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Title: Effects of leaning grapevine canopy to the West on water use efficiency and yield under Mediterranean conditions

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Abstract

This study tested the possibility of improving whole-canopy water use efficiency (WUE) of grapevines (cv. Bobal) by maximizing radiation interception during the mornings and limiting this during the afternoons, when the vapour pressure deficit and the evaporative demand are higher. The three-year study consisted of two trials conducted in parallel on North-South row oriented potted- and field-grown grapevines. In both trials, performance in terms of vine water use, yield and WUE in a vertical shoot positioned (VSP) system was compared with that of vines leaned 30º towards West (WSP). Potted vines were fully irrigated, whereas field-grown vines were submitted to rain-fed and deficit irrigation conditions. In potted plants, there was no difference in daily transpiration between vines from the WSP and VSP treatments, but transpiration in the mornings was higher in WSP vines. Dry matter and berry size increased in WSP compared to VSP vines. In the field, watering regime had a greater effect than canopy inclination on vine performance. Nonetheless, the WSP system increased leaf area by 13%, yield by 12% and WUE by 11% compared to VSP, although differences in WUE were not statistically significant and the effect on yield was negligible under rain-fed conditions. In both trials, the WSP system did not have a major effect on grape composition (soluble solids, pH, total acidity, concentrations of anthocyanins and polyphenols). In conclusion, this pioneering three-year study proved that leaning vine canopies to the West increased grapevine performance despite the great effect that environmental conditions exerted each year on the data obtained. Further research is required to study the effects of different patterns of light interception on carbon balance and grape biochemical composition.

Keywords: Canopy management; intercepted radiation; *Vitis vinifera* (L.); water relations; gas exchange.

1. Introduction

In semiarid areas, water scarcity is a major limitation for crop production and the increase in drought events in the near future poses a huge challenge to agriculture (Schwabe et al. 2015). In this context, where supplementary water applications are needed for obtaining profitable yields (Fereres et al. 2011), research on strategies aimed at improving crop water use efficiency (WUE; dry matter produced per unit of water used) must be considered key goals for ensuring the sustainability of agriculture (Bhattacharya, 2019).

This is well known within the wine and viticulture industry where agronomic techniques have been studied to increase WUE (Flexas et al., 2010). Deficit irrigation (DI) strategies where vine water consumption is reduced by limiting water supply, and partial root zone drying (PRD) that induces partial stomatal closure in plants (and then reduces the water use) are among the techniques studied (Chaves et al., 2007; Intrigliolo and Castel, 2008; Romero and Martínez-Cutillas, 2012). Both techniques can improve WUE although their use at commercial scale has not been widely embraced by farmers for different reasons, including the cost and management complexity of implementing PRD (Sadras, 2009). On the other hand, water stress in vines under DI can have beneficial effects on grape composition (Intrigliolo et al., 2012; Buesa et al., 2017) but also a reducing effect on yield (Fereres and Soriano, 2007).

In viticulture, growers can manage canopy architecture to modulate sunlight exposure and regulate the microclimate surrounding the vines to favor yield and WUE, as well as berry composition (Smart, 1985; Reynolds and Vanden Heuvel, 2009; Van Leeuwen and Destrac-Irvine 2017). This is important because, apart from soil water availability, interception of solar radiation, which is determined by the architecture of the trellising and canopy training systems, has also a significant effect on grapevine water consumption and yield (Heilman et al., 1996; Williams and Ayars, 2005; Medrano et al., 2012). A wide range of training systems and trellis configurations exists and the choice of which to use depends on many factors such as grapevine variety, terrain, soil type, vineyard management, etc. For instance, training systems such as the open Lyre (Carbonneau, 1982) or the Y-shape (Palliotti, 2011) increase sunlight interception in vigorous vines while stratifying the canopy density, which makes these training systems useful for improving grape quality and its sanitary status under humid and cool regions. However, a higher canopy light interception increases vine water use and might lead vines to experience water stress (Intrigliolo and Lakso, 2011).

