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1 2 Row orientation effects on potted-vines performance and water-use efficiency 3 4 Ignacio Buesa^{a,b*}, José M. Mirás-Avalos^{b,c} and Diego S. Intrigliolo^b 5 7 ^a Centro para el Desarrollo de la Agricultura Sostenible del Instituto Valenciano de 8 Investigaciones Agrarias (CEDAS-IVIA), 46113, Moncada, Valencia, Spain 9 ^b Departamento de Riego, Centro de Edafología y Biología Aplicada del Segura 10 (CEBAS-CSIC), 30100, Murcia, Spain ^c Current address: Unidad de Suelos y Riegos (asociada a EEAD-CSIC). Centro de 11 12 Investigación y Tecnología Agroalimentaria de Aragón (CITA), 50059 Montañana, 13 Zaragoza, Spain. 14 15*Ignacio Buesa 16e-mail: igbuepue@gmail.com 17Address: Instituto Valenciano de Investigaciones Agrarias (IVIA) 18CV-315, Km 10, 46113 Moncada, Valencia, Spain. 19Phone: +34 650578152

AGRICULTURAL AND FOREST METEOROLOGY

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21

22**Abstract**

23 The relation between water-use and intercepted solar radiation depends on 24many factors involved in vine canopy architecture and physiology. In addition, vine 25productivity is related to the efficiency with which the intercepted photosynthetically 26active radiation (IPAR) is used, which in turn depends mainly on water availability and 27transport. In hedgerow-managed vines it exists the possibility to modulate IPAR by 28 orienting their rows, influencing water-use efficiency (WUE), defined as dry matter 29produced by water used. Aiming to unravel the effects of row orientation on WUE, a 30three-year experiment was carried out in Valencia (Spain) on potted Vitis vinifera (L.) 31cv. Bobal and Verdejo with vine rows oriented either north-south (NS) or east-west 32(EW), under no-water restrictions. Simulated radiation interception over the growing 33seasons at the experimental plot showed an average 39% reduction in daily IPAR 34when EW was compared to NS. Vine transpiration was quantified by water balance, 35decreasing by 16% in Bobal and 8% in Verdejo when comparing EW against NS. In 36both cultivars, this reduction was 18% when considered relative to the total leaf area. 37Carbon assimilation was not markedly affected by row orientation. Therefore, since in 38both cultivars minor differences in vine performance occurred between orientations, 39WUE tended to increase by orienting the rows to the EW compared to NS. This 40resulted in most of the seasons an increase in water productivity calculated as grape 41 yield/water-use ratio. Leaf gas exchange measurements partially agreed with the 42radiation interception simulations, suggesting a more complex regulatory mechanism 43and highlighting the importance of canopy microclimatic conditions in the physiological 44processes of hedgerow-managed crops. These findings encourage further research 45under field conditions and different soil water availabilities, aiming to optimize 46grapevine water productivity.

47**Keywords:** Canopy sunlight interception; Hedgerow; *Vitis vinifera* (L.); Water 48productivity; Yield.

491. Introduction

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50 Vineyards are complex agro-ecosystems where in general grapevine water-use 51(WU) is a function of the sunlight intercepted by the canopy (Trambouze et al. 1998, 52Williams and Ayars 2005), which influences the amount of dry matter produced 53(Grappadelli et al. 1994, Steduto et al. 2007). Grapevine canopies, regardless of the 54training system employed, are comprised of both sun-exposed and interior leaves. 55Shaded leaves contribute less to vine carbon assimilation than those sun-exposed 56because they receive less energy, which mostly comes from diffuse radiation (Spitters 57et al. 1986, Escalona et al. 2003). Canopy sunlight interception relates to the external 58leaf area, but also to leaf position and canopy structure (Petrie et al. 2009). As a 59consequence, the relationship between grapevine canopy radiation interception and 60leaf gas exchange is extremely complex (Baeza et al. 2010, Buckley et al. 2014). Plant 61shape, size, spacing, leaf inclination and position within the canopy, row orientation, 62 cloudiness and latitude determine sunlight interception by vineyards and, consequently, 63grapevine microclimate and physiology (Smart and Barrs 1973, Carbonneau 1979). For 64a given training system, Poni et al. (2003) observed that the sunlight intercepted by 65vineyard canopy increases with leaf area until a threshold in which leaf mutual shading 66impedes the increase of carbon assimilation potential.

Regions with suitable climate for viticulture are located from 30° to 50°N and 6830° to 40°S, where solar radiation does not usually restrict grapevine photosynthetic 69capacity. Hence, in the absence of water stress and limitations in source–sink 70relationships, photosynthetically active radiation (PAR) increases vine photosynthesis 71until a given saturation point (Iacono and Sommer 1996, Flexas et al. 2002). Since the 72response of canopy photosynthesis to radiation follows a convex asymptotic

73relationship (Spitters 1986), under high light intensity (> 1000 μ mol m⁻² s⁻¹), the limiting 74factor for carbon assimilation is the leaf internal concentration of CO₂ along with 75photoinhibition processes (Escalona et al. 2000, Long and Bernacchi 2003. In view of 76the predicted climate change, with an expected lower water availability (Vicente-77Serrano et al. 2014, Fraga et al. 2016), a reduction in solar radiation interception by the 78vineyard can be used to mitigate the effects of reduced soil water availability (Moratiel 79et al. 2010).

- Viticultural practices, such as training systems and canopy management, are 81tools for modulating the efficiency of grapevine radiation interception (Mabrouk and 82Sinoquet 1998). However, vineyard canopy orientation plays a determinant role in PAR 83and ultraviolet (UV) radiation interception and, consequently, in vine physiological 84processes (Palmer 1989, Intrieri et al. 2015). Many training systems have evolved 85towards maximizing the amount of sunlight intercepted and its distribution within the 86canopy. Among them, vertical shoot-positioning and Lyre systems, which allows 87exposing most leaves to high light levels leading to an increase in vine transpiration 88(Kliewer and Dokoozlian 2005, Reynolds and Vanden-Heuvel 2009, Albasha et al. 892019), aiming to maximize yield in the absence of water stress (Baeza et al. 2005).
- Many experiments have focused on the influence of solar radiant energy 91intercepted by fruit trees on their photosynthesis and transpiration (Smart 1973, 92Mariscal et al. 2000, López-Lozano et al. 2011), but less research effort has been 93devoted to the specific effect of row orientation on the grapevine water-use efficiency 94(WUE) (Annandale et al. 2004, Campos et al. 2017). Some studies reported that the 95east-west (EW) row orientation tended to reduce growth, yield and total dry matter per 96vine as compared to NS, NE-SW and NW-SE orientations (Intrieri et al. 1996). 97Moreover, in potted vines, Intrieri et al. (2015) observed that the intrinsic water-use 98efficiency (WUE_i) at midday was higher in NS than in EW rows; however, on a daily 99basis, little variations between both orientations were detected. Indeed, PAR

100absorption regulates the rate of carbon assimilation but also the canopy microclimate 101conditions within the vine, both affecting grape ripening (Jackson and Lombard 1993, 102Bergqvist et al. 2001). In this sense, Hunter et al. (2016) observed that EW oriented 103rows intercepted lower radiation inside the canopy than those oriented NS, but EW 104captured a largest portion of total radiation in the cluster zone due to the higher soil 105reflected radiation. These authors measured higher overall gas exchange rates at the 106leaf level in vines EW-oriented compared to those NS-oriented, with less negative leaf 107water potential, pointing out to differences in energy balance and physiology induced 108by row orientation (Hunter et al. 2016). Nonetheless, in order to achieve a balanced 109ripeness in all clusters, the NS orientation of the vine rows is preferred to the EW, 110seeking for a uniform light distribution in the fruiting zone (Naylor et al. 2000, Tarara et 111al. 2005).

