot (*Vitis vinifera* L.) to differential water stress. *Am. J. Enol. Vitic.* **2006**, *57*, 514–518.
48. Basile, B.; Marsal, J.; Mata, M.; Vallverdú, X.; Bellvert, J.; Girona, J. Phenological Sensitivity of Cabernet Sauvignon to Water Stress: Vine Physiology and Berry Composition. *Am. J. Enol. Vitic.* **2011**, *62*, 452–461. [CrossRef]
49. Munitz, S.; Netzer, Y.; Schwartz, A. Sustained and regulated deficit irrigation of field-grown Merlot grapevines. *Aust. J. Grape Wine Res.* **2016**, *23*, 87–94. [CrossRef]
50. Matthews, M.A.; Anderson, M.M.; Schultz, H.R. Phenolic and growth responses to early and late season water deficits in Cabernet Franc. *Vitis* **1987**, *26*, 147–160.
51. Poni, S.; Lakso, A.N.; Turner, J.R.; Melious, R.E. The effects of pre- and post-veraison water stress on growth and physiology of potted Pinot Noir grapevines at varying crop levels. *Vitis* **1994**, *32*, 207–214.
52. Kliewer, W.M.; Wolpert, J.A.; Benz, M. Trellis and vine spacing effects on growth, canopy microclimate, yield and fruit composition of cabernet sauvignon. *Acta Hortic.* **2000**, *526*, 21–32. [CrossRef]
53. Gómez-del-Campo, M.; Ruiz, C.; Lissarrague, J.R. Effect of Water Stress on Leaf Area Development, Photosynthesis, and Productivity in Chardonnay and Airén Grapevines. *Am. J. Enol. Vitic.* **2002**, *53*, 138–143.
54. Ojeda, H.; Andary, C.; Creaba, E.; Carbonneau, A.; Deloire, A. Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* var. Shiraz. *Am. J. Enol. Vitic.* **2002**, *53*, 261–267.
55. Bellvert, J.; Marsal, J.; Mata, M.; Girona, J. Yield, Must Composition, and Wine Quality Responses to Preveraison Water Deficits in Sparkling Base Wines of Chardonnay. *Am. J. Enol. Vitic.* **2015**, *67*, 1–12. [CrossRef]
56. Buesa, I.; Pérez, D.; Castel, J.; Intrigliolo, D.S.; Castel, J.R. Effect of deficit irrigation on vine performance and grape composition of *Vitis vinífera* L. cv. Muscat of Alexandria. *Aust. J. Grape Wine Res.* **2017**, *23*, 251–259. [CrossRef]
57. Gambetta, G.A.; Herrera, J.C.; Dayer, S.; Feng, Q.; Hochberg, U.; Castellarin, S.D. The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. *J. Exp. Bot.* **2020**, *71*, 4658–4676. [CrossRef]
58. Poni, S.; Intrieri, C. Grapevine photosynthesis: Effects linked to light radiation and leaf age. *Adv. Hortic. Sci.* **2001**, *15*, 5–15.
59. Kliewer, W.M.; Dokoozlian, N. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* **2005**, *56*, 170–181.

60. FAO-ISRIC-ISSS. *World Reference Base for Soil Resources*; FAO: Rome, Italy, 2006; p. 142.
61. Soil Survey Staff. *Key to Soil Taxonomy*, 12th ed.; USDA-Natural Resources, United States Department of Agriculture, Conservation Service: Washington, DC, USA, 2014; p. 379.
62. Pavloušek, P. Preliminary results of tests of grapevine rootstocks resistance to lime-induced chlorosis. *Acta Univ. Agric. Silvic. Mendel. Brun.* **2008**, *56*, 299–302. [CrossRef]
63. Scholander, P.F.; Bradstreet, E.D.; Hemmingsen, E.A.; Hammel, H.T. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. *Science* **1965**, *148*, 339–346. [CrossRef]
64. Johnson, L.F.; Pierce, L.L. Indirect Measurement of Leaf Area Index in California North Coast Vineyards. *HortScience* **2004**, *39*, 236–238. [CrossRef]
65. Stewart, A.M.; Edmisten, K.L.; Wells, R.; Collins, G.D. Measuring Canopy Coverage with Digital Imaging. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 895–902. [CrossRef]
66. Hsiao, T.C. Plant Responses to Water Stress. *Annu. Rev. Plant Physiol.* **1973**, *24*, 519–570. [CrossRef]
67. Sommer, K.J.; Clingeleffer, P.R. Vine canopy development and carbohydrate partitioning as influenced by pruning. In Proceedings of the Ninth Australian Wine Industry Technical Conference, Adelaide, Australia, 16–19 July 1995; pp. 123–127.
68. Rubio, J.A. Riego y Aclareo de Racimos: Efectos en la Actividad Fisiológica, en el Control del Rendimiento y en la Calidad de la Uva del cv. Tempranillo (*Vitis vinifera* L.). Ph.D. Thesis, Departamento de Producción Vegetal: Fitotecnia, Universidad Politécnica de Madrid, Madrid, Spain, 2002; 218p.
69. Williams, L.E.; Grimes, D.W. Modelling vine growth development of a data set for a water balance subroutine. In Proceedings of the VI Australian Wine Industry Technical Conference, Adelaide, Australia, 14–17 July 1986; pp. 169–174.
70. Acevedo-Opazo, C.; Ortega-Farias, S.; Fuentes, S. Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. *Agric. Water Manag.* **2010**, *97*, 956–964. [CrossRef]
71. Smart, R.E.; Turkington, C.R.; Evans, J.C. Grapevine response to furrow and trickle irrigation. *Am. J. Enol. Vitic.* **1974**, *25*, 62–66.