Intrigliolo and Lakso (2011) studied, over a single growing-season, the effects of leaning the canopies of North-South oriented vines (cv. Riesling) to the East and to the West on canopy light interception and whole-canopy gas exchange. Compared to a vertically shoot positioned (VSP) system, the West shoot positioned (WSP) system intercepted less light in the afternoon and increased the daily photosynthesis rate of the whole canopy. The result obtained by Intrigliolo and Lakso (2011) was in line with that of Poni et al. (1999, 2003), who indicated that gas exchange of the whole plant is a function of both total amount of light intercepted and time of the day when the maximum light interception occurs.

In grapevines, VSP is the canopy management system most widely used, preferentially in vineyards with a North-South row orientation, to ensure that light is intercepted uniformly on both sides of the canopy (Baeza et al., 2005). Moreover, canopy management practices have a relevant influence on berry composition (Spayd et al., 2002; Trought et al., 2017) and can play an important role as adaptation practices to the alterations that climate change may exert on grape composition, wine quality and typicity (Van Leeuwen and Destrac-Irvine 2017, Poni et al., 2018, Buesa et al. 2019).

The effects of canopy inclination, in comparison to VSP as a control, on WUE have seldom been studied and never for more than two growing seasons (Carbonneau, 2009; Intrigliolo and Lakso, 2011). The main objective of the present work was to study the mid-term (3 years) effects of a WSP system, in which canopies were leaned 30° to the West, using a VSP system as a control for comparison, on grapevine (cv. Bobal) performance in terms of water use, yield and WUE. Considering the great importance of grape composition in wine prices, the effect of the WSP system on berry composition was also studied. The hypothesis was that in North-South row-oriented vineyards, a WSP system could have a positive effect on WUE, in relation to the VSP control, due to a reduction of the solar radiation interception in the afternoon when vapor pressure deficit (VPD) is usually higher. To test this, two parallel trials were conducted over three growing seasons, on potted and field-grown vines. This research is of interest considering the projected scenario of limited water availability in the Mediterranean-like wine-growing regions and, particularly, of Spain (Fraga et al., 2016; Lorenzo et al., 2016).

2. Materials and Methods

This work consisted of two separate, but complementary trials conducted from 2012 to 2014 on grapevines (*Vitis vinifera* L.) cv. Bobal grafted onto the rootstock 110 Richter (110-R). One trial was carried out on potted vines while the other was performed in the field on plants from a commercial vineyard. Both vineyards had training systems in North-South oriented rows with 2.5 meters separation between them. The WSP training system was established at the beginning of the trials by adding leaned posts, which supported the catching wires and the canopy, to the existing vertical trellis system (Figure S1). For clarity purposes, the trials will be described independently.

2.1. Potted vines: trial location, experimental design and measurements

This trial was conducted in an experimental farm at the Valencian Agriculture Research Institute (IVIA) located in Moncada, Valencia, Spain (39º 30’ N, 0º 24’ E, elevation 68 m). The climate in this area is Mediterranean, with an average annual rainfall and reference evapotranspiration (ET_o) of 396 and 1064 mm (2001-2012), respectively.

Performance of vines wit shoots leaned 30º to the West (WSP) was compared with that of vines managed in a traditional VSP system (vertical-trained shoots). Each treatment consisted of 10 vines each. Vines were grown outdoors in a 2.5 m x 2 m grid with border vines surrounding the ones that were monitored during the study.

In 2012, one-year-old unfruited grapevines were planted in 40-L pots. Coconut fibre mixed with compost was used as a substrate, which was covered with plastic in order to minimize evaporation. Vines had a total of two to four shoots, which were hedged once in July. Plants were hand-watered two to three times per day to ensure that no soil water limiting conditions occurred. Since unfruited vines were used during this first year of study, roots and the aboveground biomass of sampled vines were collected for dry matter determinations.