In this context, the objective of the current work was to unravel the effects of 113 orienting row trellises EW, instead of the more usual NS, on vine WU and WUE. Taking 114 into account that orienting rows EW reduces sunlight interception of hedgerow fruit 115 trees, such as apple and olive, when compared to the NS orientation (Palmer et al., 1161989; Connor et al., 2014; Trentacoste et al., 2015), our hypothesis was that this would 117 also occur in grapevines, reducing canopy transpiration. At Mediterranean latitudes, 118 solar radiation is not a limiting factor; therefore, this reduction in the intercepted 119 photosynthetically active radiation (IPAR) might not compromise obtaining high yields, 120 leading to the improvement of WUE. In order to confirm the hypothesis that row 121 orientation can cause a reduction in transpiration without impairing vine performance, 122 an experiment was carried out during three consecutive seasons in fully-irrigated 123 potted-vines (both red and white cultivars) oriented NS and EW.

1262. Materials and Methods

1272.1 Description of the site and plant material

A pot experiment was carried out from 2014 to 2016 in *Vitis vinifera* (L.) cv. 129Bobal and Verdejo grafted onto rootstock 110 Richter at the Valencian Agriculture 130Research Institute (IVIA) experimental farm (Valencia, Spain, 39°35′13.952″N, 0°23 131′54.005″W). At the beginning of the experiment, vines were three years old. The 132climate at the site was Mediterranean, with annual rainfall and reference 133evapotranspiration (ET_o) of 370 and 1125 mm (1999-2016), respectively. The prevailing 134wind direction at the experimental site was south, with an average speed of 4.65 m s⁻¹. 135Weather data were recorded at an automated meteorological station located at 100 m 136from the experimental plot and ET_o was calculated with the Penman-Monteith approach 137(Allen et al. 1998). According to the climatic classification system for grape-growing 138regions (Tonietto and Carbonneau, 2004), the experimental area showed a warm 139climate, with temperate nights, while being moderately dry.

Seventy liter pots, of 0.45 m height and 0.55 m width, filled with a mix of coco 141 fiber substrate and compost, were used. Pots were covered with silver plastic in order 142 to minimize heating and water evaporation from the substrate. Vines were placed 143 outdoors in rows with spacing of 2 m between vines and 2.5 m between rows, 144 simulating a common spacing in the study region. The vines were winter pruned to 145 approximately 10 spurs per vine and 2 nodes per spur on a bilateral Royat cordon and 146 trained to a vertical trellis system consisting of a forming wire and three double catch 147 wires spaced 0.4 m apart. These vine arrangements reflects the general viticulture 148 practices carried in the region when vertically shoot positioning is employed as training 149 system (Buesa et al., 2019). Cluster thinning was applied during flowering in order to 150 standardize the number of clusters per vine and to balance leaf area to yield (7 and 12 151 clusters per linear meter of trellis in Bobal and Verdejo, respectively), since initial shoot

152fruitfulness (number of clusters per shoot) was too high. Shoot thinning was performed 153before flowering and shoot trimming when shoots reached nearly 2 m height. When 154secondary shoots reached the double catch wires, they were manually distributed to 155form a homogeneous canopy. The final hedgerow was continuous of 0.2-0.3 m wide 156(Supplemental Figure). The pots were drip irrigated to avoid water restrictions during 157the whole season and not to add an additional experimental factor to the responses to 158be determined. Irrigation scheduling was the same for both cultivars and aimed to 159ensure some drainage after each water application. Irrigation was applied 3 to 5 times 160per day (10 to 15 minutes per event) through 3 pressure-compensated emitters of 3.8 L 161h⁻¹ per plant. Vines were seasonally fertigated at a rate of 30-20-60-7.5 kg·ha⁻¹ of N, 162P₂O₅, K₂O, and MgO, respectively, as well as with iron chelates and other 163microelements at specific times.

1642.2. Row orientation treatments

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Two orientations were tested in each cultivar 1) north-south (NS) and 2) east-166west (EW). Each orientation consisted of 4 rows, and each row consisted of 12 vines. 167The 10 vines in the middle of the rows were used for measurements and samplings 168while the remaining 2 vines at each end acted as buffers. Each experimental unit (EU) 169consisted of 5 vines and each cultivar was in every row. During the first season (2014), 170each treatment consisted of 10 vines per treatment (2 EU), and 20 vines per treatment 171(4 EU) during the subsequent seasons (2015 and 2016).

1722.3. Modelling grapevine radiation interception

In order to estimate the differences in the radiation interception by a grapevine 174hedgerow-oriented NS and EW, the simple approach proposed by Oyarzun et al. 175(2007) was used. This model calculates radiation interception based on the portion of 176the floor shaded by the vines at any given time using geometric relationships of the 177length of the shadow cast by the vines and the configuration of the plantation. Inputs

178required by this model include hedgerow parameters, location data and meteorological 179variables.

In the current study, the values employed for the hedgerow parameters referred 181to the experimental vines and were the following: vine height (1.9 m), height of the 182insertion of the lower branches (0.8 m), canopy width perpendicular (0.3 m) and along 183the row direction (2.5 m), spacing between vines along the row (2 m), noon light 184porosity (0.44) and the row azimuth (0° for NS and 90° for EW). Location data include 185altitude (68 m), latitude (39.5 °), longitude (0.4 °), standard meridian (1 °), slope (0 °) 186and aspect of the terrain (0 °). The only meteorological input required is the daily global 187solar radiation (MJ m⁻² s⁻¹), taken from the weather station located at the study site.

Outputs from the model include the fraction of intercepted PAR (fIPAR), the 189PAR intercepted by the canopy (IPAR), and the PAR intercepted daily (DIPAR). 190Simulations were carried out from June 1st until August 31st for each experimental 191season.