72. Pellegrino, A.; Gozé, E.; Lebon, E.; Wery, J. A model-based diagnosis tool to evaluate the water stress experienced by grapevine in field sites. *Eur. J. Agron.* **2006**, *25*, 49–59. [CrossRef]
73. Hidalgo, L. *La Calidad del Vino Desde el Viñedo*, 1st ed.; Mundi-Prensa, S.A., Ed.; Artes Gráficas Cuesta: Madrid, Spain, 2006; 390p.
74. Kizildeniz, T.; Mekni, I.; Santesteban, H.; Pascual, I.; Morales, F.; Irigoyen, J.J. Effects of climate change including elevated CO2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. *Agric. Water Manag.* **2015**, *159*, 155–164. [CrossRef]
75. Shellie, K. Water Productivity, Yield, and Berry Composition in Sustained versus Regulated Deficit Irrigation of Merlot Grapevines. *Am. J. Enol. Vitic.* **2014**, *65*, 197–205. [CrossRef]
76. Baeza, P.; Sánchez-De-Miguel, P.; Centeno, A.; Junquera, P.; Linares, R.; Lissarrague, J.R. Water relations between leaf water potential, photosynthesis and agronomic vine response as a tool for establishing thresholds in irrigation scheduling. *Sci. Hortic.* **2007**, *114*, 151–158. [CrossRef]
77. Martínez, J.; Romero, R.; Chacón, J.L. Efectos del rigor del deficit hídrico de las plantas sobre el peso unitario de la uva madura en cv. Merlot. XI Congreso de la Sociedad Española de Ciencias Hortícolas (SECH). *Actas Hortic.* **2007**, *48*, 150–153.
78. Martínez, E.M.; Rey, B.J.; Fandiño, M.; Cancela, J.J. Impact of water stress and nutrition on *Vitis vinifera* cv. "Albariño": Soil-plant water relationships, cumulative effects and productivity. *Span. J. Agric. Res.* **2016**, *14*, e1202. [CrossRef]
79. Camps, J.O.; Ramos, M.C. Grape harvest and yield responses to inter-annual changes in temperature and precipitation in an area of north-east Spain with a Mediterranean climate. *Int. J. Biometeorol.* **2011**, *56*, 853–864. [CrossRef] [PubMed]
80. Cooley, N.; Clingeleffer, P.; Walker, R. Effect of water deficits and season on berry development and composition of Cabernet Sauvignon (*Vitis vinifera* L.) grown in a hot climate. *Aust. J. Grape Wine Res.* **2017**, *23*, 260–272. [CrossRef]
81. Freeman, B.M.; Lee, T.H.; Turkington, C.R. Interaction of irrigation and pruning level on growth and yield of Shiraz vines. *Am. J. Enol. Vitic.* **1979**, *30*, 218–223.

82. Girona, J.; Mata, M.; Del Campo, J.; Arbones, A.; Bartra, E.; Marsal, J. The use of midday leaf water potential for scheduling deficit irrigation in vineyards. *Irrig. Sci.* **2005**, *24*, 115–127. [CrossRef]
83. Santesteban, L.; Miranda, C.; Royo, J. Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv. "Tempranillo". *Agric. Water Manag.* **2011**, *98*, 1171–1179. [CrossRef]
84. Ferreyra, R.; Sellés, G.; Ruíz, R.; Sellés, I. Effect of water stress applied at different development periods of grapevine cv. Chardonnay on production and wine quality. *Agric. Técnica* **2003**, *63*, 277–286.
85. Williams, L.E.; Matthews, M.A. Grapevine. In *Irrigation of Agricultural Crops*; Stewart, B.A., Nielsen, D.R., Eds.; UC Davis Publications: Madison, WI, USA, 1990; pp. 1019–1055.
86. Nadal, M.; Arola, L. Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis* **1995**, *34*, 151–154.
87. García-Escudero, E.; López, R.; Santamaría, P.; Zaballa, O.; Arroyo, M.C. Ensayos de riego localizado en viñedos productivos de cv. Tempranillo. *Vitic. Enol. Prof.* **1997**, *50*, 35–47.
88. Esteban, M.A.; Villanueva, M.J.; Lissarrague, J.R. Effects of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids and mineral elements. *Am. J. Enol. Vitic.* **1999**, *50*, 418–434.
89. Tandonnet, J.; Ollat, N.; Neveux, M.; Renoux, J. Effect of three levels of water supply on the vegetative and reproductive development of merlot and cabernet sauvignon grapevines. *Acta Hortic.* **1999**, *493*, 301–308. [CrossRef]
90. Ojeda, H.; Deloire, A.; Carbonneau, A. Influence of water deficits on grape berry growth. *Vitis* **2001**, *40*, 141–145.
91. Castellarin, S.D.; Degan, M.; Di Gaspero, G.; Peterlunger, E. Impact of water deficit on the synthesis of phenolic compounds during berry ripening of *Vitis vinifera* L. cv. Merlot. In Proceedings of the XIV International Symposium of GESCO, Geisenheim, Germany, 23–27 August 2005; pp. 173–179.
92. Niculcea, M.; Martínez-Lapuente, L.; Guadalupe, Z.; Sánchez-Díaz, M.; Ayestarán, B.; Antolín, M.C. Characterization of phenolic composition of *Vitis vinifera* L. 'Tempranillo' and 'Graciano' subjected to deficit irrigation during berry development. *Vitis* **2015**, *54*, 9–16.
93. Ferlito, F.; Nicolosi, E.; Gentile, A.; Lo Piero, A.R.; Squadrito, M.; Continella, A. Responses of four winegrape varieties to managed water stress and partial defoliation in an arid environment. *Vitis* **2014**, *53*, 73–80.