In 2013, two-year-old fruiting vines were purchased and grown in 70-L containers with the same type of substrate used in the previous growing season. The same vines were used the subsequent, and last, year of study. Vines were winter pruned on a bilateral cordon system to leave 11 to 15 shoots in 2013 and 16 to 20 shoots in 2014. Canopy was supported by means of three pair of steel catch wires. Irrigation was supplied by means of a drip irrigation system with three emitters (4 L h^{-1}) per pot. Plants were watered three to four times per day to ensure that no water limiting conditions occurred.

Weather data during the study were collected from an automatic meteorological station located 100 m from the experimental site. The ET_o was calculated according to Allen et al. (1998). During the three years of study and throughout the growing season, April to September, rainfall ranged from 80 to 165 mm. Rainfall was similar in the first and third years of study and 2013 was the wettest year. The ET_o ranged between 832 and 892 mm (Table 1). Vines were fertigated every growing season with 30-20-60-7.5 kg ha^{-1} of N, P_2O_5, K_2O, and MgO, respectively, to avoid nutritional deficiencies.

2.2. Field trial: location, experimental design and measurements

The field trial was carried out in a commercial farm located in Requena, Valencia, Spain (39º 29' N, 1º 13' W, elevation 750 m). Vines were planted in 2002 at a spacing of 2.5 m x 1.45 m (3625 vines ha^{-1}). The soil at this site is a Typic Calciorthid, common in the region, with a clay loam to light clay texture, highly calcareous and with low fertility (0.66% organic matter, 0.04% nitrogen). The soil profile is more than 2 m deep, bulk density ranges from 1.43 to 1.55 t m^{-3} and available water capacity is 180 mm m^{-1}, approximately. This area has a semiarid continental climate with 394 mm of average annual rainfall (65% falls during the dormant period) and ET_o of 1108 mm (2001-2012). During the studied vine-growing seasons, April to September, rainfall ranged from 96 to 249 mm, while ET_o ranged between 861 and 946 mm (Table 1).

The combination of two factors, water regime and canopy management, was tested in this trial. The watering regimes tested were no irrigation (rain-fed) and a regulated deficit irrigation (DI) strategy. The canopy management techniques tested were a VSP system and a WSP system where shoots were leaned 30º to the West.

The experimental setup was a two by two factorial randomized split-plot design with four replicates per treatment and the watering regime as the main factor. The experimental units consisted of five rows with 7-9 vines each (35-45 vines per plot). Only vines from the three central rows were selected for the measurements while the remaining rows were used as buffers.

Irrigation in the DI regime occurred when vines reached a midday stem water potential (Ψ_{stem}) value of -1.0 MPa. Vines were irrigated at 35% of the crop evapotranspiration (ET_c), computed as the product of ET_o and a crop coefficient (k_c) that increased gradually from June (0.25) to August (0.55) according to López-Urrea et al. (2012). Water was applied by means of drippers (4 L h^{-1}) spaced 1.25 m apart. Irrigation in 2012, 2013 and 2014 amounted to 90.5, 64.5 and 126.6 mm, respectively (Table 2).

In all the seasons, vines were pruned in winter and trained to a bilateral cordon system leaving six two-bud spurs per vine, which is the usual practice among vine-growers in the region, and fertigated with 30-20-60-16 kg ha^{-1} of N, P_2O_5, K_2O, and MgO, respectively.