1922.4. Field determinations

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1932.4.1. Vine phenology and vegetative development

Phenological stages were monitored by visual inspection in two shoots per vine 195 on a weekly basis during the whole season. At veraison, the percentage of coloured 196 berries was determined in two clusters per vine. Vegetative growth was determined 197 monthly in a sample of 4 shoots per vine by measuring, non-destructively, the length of 198 primary and secondary shoots. Removed vegetative fresh mass was weighted for each 199 vine after thinning (clusters), trimming (shoot tips) and pruning (wood) operations at the 200 usual times of vineyard management, previously indicated in this section. Dry matter 201 was calculated as the sum of the fresh mass of each plant organ after oven-drying it at 20260 °C for one week. Leaf area was estimated from allometric relations between shoot 203 length and leaf area per shoot, using a LI-3100C Area Meter (LI-COR Bioscience,

204Lincoln, NE, USA) and separating main shoots and laterals. These relations were 205obtained from samples of 10 shoots of different vigor coming from the buffer vines for 206each cultivar and season. After harvest, total leaf area (LA) was estimated in each 207experimental vine by measuring the length of all main and secondary shoots. The ratio 208LA-to-yield was calculated for all experimental vines.

2092.4.2. Vine water-use (WU)

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210 In 2014, daily vine WU was obtained by water balance in all pots on 5 211occasions during the early spring and summer time of the season (coinciding with the 212end of the rapid leaf area growth). This was calculated by subtracting the weight at 2137:00 solar time during two consecutive days. Additionally, pots were weighted at solar 214noon in order to obtain morning and afternoon transpiration separately. During these 215determinations, the irrigation applied (1 L in the morning and 1 L in the afternoon) was 216taken into account. This amount of water was accurately supplied by hand to each pot 217in order to prevent vine water stress, while controlling the absence of drainage. For 218total vine WU estimation, the daily transpiration values obtained by weighting were 219extrapolated to the subsequent days until the next measurement, assuming a constant 220transpiration rate between consecutive actual determinations. It should be noted that 221evaporation from the substrate was minimized by covering the pots with silver plastic. 222In 2015, a drainage collector system was built with a tank and a water meter per EU. 223Therefore, during the 2015/16 seasons, WU was estimated weekly by water balance 224(discarding the periods with rainfall), calculated as the subtraction between the 225irrigation volume registered by the water meters and the water volume drained to the 226tanks. However, in 2015 because of some episodes of heavy rainfall, WU 227measurements started later in the season than in 2016. The possible variation in 228substrate water content between determinations was disregarded since the measure 229was carried out immediately after the end of the occurrence of drainage after an 230irrigation event.

2312.4.3. Vine water relations and physiology

232 Vine water status was determined monthly before dawn in un-bagged leaves $233(\Psi_{pd})$ and at midday in bag-covered leaves (Ψ_{md}) during the three experimental years 234(Santesteban et al. 2019). For the Ψ_{md} determination, leaves were enclosed in hermetic 235plastic bags covered with aluminum foil for at least 1 h prior to the measurement. Water 236potential was measured with a Scholander pressure chamber (Model 600, PMS 237Instrument Company, Albany, OR, USA) on 1 basal leaf per plant from 2 vines per EU. 238Leaves were taken from the west side of the NS rows and from the north side of the 239EW ones. Additionally, stomatal conductance (g_s), net photosynthesis (A) and 240transpiration rate (E) were determined at the leaf level by means of a portable 241photosynthesis analyzer (LCpro+, ADC BioScientific Ltd., Hoddesdon, England) (Long 242and Bernacchi 2003). Gas exchange was assessed in 2 vines per EU and cultivar, on 243the same vines and dates in which water potential determinations were conducted. In 2442014, these measurements were carried out coinciding with the date of pot-weighting. 245Gas exchange measurements were taken under ambient conditions in two basal and 246mature leaves per vine of the sun-exposed side of the canopy, avoiding the 247modification of the natural leaf arrangement (without fixing CO₂, light intensity, relative 248humidity or leaf disposition). Gas flow was set not to exceed 2-3 °C the ambient 249temperature. Determinations were carried out in the following time intervals 7:00-9:00, 25011:00-13:00 and 16:00-18:00 during the morning, midday and afternoon, respectively. 251In addition, intrinsic water-use efficiency (WUE_i) at the leaf level was calculated as the 252ratio of A to g_s.

2532.4.4. Water-use efficiency (WUE) and water productivity (WP)

The whole-vine WUE was estimated at harvest as the ratio between total dry 255mass (cluster thinning, shoot trimming, pruning wood and grape clusters) and the 256amount of water used (transpired). Additionally, water productivity (WP) was calculated

257as yield (fresh grape mass) and the amount of WU. Since the measuring periods of 258water balance do not comprise the whole growing season and the data collected were 259not exactly the same in all seasons, the absolute values of WU, WUE and WP will not 260be comparable among seasons. In any case, our goal was to quantify the differences in 261between the two treatments explored and, within each season, the same procedure 262was followed in all experimental treatments. In 2014, the water balance was calculated 263for each experimental vine from June 1st to the end of July. In 2015-2016 it was 264calculated for each EU (5 vines), from July 7th to September 7th in 2015, and from April 2651st to the end of August in Bobal, and until September 23rd in Verdejo in 2016.

2662.4.5. Vine performance

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Vine yield was determined at harvest. Harvest was performed when grapes 268attained 16 and 21.5 °Brix respectively for cv. Bobal and Verdejo. The low value of total 269soluble solids in Bobal was caused by the early harvests performed in order to avoid 270yield losses caused by *Botrytis cinerea* attacks. Cluster weight was obtained by 271dividing the yield of each vine by its number of clusters. Clusters per vine were counted 272both before applying cluster thinning (initial) and at harvest (final). Additionally, the 273number of berries per cluster was determined from samples of one average-size 274cluster per vine. At harvest, fresh berry mass was determined from samples of 200 275berries collected randomly per EU.

2762.5. Statistical analysis

Data analysis was performed using "Statgraphics Centurion XVI" package 278version 16.0.07 (StatPoint Technologies, Inc., Warrenton, VA, USA). The significance 279of the treatment, season and their interaction on vine traits were assessed by two-way 280analysis of variance (ANOVA). Additionally, transpiration data were analyzed in two 281steps (Sadras et al. 2009). First, we plotted actual transpiration rate for each orientation 282against the environmental mean. This procedure has been widely used to capture the

283aggregated effects of multiple driving factors on a trait (Lacaze et al. 2009, Peltonen-284Sainio et al. 2011, Trentacoste et al. 2011, Sadras et al. 2012), such as transpiration 285which is driven by multiple soil, weather, management and crop factors. Second, we 286calculated the deviations relative to the 1:1 line and used Fisher's LSD multiple range 287test (p < 0.05) to separate means when ANOVA indicated significant differences 288among treatments.