2.3. Physiological measurements

2.3.1 Leaf area and dry matter

Leaf area (LA) was estimated using a linear equation relating LA to shoot length (main plus laterals shoots), following the procedure described by Buesa et al. (2019). Briefly, this relationship was established by measuring LA (LI-3100 Area Meter, LI-COR

Inc., Lincoln, NE, USA) and total shoot length in 10 shoots. In the field, shoots were collected from border vines of each canopy inclination (five from each water regime) after veraison when shoot growth was minimal. Afterwards, shoot length was determined in five vines per experimental unit. In the potted vines, shoots were collected from the border vines and shoot length was measured six times within each season. On each date, shoot length was determined in all experimental vines. At the end of the first growing season (2012), in the potted vines, roots and the aboveground biomass of 10 vines per treatment were collected, oven-dried at 70°C for 48 h and re-weighed to determine dry matter.

2.3.2 Water use

Daily water use in potted vines was determined on selected days by weighing all the pots at 5am (solar time) on consecutive days (24 hours). In addition, pots were also weighed at 12pm to assess separately morning (5am to 12pm) and afternoon (12pm to 5am) water use. During the first season of study, pots were weighed weekly (14 times/season). No irrigation was applied during the 24 hours when pot weight determinations were conducted. In the subsequent seasons (2013 and 2014), pots were weighed fortnightly (7 times/season). Vines were more vigorous than in 2012 and were hand-watered with 2 L (ensuring the absence of drainage) between weight determinations in order to prevent them from experiencing water stress. Seasonal water use was obtained from the sum of water consumed at each of the measurement periods. These were estimated by multiplying the number of days between measurements by the average daily water use in that period.

In the field, water flow meters (Model CZ3000, Contazara S.A., Zaragoza, Spain) were used to monitor the amount of water applied to each experimental unit.

2.3.3 Plant water status

Midday Ψ_{stem} (Choné et al., 2001; Santesteban et al., 2019) was determined fortnightly in both trials on a single leaf per vine using a pressure chamber (Model 600, PMS Instruments Inc., Albany, OR, USA) to monitor plant water status. In order to assess whether differences in vine performance among canopy orientations could be explained by differences on vine water status over the day, diurnal patterns of Ψ_{stem} were assessed from pre-dawn to evening. In potted vines, these measurements were carried out on 26 June 2013 and 24 July 2014. In the field trial, the Ψ_{stem} diurnal patterns were conducted on 17 July and 22 August 2012. In potted vines, Ψ_{stem} was measured on a single leaf per vine in four vines per treatment, whereas in the field trial, determinations were carried out in eight vines per treatment combination (water regime and canopy management).

Whole-canopy gas exchange measurements were simultaneously conducted in six potted vines (three per canopy inclination, Figure S2) on 07 August 2013 during 48 hours. The gas exchange chambers were designed according to Poni et al. (1997) and its functioning is detailed elsewhere (Ballester et al., 2011). Briefly described, the chamber encompasses the entire plant and a fan provides a constant airflow that enters the chamber from the bottom and exits through a chimney located on the top. The whole-canopy net CO_2 exchange rate (NCER) and whole-canopy transpiration were determined by calculating the difference in CO_2 and water vapor partial pressure between the output and the input of each single chamber using an infrared gas analyzer (LI-840, LI-COR, Lincoln, NE, USA). Instantaneous water-use efficiency (WUE_i) of the canopy was computed as the ratio of NCER to transpiration. This methodology has been previously used in several studies for the assessment of WUE (Merli et al., 2016, Douthe et al., 2018).

2.3.4 Yield, WUE and berry composition

Total yield per vine, number of clusters per vine and average cluster weight were determined on all vines from each replicate in both trials. These data were not available for the one-year-old potted vines in the 2012 growing season. The number of berries per cluster was determined from one randomly collected cluster per vine. Samples of 200 berries per replicate were randomly collected and the average berry weight determined.

Water use efficiency in potted vines was determined as the ratio between dry matter produced in 2012 and yield in subsequent seasons, and the total amount of irrigation applied (g L^{-1}). In the field trial, WUE was estimated as the ratio between yield and the total amount of irrigation applied plus rainfall.

Berry composition was assessed at harvest on the same 200 berries sampled to determine berry weight. Half of the sample (100 berries) was crushed for 30 s at speed 6 with a blender (Thermomix, Vorwerk, Wuppertal, Germany) and hand-pressed through a metal screen filter to obtain the juice for the evaluation of technological maturity (total soluble solids content, TSS; titratable acidity, TA; and pH). Juice TSS was determined by refractometry (PR-101 Series Palette, Atago Co. LTD, Tokyo, Japan); pH and TA were measured with an automatic titrator (Metrohm, Herisau, Switzerland). Juice was titrated with a 0.1 N solution of NaOH to an endpoint of pH 8.2 and results were expressed in tartaric acid equivalents. The other half of the sample was homogenised for 60 s at speed 3 with a blender (Ultraturrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany) to assess the phenolic composition. One gram of the resultant paste was diluted in 10 mL of ethanol 50% for measuring the optical density (nm) by UV/VIS spectrophotometry (Lambda 35, PerkinElmer Inc., MA, USA). Total anthocyanins and total phenolic compounds were calculated according to Ribereau-Gayon et al. (2000). All determinations were replicated twice and results averaged.

2.4. Statistical analysis

The statistical analysis was performed with R v.3.4.1 software (R Core Team, 2017). Two and three-way analyses of variance (ANOVA) were used to determine the existence of significant differences among treatments in the parameters measured, as well as to examine the interactions among factors in the potted vine and field trials. In the trial conducted on potted vines, year of study and canopy inclination were the factors considered. In the field trial, the watering regime was the third factor considered. Mean values of the parameters assessed in the field trial were separated using the Tukey Honest Significant Difference (HSD) test at a significance level of 0.05.

3. Results

3.1. Potted vines

3.1.1 Vegetative growth

In 2012, LA tended to be greater (p = 0.065) in the WSP (3.20 m^2) treatment than in the VSP (2.88 m^2) due to a greater growth of lateral shoots. This result together with the greater root and trunk dry weights observed in vines trained with the WSP system (920 g per vine) compared to VSP (841 g per vine) resulted in statistically significant differences in the total vine dry matter production between treatments (9%). For the subsequent growing seasons, when the vines were productive and they were not replaced at the end of the season, the significance of canopy inclination and year on the different parameters assessed and their interaction was analyzed (Table 3). There was no interaction between year and canopy inclination for the vegetative growth, and thus these results along with the yield components and WUE were analyzed and presented on average for the two growing seasons. No differences in LA and pruning weight were observed between treatments.

3.1.2 Plant water status and water use

The effect of canopy inclination on plant water status was negligible and only occurred during the afternoon, Ψ_{stem} was significantly more negative in the VSP treatment than in the WSP (Figure 1). In fact, in most of the measurement dates in all three seasons, midday Ψ_{stem} was > -0.8 MPa in both treatments (data not shown).

In 2012, water use of WSP and VSP vines was similar (175 L and 164 L, respectively), as it was WUE (5.27 g L^{-1} in WSP versus 5.12 g L^{-1} in VSP). There was a significant interaction between canopy inclination and year for the morning water use indicating not consistent results for this parameter (Table 3). Morning transpiration was higher in vines with a WSP system than in vines with a VSP system in the three growing seasons (16%, 8% and 13%, respectively) although differences in transpiration were only statistically significant in 2012 and 2014 (Table 4). In the afternoon, water use in the WSP treatment was on average 6% lower than in the VSP, but differences were not statistically significant. On a daily basis, WSP vines transpired more ($p < 0.05$) than those from VSP only in 2012 (Table 4). However, differences between treatments disappeared when daily water use was expressed per unit of LA.