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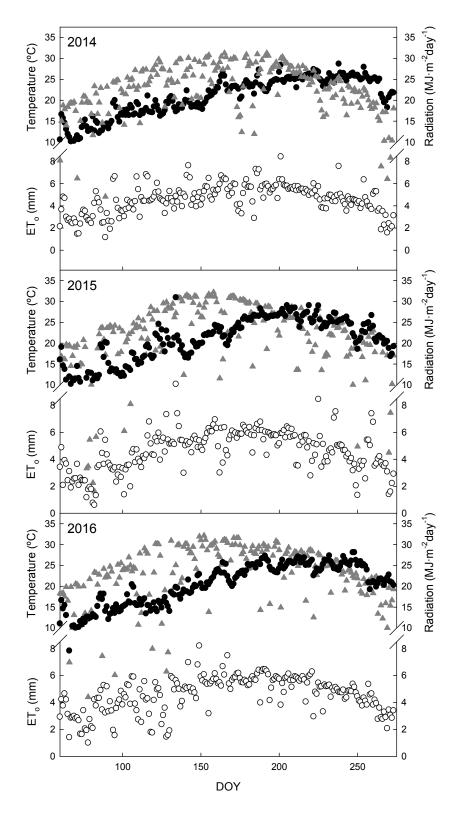
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291**3. Results**

2923.1. Weather conditions

The annual ET_o at the study site was 1320, 1268 and 1268 mm in 2014, 2015 294and 2016, respectively. The daily evolution of ET_o, mean temperature and solar 295radiation during each growing season (1st of March to 30th September) is depicted in 296Figure 1. The maximum daily ET_o values were 8.4, 10.2 and 8.7 mm in 2014, 2015 and 2972016, respectively. These days of high evaporative demand, corresponded with 298maximum temperatures over 33-40 °C, westerly winds of 2.8-3.3 m s⁻¹ and relative 299humidity of 30-40%. Radiation during the three growing seasons was fairly 300homogeneous, with most days with a clear sky and 25 MJ m⁻² on average each 301season, corresponding to 51.5 mol PAR m⁻².

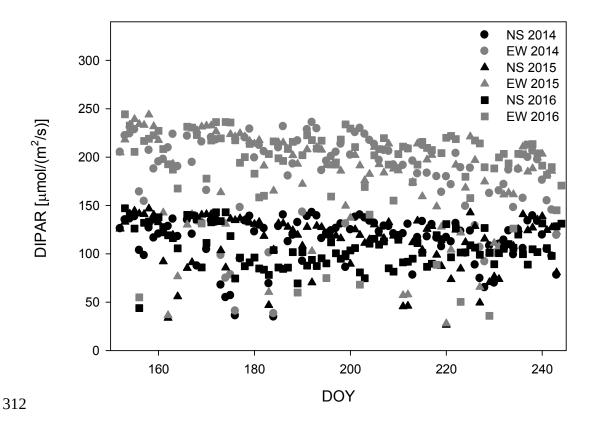


303Figure 1. Seasonal patterns of daily reference evapotranspiration, ET $_{\circ}$ (\circ); mean air 304temperature (\bullet); and incoming solar radiation (\blacktriangle) in Moncada, Valencia, Spain. The 305day of year (DOY) follows a continuous annual-time scale from March 1st to September 30630th.

3083.2. Modelling canopy radiation interception

In all seasons the EW orientation decreased with respect to NS by an average 310of 39% the amount of PAR intercepted daily by the vines (Figure 2).

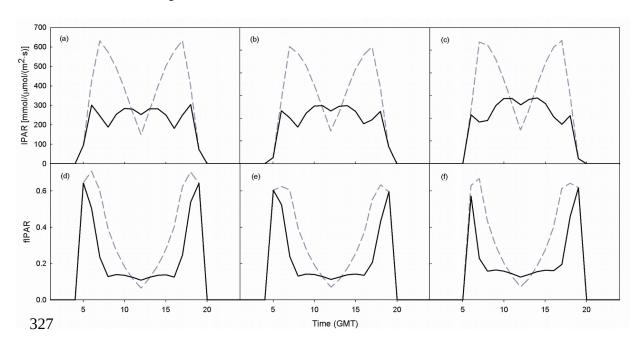
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313Figure 2. Daily averages of photosynthetically active radiation intercepted by grapevine 314canopies (DIPAR) as simulated during summer for the north-south (NS; •■▲) and east-315west (EW; •■▲) row orientations in Valencia (Spain). Data are averages of the 316estimations from the model proposed by Oyarzun et al. (2007) for the 2014 (\circ), 2015

 $317(\Box)$ and 2016 (\triangle) seasons. The day of year (DOY) follows a continuous annual-time 318scale from June 1st to August 31st.

The differences observed on the hourly fraction of PAR intercepted (fIPAR) for 320each orientation explain this reduction in DIPAR for EW. Figure 3 shows the daily 321pattern of fIPAR for both orientations on three different dates. During most hours of the 322morning and afternoon, fIPAR is greater in NS than in EW (Figure 3). In contrast, EW 323orientation intercepts more PAR at noon. As shown in Figure 3d, 3e and 3f, IPAR is 324also higher for the NS orientation during the morning and afternoon, but the contrary 325occurs at noon. Reductions in DIPAR caused by EW orientation were 35-40% for the 326dates shown in Figure 3.

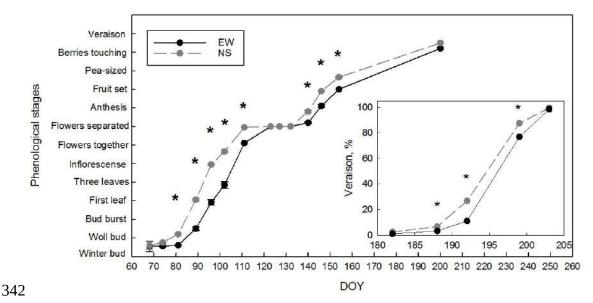


328Figure 3. Hourly evolution of the fraction of intercepted photosynthetically active 329radiation (flPAR) [a-c], and the intercepted PAR (IPAR) [d-f], simulated for the 330hedgerow orientation EW (—) and NS (— —) in Valencia, Spain. Simulated data 331corresponded to a day in June (Day of the year, DOY, 153) in 2016 [a, d], a day in July 332(DOY 201) in 2015 [b, e] and a day in August (DOY 217) in 2014 [c, f].

3333.3. Vine phenology and vegetative development

Budburst took place, approximately, in the second week of March in the case of 335Verdejo (DOY 67-73) and during the fourth week in Bobal (DOY 88-94). Anthesis 336occurred in the second week of May (DOY 130-137) and during the subsequent (DOY

337137-145), in Verdejo and Bobal, respectively. Veraison took placed during the first 338fortnight of July in both cultivars. The phenological stages of Verdejo were not 339significantly affected by the treatments imposed, but those of Bobal were delayed in 340each season by the EW row orientation compared to NS (Figure 4). However, this 341delay between orientations was recovered as the season progressed.



343Figure 4. Evolution of the different phenological stages in Bobal in north-south (NS; ●) 344and east-west (EW; ●) hedgerow orientations during 2016. The percentage of colored 345berries (veraison) is depicted for the 2014 season. Data are averages and standard 346errors of two shoots per vine (n=40). Asterisks denote significant differences between 347orientations at p<0.05. DOY, day of the year.

In agreement with the evolution of phenology in Bobal vines, shoot growth and 349development was delayed in EW compared to NS (data not shown). In contrast, 350Verdejo vines did not show significant differences in shoot growth between the two 351orientations and the total dry matter produced by the vine was not affected by row 352orientation (Table 1). However, in Bobal vines, the NS orientation increased the total 353dry matter produced.

The number of shoots per treatment did not differ between treatments since the 355pruning and thinning criteria were the same (Table 1). Total leaf area (LA) was not 356consistently affected by row orientation in any cultivar during the three seasons

357studied. In 2015, LA was significantly increased by EW in comparison with NS for 358Verdejo. On the contrary, in 2016, LA of Bobal vines was significantly reduced by EW 359orientation compared to NS. In fact, in both cultivars, an interaction between season 360and treatment was detected for LA. Additionally, the LA-to-yield ratio was differently 361affected by the treatments imposed depending on the season.

Table 1. Vine shoot number, total dry matter, leaf area (LA) and LA-to-yield ratio in 363north-south (NS) and east-west (EW) oriented *Vitis vinifera* cv. Bobal and Verdejo 364potted-vines during three seasons.

Cultivar	Season	Orientation	Shoot number vine ⁻¹	Dry matter, g vine	LA, m ² vine ⁻¹	LA-to- yield, m² kg ⁻¹
	2014	NS	17.0	482	6.3	2.6
	2014	EW	17.6	453	6.7	2.4
	2015	NS	17.2	635a	5.0	0.5a
	2015	EW	18.0	572b	4.9	0.4b
	2016	NS	18.2	893a	7.4b	1.8
Bobal	2016	EW	18.4	671b	6.2a	1.4
		NS	17.5	670a	6.2	1.6
	Average	EW	18.0	565b	5.9	1.4
		Treatment	0.09	< 0.01	0.14	0.17
		Season	0.03	< 0.01	< 0.01	< 0.01
		TxS	0.69	< 0.01	< 0.01	0.59
	2014	NS	17.7	503	6.1	3.5
	2014	EW	17.5	579	6.7	4.1
	2015	NS	17.9	755	5.3b	1.2b
	2015	EW	16.9	782	7.1a	1.7a
	0040	NS	18.2	1019	8.4	0.5a
Verdejo	2016	EW	18.0	1025	8.0	0.4b
	Average	NS	18.0	852	6.9b	1.2
		EW	17.6	873	7.5a	1.3
		Treatment	0.08	0.59	< 0.01	0.49
		Season	0.05	< 0.01	< 0.01	< 0.01
		TxS	0.33	0.52	< 0.01	0.07

365For each parameter, data are average values of each treatment. Within each season and cultivar, different 366letters indicate significant differences at p<0.05. The statistical significances of the treatment (T), season 367(S) and their interaction are also indicated by means of p-values. Dry matter, total vegetative dry mass 368(cluster thinning, shoot trimming and pruning wood); LA, leaf area.

370**Table 2.** Water-use (WU), water-use relative to leaf area, water-use efficiency (WUE) 371and water productivity (WP) in north-south (NS) and east-west (EW) oriented *Vitis* 372*vinifera* cv. Bobal and Verdejo potted-vines during three seasons.

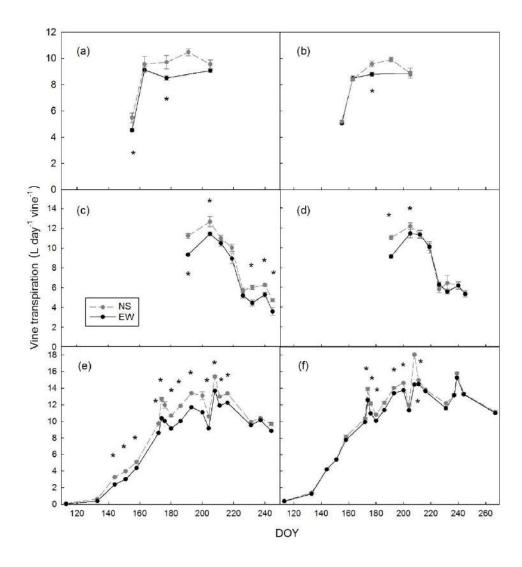
Cultivar	Season	Orientation	WU, L vine ⁻¹ day ⁻¹	Relative WU, L m ⁻² day ⁻¹	WUE, g L	WP, kg m ⁻³
	2014	NS	9.2a	1.46a	0.15b	4.3b
	2014	EW	7.8b	1.16b	0.22a	6.4a
Bobal	2015	NS	9.4a	1.91a	0.51b	19.7b
- -	2015	EW	7.6b	1.56b	0.60a	25.2a
	2016	NS	7.4a	1.01	0.13	4.9
		EW	6.5b	1.03	0.12	5.0
	2014	NS	9.4a	1.54a	0.18b	4.1b
- Verdejo	2014	EW	8.2b	1.22b	0.29a 5.3a	5.3a
	2015	NS	8.8a	1.67a	0.07	2.0
	2015	EW	8.2b	1.16b	0.07 1.9	
	2016	NS	9.1	1.08	0.12	2.9
	2010	EW	8.7	1.10	0.13	2.5

³⁷³For each parameter, data are average values of each treatment. Within each season and cultivar, different 374letters indicate significant differences at p<0.05. WU, water-use; WUE, water-use efficiency (total 375vegetative and grape cluster dry mass/water-use); WP, water productivity (grape fresh weight/water-use).

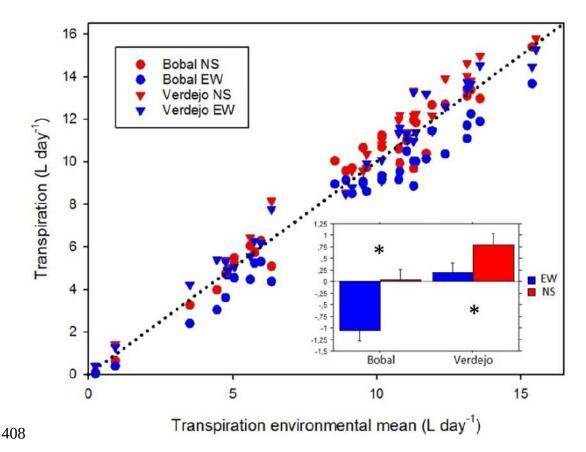
3773.4. Vine water-use (WU)