Whole-canopy gas exchange measurements showed that in both treatments (VSP and WSP) there was a greater gas exchange in the morning than in the afternoon. Transpiration in vines from the WSP treatment in the morning (2.62 mmol m^{-2} s^{-1}) was twice that in the afternoon (1.42 mmol m^{-2} s^{-1}). In the VSP system, however, transpiration in the morning was 50% greater than in the afternoon (Figure 2). These results led the vines from the WSP treatment to have a higher (p < 0.05) WUE_i (6.40 mmol mol^{-1}) in the afternoon than those from the VSP treatment (5.28 mmol mol^{-1}), while no differences were observed in the morning.

3.1.3 Yield and grape composition

Canopy inclination did not have an effect on yield and most of its components (cluster per vine, cluster weight and berries per cluster). Only the berry weight was significantly increased in vines from the WSP system (Table 5).

Vines from the WSP treatment reached veraison earlier than vines from the VSP treatment and, consequently, berries from the WSP system had a higher (p <0.05) TSS and pH, and a lower TA (Table 6). No statistically significant differences were observed in polyphenols and anthocyanins between treatments.

3.2. Field trial

3.2.1 Plant water status

Because of the dry environmental conditions in 2012 (Table 1), severe water stress was observed in vines from both water regimes, and particularly in the rain-fed vines, as indicated by the Ψ_{stem} measurements (Figure 3). During that year, rain-fed and DI vines reached on average midday Ψ_{stem} values around -1.5 MPa and -1.2 MPa, respectively. Less negative midday Ψ_{stem} values were reached in rain-fed and DI vines in 2013 and 2014. Within each watering regime, there were not significant differences in Ψ_{stem} between the treatments, although vines from the WSP treatment tended to show less negative values (reduced water deficit) than those from the VSP vines in the driest seasons, 2012 and 2014 (Figure 4).

The diurnal patterns of Ψ_{stem} showed that, before noon, similar values were observed in vines from the WSP and VSP treatments in both irrigation treatments (Figure 4). In the afternoon, the Ψ_{stem} of rain-fed vines was 0.10 to 0.15 MPa less negative in vines with a WSP system than in those with a VSP system. A similar trend was observed for DI vines in the afternoon in the measurements conducted on 17 July 2012, but not later in the season.

3.2.2 Vegetative growth, yield and its components and WUE

Table 3 shows the significance of the effects that year, watering regime and canopy inclination had on the traits assessed in the field trial. The year of study had a significant influence on most of the traits with the exception of clusters per vine, berries per cluster and yield (Table 3). Significant interactions between year and watering regime were detected for LA, clusters per vine, cluster weight, yield, berries per cluster, berry weight, WUE and pH.

The watering regime had a significant effect on all the vegetative growth, yield and its components. DI vines had the highest values in all the cases (Table 5). LA, pruning weight, yield and berry weight were respectively 25%, ~40%, 59% and 25% higher ($p < 0.05$) in the DI treatment than in the rain-fed. Nonetheless, the watering regime did not significantly affect WUE.

Canopy inclination had a significant effect on LA, cluster weight, yield and number of berries per cluster. LA increased by 13% in vines from the WSP treatment compared to those of the VSP (Table 5). Over the three years, WUE was 11% higher in vines trained in a WSP system than in those trained in a VSP system (both treatments received on average 93.9 mm season^{-1}: Table 2), although this difference was not statistically significant. When assessing the effects of each combination of watering regime and canopy inclination with more detail (Table 5), the biomass pruned from the WSP vines was 17% higher ($p < 0.05$) than that of the VSP vines only for the rain-fed treatment. In contrast, cluster weight and yield were respectively 32% and 18% higher ($p < 0.05$) in the WSP treatment than in the VSP only under DI. The number of berries per cluster was greater ($p < 0.05$) in the WSP treatment than in the VSP in both water regimes (Table 5).

3.2.2 Berry composition

The results obtained suggest that watering regime exerted a greater effect on berry composition than canopy inclination (Table 3). In fact, TSS was 7% higher in berries from the rain-fed treatment than in berries from the DI treatment (Table 7). Berries from rain-fed vines had also higher values of TA than those of DI vines. This was particularly noticeable in vines from the WSP treatment. In contrast, juice pH did not differ among treatments (Table 7). The concentrations of phenolic compounds were also higher in berries from the rain-fed treatment than in those from the DI. Moreover, polyphenols content was significantly higher in berries from the WSP treatment than in the VSP treatment regardless of the watering regime.