In all seasons, the peak transpiration rates were recorded in July (Figure 5), 379coinciding with the maximum ET_o (Figure 1). In both cultivars, vine WU was 380significantly higher in NS than in EW in most measurements (Figure 5). During the first 381season, when morning and evening transpiration were determined separately, 382differences between orientations occurred in both periods of the day (data not shown). 383In the Bobal cultivar in all the three seasons EW reduced transpiration in comparisons 384to NS by 12 to 23%. In the Verdejo cultivar reductions in water use brought by the EW 385rows orientation were less important and in 2016 there were not statistically significant 386differences between the two experimental treatments (Table 2). Nonetheless, the 387analysis of deviations relative to the 1:1 line from each treatment with the daily average 388of transpiration across seasons, cultivars and orientations (environmental mean),

389showed that EW significantly reduced grapevine transpiration compared to NS in both 390cultivars (Figure 6). The null hypothesis was that, for each cultivar, treatments are not 391different; hence, transpiration for individual treatments should align with the 1:1 line. 392Under our experimental conditions combining seasons, cultivars and row orientation, 393transpiration environmental mean ranged from almost zero in vines at the beginning of 394the growing season to 15 L day⁻¹ in vines fully developed under high ET_o conditions. In 3952014 and 2015 in both cultivars, vine WU relative to leaf area was significantly higher in 396NS than in EW (Table 2). Moreover, in both cultivars, EW increased WUE in 397comparison to NS, by 18% and 23% in Bobal and Verdejo, respectively. In terms of 398WP, in the Bobal variety, there was a clear increase in the EW compared to NW in the 399first two experimental seasons (Table 2). In the Verdejo cultivar, significant and clear 400differences in WP were obtained only during the first experimental seasons.



402Figure 5. Seasonal transpiration measured by water balance in Bobal (left) and Verdejo 403(right) potted vines oriented north-south (NS; ●) and east-west (EW; ●) during 2014 [(a) 404and (b)], 2015 [(c) and (d)] and 2016 [(e) and (f)]. Data are averages and standard 405errors of each treatment, consisting of 5 vines per experimental unit and date. Asterisks 406denote significant differences between treatments at p<0.05. DOY, day of the year.

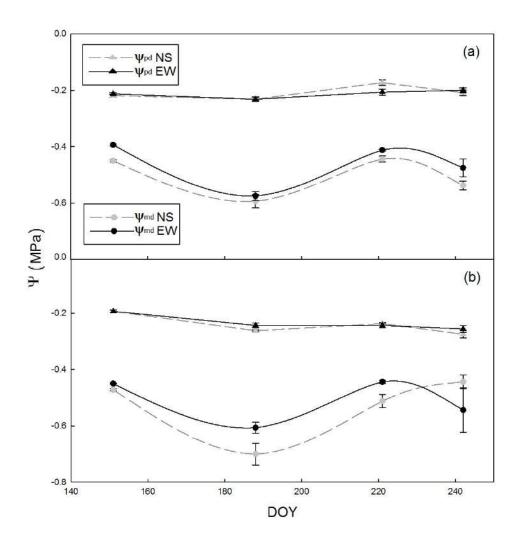


409Figure 6. Scatter plot relating potted-vines transpiration environmental mean to daily 410transpiration of Bobal in north-south (NS; ●) and east-west (EW; ●) and of Verdejo in 411north-south (NS; ▼) and east-west (EW; ▼) hedgerow orientation in 2014/16. Data are 412averages of every experimental unit of each treatment. Bar chart shows significant 413deviation of each treatment and cultivar from the 1:1 line of the daily average of 414transpiration across seasons, cultivars and orientations. Asterisks indicate significant 415differences between orientations for each cultivar.

4173.5. Vine water relations and physiology

Vines were over-irrigated during the whole season, ensuring water leaching 419after each irrigation event, and vines did not suffer from water deficit (Intrigliolo and 420Castel 2006, Williams and Baeza 2007). As an example, the Ψ_{pd} and Ψ_{md} evolution 421during 2016 is depicted for both cultivars in Figure 7. Values of Ψ_{pd} and Ψ_{md} never went 422below -0.25 MPa and -0.75 MPa, respectively. Moreover, the treatments imposed did 423not cause significant differences in vine water status. In 2014, the Ψ_{md} in Bobal had a

424seasonal average of -0.56 MPa in NS and -0.55 MPa in EW and, in Verdejo, -0.61 MPa 425and -0.59 MPa, respectively. In 2015, Ψ_{md} in Bobal was on average -0.50 MPa in NS 426and -0.43 MPa in EW and, in Verdejo, -0.47 MPa and -0.46 MPa, respectively.



427

428Figure 7. Evolution of vine water status at predawn (Ψ_{pd}) and at midday (Ψ_{md}) during 429the 2016 season in north-south (NS) and east-west (EW) oriented *Vitis vinifera* cvs. 430Bobal [(a)] and Verdejo [(b)] potted vines. Data are averages and standard errors of 2 431leaves per experimental unit (n=8) for predawn leaf water potential (\blacktriangledown , \blacktriangledown) and midday 432stem water potential (\blacktriangledown , \blacktriangledown). DOY, day of the year.

Regarding gas exchange determinations in both cultivars (Table 3), pooling 434data across seasons, daily transpiration (E) was slightly higher in NS compared with 435EW. This trend was consistent with the water balance measurements (Figure 5). 436However, the differences in E found on a leaf basis resulted significant only in Bobal

437during the afternoon. The stomatal conductance (g_s) and net photosynthesis (A) were 438significantly higher in NS than EW during the morning in Verdejo, and during the 439afternoon in Bobal. At midday, leaf-gas exchange parameters did not show significant 440differences between treatments. On a daily scale, g_s and A were significantly higher in 441NS than in EW in both cultivars (Table 3). The WUE_i was not significantly affected by 442row orientation, although in both cultivars it tended to decrease in NS in the morning 443and to increase in the afternoon when compared to that of EW. On a daily basis, these 444effects were mostly offset. The effect of the season was significant in most gas-445exchange parameters and times of the day, but not the interaction between year and 446treatment.

448**Table 3.** Leaf gas exchange parameters of Bobal and Verdejo potted-vines oriented 449north-south (NS) or east-west (EW). Data were pooled across three seasons.