4. Discussion

The study here presented provides new insights into the medium-term (three years) effects of intercepting the highest solar radiation in the morning hours (by leaning canopies towards the West) on the vegetative and reproductive growth, yield and WUE of grapevines cv. Bobal. Very often, vine growers decide architecture of trellising systems considering manageability factors rather than agronomic or physiological criteria (a decision that is permanent for the whole life of the vineyard) and accepting the possible negative consequences on yield (Hunter et al., 2016). This is so, notwithstanding that the training system and row orientation are considered the main agronomic practices that can influence the canopy radiation interception (Reynolds and Vanden Heuvel, 2009; Hunter et al., 2016).

In potted vines where no water restrictions were applied, total dry matter production was higher ($p < 0.05$) in the WSP system than in the VSP. This increase in dry matter production was most likely caused by the greater water use observed in vines from the WSP treatment during the morning hours (Table 4) when WUE is highest (Baeza et al., 2005). Daily water use, however, was similar between vines from both canopy inclinations. Our results are in line with those reported by Intrigliolo and Lakso (2011) in a study conducted on cv. Riesling vines grafted onto 101-14 under cooler climatic conditions than in the present study. Intrigliolo and Lakso (2011) observed that leaning vines to the West increased transpiration and NCER during the morning hours, while improved WUE_i in the afternoon. A similar response, although to a lesser extent, was observed here for the cv. Bobal (Figure 2). Among the reasons for the lower effect of canopy inclination on WUE_i in our study could be the different atmospheric demands in the locations where the studies were conducted (higher ET_o and VPD in our research), different soil characteristics and the different combinations of cultivar-rootstock used, which may have distinct sensitivities to the modified pattern of direct and diffuse radiation interception (Petrie et al. 2009; Tomás et al. 2014; Hunter et al., 2016).

In the field trial, there was an interaction between the year of study and the watering regime applied, which highlights the relevance that rainfall and plant water status have in the Mediterranean viticulture (Mirás-Avalos and Intrigliolo, 2017). On the contrary, the effect of intercepting the highest solar radiation in the morning hours on vine performance was consistent among growing seasons and between the watering regimes (Table 3). Over the three years, the WSP treatment increased LA by 13% and yield by 12% compared to the VSP treatment (Table 5) in concordance with the increase in total dry matter reported for potted vines. Training systems affect sunlight interception and grapevine water status via effects on exposed leaf area (Kliewer and Dokoozlian, 2005; Williams and Ayars, 2005). For instance, minor canopy modifications such as an increase in trellis height rises the plant water needs (Mirás-Avalos et al., 2017). In the current study, despite both potted and field-grown vines showed similar midday Ψ_{stem} values regardless of canopy inclination (Figures 1 and 3), vines trained to WSP system in the pot trial showed a slightly greater transpiration than those trained to VSP system only on specific dates (Table 4). In the field trial, the moderate and severe water stress levels reached in the DI and rain-fed treatments, respectively, limited the vigour of the plants (Intrigliolo et al., 2012, Buesa et al., 2017). Regardless, slight improvements in vine water status in response to leaning vines to the West were only observed in both trials during the afternoon hours (Figures 1 and 4), in response to variations in sunlight interception (Carbonneau and Constanza, 2004).