Cultivar	Time of the day	Orientation	E, mmol H ₂ O m ⁻² s ⁻¹	g _s , mol m ⁻² s ⁻¹	A, µmol CO ₂ m ⁻² s ⁻¹	WUE _i µmol CO₂ mmol ⁻¹ H₂O
		NS	3.1	197	11.5	49.3
		EW	3.4	211	11.3	51.4
	Morning	Treatment	0.29	0.01	0.20	0.57
		Season	< 0.01	< 0.01	0.37	< 0.01
		TxS	< 0.01	0.14	0.03	0.15
		NS	3.4	205	10.1	52.3
		EW	3.3	211	10.2	53.5
	Midday	Treatment	0.76	0.74	0.92	0.79
Bobal -		Season	< 0.01	< 0.01	< 0.01	< 0.01
		TxS	0.27	0.25	0.85	0.76
		NS	2.6a	208a	10.0a	50.5
	Afternoon	EW	2.0b	146b	6.7b	46.6
		Treatment	< 0.01	< 0.01	< 0.01	0.51
		Season	0.02	0.14	< 0.01	0.06
-		TxS	0.46	0.33	0.97	0.54
		NS	3.2	257a	10.8a	50.0
		EW	3.0	207b	9.7b	51.2
	Daily	Treatment	0.11	< 0.01	0.03	0.65
		Season	< 0.01	< 0.01	0.04	< 0.01
		TxS	0.37	0.38	0.47	0.75
Verdejo	Morning	NS	3.1	290a	11.8a	48.0

-	EW	3.3	227b	10.5b	53.4
	Treatment	0.39	< 0.01	0.02	0.09
	Season	< 0.01	< 0.01	< 0.01	< 0.01
	TxS	< 0.01	0.51	0.81	0.10
	NS	3.3	200	10.3	56.7
	EW	3.0	160	9.4	60.1
Midday	Treatment	0.13	0.22	0.83	0.47
	Season	< 0.01	0.83	< 0.01	< 0.01
	TxS	0.11	0.03	0.10	0.92
	NS	2.5	185	9.2	50.7
	EW	2.2	163	7.9	48.5
Afternoon	Treatment	0.08	0.17	0.10	0.51
	Season	< 0.01	< 0.01	< 0.01	< 0.01
	TxS	0.89	0.59	0.23	0.01
	NS	3.1	234a	10.8a	52.5
	EW	3.0	199b	9.9b	54.8
Daily	Treatment	0.30	< 0.01	0.04	0.32
	Season	< 0.01	< 0.01	0.08	< 0.01
	TxS	0.14	0.04	0.06	0.04

451For each parameter, data are averages foreach treatment, consisting in 4 leaves per experimental unit and 4523 dates of measurement during each season. Within each time of the day and cultivar, different letters 453mean significant differences at p<0.05. The effects of the treatment (T), season (S) and their interaction 454are also shown by means of p-values. E, transpiration; g_s , stomatal conductance; A, net photosynthesis; 455WUE, intrinsic water-use efficiency (A/ g_s).

4563.6. Yield components

457Yield was rather variable across seasons in both cultivars, without significant effects, 458on average for the study period, in response to the treatments imposed (Table 4). 459Exceptionally, yield was increased by EW compared with NS in Bobal in 2015, whereas 460it was reduced in Verdejo in 2016. Number of clusters per vine, berries per cluster or 461cluster mass were similar between treatments in both cultivars. The initial number of 462clusters per vine, before thinning, did not differ between treatments (Table 4). Finally, 463berry mass significantly decreased under NS when compared with EW in Verdejo, 464whereas in Bobal this effect was only significant in the first experimental season.

465**Table 4.** Yield components of potted-vines in rows oriented north-south (NS) and east-466west (EW) in Bobal and Verdejo cultivars during three seasons.

Cultiva r	Season	Orientation	Initial clusters vine ⁻¹	Final clusters vine ⁻¹	Cluster mass, g	Berries cluster ⁻¹	Berry mass, g	Yield, kg vine ⁻¹
	2014	NS	7.2	6.3	421	120	3.0b	2.9
		EW	6.7	6.6	422	135	3.2a	2.9
	2015	NS	17.2	13.3	785	287b	2.7	10.5b
		EW	17.3	14.4	813	319a	2.5	11.8a
	2016	NS	9.5	8.1	613	193	3.1	5.3
Bobal		EW	11.0	8.9	540	170	3.2	4.8
	Average	NS	11.3	9.2	608	200	3.0	6.3
		EW	11.7	10.0	592	208	3.0	6.5
		Treatment	0.57	0.10	0.58	0.41	0.85	0.49
		Season	<0.01	< 0.01	< 0.01	< 0.01	<0.01	< 0.01
		TxS	0.37	0.78	0.20	0.02	0.25	0.06
Verdejo	2014	NS	21.0	16.7	92	32	2.8b	1.8
		EW	22.3	16.2	113	34	3.3a	2.0
	2015	NS	30.3	24.9	178	70	2.6b	4.4
	2015	EW	27.8	24.5	179	64	2.8a 4.4	4.4
	2016	NS	29.5	24.5	173	87	2.0b	4.5a
		EW	27.3	23.6	167	77	2.2a	3.6b
	Average	NS	26.9	23.6	164	73	2.4b	4.1
		EW	25.8	23.0	166	67	2.7a	3.7
		Treatment	0.13	0.63	0.49	0.15	<0.01	0.411
		Season	<0.01	< 0.01	< 0.01	< 0.01	<0.01	< 0.01
		TxS	0.05	0.95	0.41	0.31	0.05	0.06

 467 For each parameter, data are average values of each treatment. Within each season and cultivar, different 468 letters mean significant differences at 69 Co.05. The statistical significance effect of the treatment (T), 469 Season (S) and their interaction are also indicated by means of p-values.

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4714. Discussion

Assessing seasonal transpiration and WUE of grapevines in response to 473hedgerow orientation in Mediterranean latitudes is important to optimize vineyard water 474balance. As light interception is related to the potential crop evapotranspiration, 475techniques that reduce canopy radiation load and therefore vine water requirements 476are needed for adapting Mediterranean viticulture to the effects of the projected 477increase in water deficit (Schultz 2000, van Leeuwen et al. 2019). In the current study, 478orienting hedgerows EW decreased grapevine transpiration when compared with rows 479oriented NS (Figure 6). The average reduction in WU (-18%) found in EW compared to 480NS rows occurred in both cultivars independently of LA (Table 2). Moreover, WUE and

481WP across seasons increased in both cultivars when orienting rows EW. When gas 482exchange was determined at the single leaf level, transpiration in the vines oriented 483EW compared to NS vines was reduced only by 7% in Bobal and by 4% in Verdejo 484(Table 3). This suggests that the row orientation effects on water-use were not only due 485to modifications in gas exchange on a leaf basis. Nevertheless, we cannot rule out the 486possibility that some modification of the conditions of temperature, humidity, radiation 487that the gas exchange measurement device causes on the leaf environment, might 488have inevitably influenced transpiration determinations carried at the leaf level. In this 489sense, Poni et al. (2009) pointed out that extrapolating single-leaf-based 490determinations to the whole-canopy level do not necessarily reflect the whole-canopy 491behavior, and even more when, as in our experiment, the treatments imposed also 492affected the whole vine micro-climate.

In the present research, in fact, the estimated seasonal IPAR reduction due to 494the EW row orientation was 39% (Figure 2) in accordance with the experimental data 495and model simulations for radiation interception in row vineyards tested by Campos et 496al. (2017). This estimated reduction in IPAR is therefore higher than the decrease in 497WU determined at the whole vine level. This might be because the regulation of canopy 498conductance depends not only on the radiation interception, but also on the 499interrelation between grapevine physiology and the microclimatic conditions within the 500canopy (Steduto et al. 2007). Under ambient conditions the higher transpiration 501induced by the NS orientation may have decreased the vapor pressure deficit in the air 502surrounding the canopy affecting therefore the evaporative demand at the vine level.

Phenological stages and shoot growth were significantly anticipated only in the 504Bobal cultivar when it was oriented towards NS compared to EW (Figure 4) suggesting 505that the thermal effect of hedgerow orientation on vine phenology is cultivar dependent. 506This effect could have been enhanced by the heating effect of radiation onto the pots 507and thus the warmer root system. Nevertheless, the advancement in phenological

508stages was attenuated during the course of the season. Notwithstanding, increments in 509Bobal dry matter were found in 2015 and 2016 in NS compared to EW. This seems to 510be related to the advancement in shoot growth due to the earlier phenology caused by 511NS. In Bobal, indeed, if shoot trimming had not been performed, differences in WU 512between NS and EW might have been higher. In Verdejo, where advancements in 513phenology caused by changing row orientation did not occur, few differences in total 514dry matter were found between treatments.

515 Vine productivity was primarily unaffected by the reductions in the estimated 516radiation load provoked by orienting grapevine trellis systems towards EW instead of 517NS (Table 4). This lack of effects disagrees with previous studies assessing row 518 orientation effects of olive (Olea europaea L.) hedgerows (Trentacoste et al. 2015), 519which can be attributed to the obvious morphological and physiological differences 520between these crops. The positive linear relationship of growth and yield of olive trees 521with IPAR (Villalobos et al. 2006) was not observed in our experiment. Grapevine 522productivity under conditions of high radiation load has been reported to be more 523dependent on the vine water relations than on the vine source capacity (Mirás-Avalos 524et al. 2017). In our experiment, the only yield component that seems to have had a 525consistent effect in response to row orientation was berry mass, which tended to 526decrease in NS (Table 4), with lower sensitivity in Bobal grapes. This might be caused 527by turgor pressure effects (Intrigliolo and Castel 2010); however, there were no 528significant differences in vine water status between treatments to support this 529hypothesis (Figure 7). Indeed, a large number of factors affects the physiology of berry 530growth (Dai et al. 2009). For instance, Hunter et al. (2016, 2017) observed a reduction 531in berry size in field-grown Shyraz grapevines not explainable by the low vine water 532potential differences due to row orientation. The authors explained this reduction in 533berry size by a lower berry transpiration in the clusters of the EW oriented vines 534compared to those NS-oriented. In addition, these authors observed that vines NS-

535oriented consistently yielded 6% more than EW vines during a seven-year trial under 536mild water stress. On the contrary, under our non-limiting water conditions, only total 537dry matter in Bobal significantly decreased by EW compared to NS (Table 1).

538 Improving WP, estimated as yield to water-use ratio, is crucial for a sustainable 539viticulture, especially in semi-arid regions (Medrano et al. 2015b). Growing different 540cultivars can be an option to improve WP because, as observed in our trial, yield 541response depends greatly on the genotype. However, for winemaking purposes, local 542cultivars determine wine typicity. In both cultivars, WUE and WP significantly increased 543in EW when compared to NS (Table 2), with the exception of 2016, when a significant 544yield reduction occurred in EW Verdejo, and no differences in Bobal. Overall, the 545higher WU and the higher photosynthesis rates of NS oriented vines compared to 546those EW (Table 3), did not involve higher yields or the initial number of clusters per 547vine (Table 4), reducing both WP. A possible explanation to this lower efficiency in NS 548compared to EW might be their lower WUE; (assimilation-to-transpiration ratio) during 549the morning hours, when grapevine is more physiologically active and NS rows were 550intercepting more radiation than those EW (Figure 3). However, this hypothesis could 551not be statistically confirmed with the single leaf gas exchange data reported in Table 5523. This, together with the limitations of extrapolating the gas exchange results from 553single-leaf level to the whole-canopy (Poni et al. 2009, Tomàs et al. 2012), although to 554a lesser extent in well-watered vines (Medrano et al. 2015a), suggest that the 555explanation for this effect may be attributed to different reasons. First, the higher direct 556radiation interception in NS rows could have affected thermal distribution within the 557canopy (Albasha et al. 2019) and thus increased transpiration, but also 558photorespiration and mitochondrial respiration in NS rows, and consequently reduced 559carbon assimilation efficiency (Amthor 1989). It should not be overlooked that daily 560respiration accounts for more than 25% of daily total photosynthesis (Poni et al. 2006, 561Escalona et al. 2012). Hunter et al. (2016) reported a greater portion of diffuse light

562intercepted by vineyard EW rows than those NS, which may be related to higher 563radiation use efficiency (Petrie et al. 2009). Moreover, the nonlinearity of the 564photosynthetic response to direct-light could be pointing to some degree of 565photoinhibition in the canopies oriented NS. Secondly, the greater photosynthesis of 566NS would have been used to synthesize other organic compounds, besides 567carbohydrates and woody structures, to a greater extent than in EW because, under 568high light intensities, alternative biochemical pathways can be stimulated to protect 569leaves and fruits from photo-oxidative damages (Grappadelli and Lakso 2007, Losciale 570et al. 2010).

In summary, the findings from the current work, encourage further studies on 572sunlight interception and canopy architecture designs as potential techniques for 573adapting vineyards to climate change. Field research under different soil water 574availability conditions and particularly under deficit irrigation should be carried out. In 575any case our research demonstrates that under no soil water limitations, grapevine 576consumptive water-use can be reduced by row orientation. In an irrigated area, 577adopting the E-W row orientation might therefore reduce the pressure on the available 578water resources by reducing the consumptive water-use. An aspect of importance in 579semi-arid environments with chronic water scarcity conditions where tools for 580decreasing water-use in addition to increase the agro-ecosystem water productivity are 581needed.

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5835. Conclusions

This experiment quantified the effects of row orientation on grapevine water-use 585on potted vines of two grapevine cultivars. Vine transpiration was reduced in EW 586compared to the NS orientation, although to a lesser extent than simulated IPAR. 587Specifically, it was decreased seasonally by 16% in Bobal and by 8% in Verdejo, 588corresponding to an 18% per unit of leaf area in both cultivars. Carbon balance was not

589markedly affected by row orientation. Consequently, WP increased significantly across 590seasons by 25% in EW compared to NS. Therefore, at Mediterranean latitudes, where 591solar radiation falls more perpendicular to the Earth's surface during the grapevine 592growing season, modifying canopy sunlight interception by orienting hedgerows EW 593instead of NS, reduces grapevine radiation load, which under no water deficit 594conditions, decreases WU and can improve WUE. Further research, especially under 595field conditions, is needed to determine the effects of vineyard row orientation under 596different degrees of water stress, paying attention to the potential effects on grape 597composition for winemaking purposes.

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855Supplementary Figure



856 857Supplementary Figure. A photograph showing the pot experiment with vines NS-oriented and 858EW-oriented.