The higher ($p < 0.05$) yield obtained in the field-grown vines under the WSP system was most likely caused by maximizing the radiation interception during the morning when leaf photosynthesis rates are generally high and minimizing this interception in the afternoon when VPD, and thus the evaporative demand, is higher. This led vines to have higher transpiration rates during the hours with lower evapotranspiration demand in the day (morning) and lower transpiration rates during the most demanding hours (Table 4). Nevertheless, it has to be noted that the effects of canopy inclination on vine performance were lower in the rain-fed treatment than in DI (Table 5). This could be explained by different factors; first, vines had a lower LA under rain-fed conditions than under DI;

second, vine water stress affects leaf angles and thus reduces leaf exposure to solar radiation (Smart et al. 1985; Wang and Li 2013); and third, rain-fed vines probably had a higher stomatal regulation and hydraulic limitations (Drake et al. 2017; Buesa et al. 2019). Therefore, rain-fed vines had more porous canopies with less-exposed leaves resulting in a less clear effect of canopy inclination on radiation interception in addition to an expected lower gas exchange.

The trend observed towards smaller berries in potted vines with a VSP system seems to be in accordance with Hulands et al. (2014), who observed that increased light intensities reduced the berry size and sugar contents. Since WSP vines received less solar radiation in the afternoon, when light intensity is higher, and this can have a cooling effect that reduces grape transpiration (thus water loss from the grape), the increase in berry size observed in WSP potted vines could be expected. However, under field conditions this increase in berry size did not occur, possibly due to the effect of temperature under water-stress conditions and more porous vine canopies (Bonada et al. 2013), and it was the number of berries per cluster the parameter that increased instead. Increments on both berry weight and number of berries per cluster could be related to the slightly better vine water status observed in both trials and in all three seasons during the afternoon (Figures 1 and 4). Because of this, fruit set could be improved and, given the great influence that minor changes in vine water status can have on cell turgor, berry growth could also be enhanced (De la Hera et al. 2006; Intrigliolo and Castel, 2008; Gambetta et al. 2010). In addition, vine development is based on reserves mobilization from budburst to flowering. It is only after flowering when vines are capable to produce enough photo-assimilates to meet their developmental requirements (Smith and Holzapfel, 2009; Intrigliolo et al. 2018; Frioni et al. 2019). During this period, the higher photosynthetic capacity of WSP vines compared to the VSP (Figure 2) could have improved fruit set. On the other hand, in the potted vines that were fully fertirrigated and therefore might have had an optimum nutritional reserves status, fruit set was not modified by the canopy inclination and the improved vine physiological performance due to the WSP training was reflected in the larger berry weight recorded at harvest. Nevertheless, the significant increases in some yield components observed in WSP vines compared to VSP in both trials did not result in a statistically significant increase in WUE despite similar water use between treatments.

Regarding the effects of treatments on berry composition, data collected over the three seasons are not fully conclusive. Contrary to Hulands et al. (2014), there was not a clear response of TSS to the canopy inclinations (Tables 6 and 7). Only grape polyphenolic content showed a slight tendency to increase in response to canopy inclination, although this was only statistically significant under field conditions. In potted vines, this effect could have been mitigated by the low levels of grape ripeness (Table 6) due to early harvesting performed to avoid yield losses caused by fungal diseases, as well as due to the increased berry weight in WSP (Table 5). The latter given the dilution effect that berry size has on TSS, TA and phenolic compounds in this cultivar (Salón et al., 2005). These effects were clearly observed in the field trial when comparing DI to rain-fed regardless of the canopy inclination (Table 7). Therefore, the trend towards an increase in polyphenols concentration in the WSP treatment might be pointing out to a slight improvement in cluster microclimate conditions for the synthesis of these compounds. Bergqvist et al. (2002) and Spayd et al. (2002) showed that phenolic compounds in grape skins are negatively affected by high temperatures but in turn these increase with light interception. It should be noted that the likely increase in shade in the WSP clusters reduces both light intensity and air temperature in the cluster zone during the afternoon, when air temperature is higher, compared to that occurring in VSP. The overall low response of sugar content and color parameters to canopy inclinations in both trials led us to conclude that leaning the canopies towards the West does not cause major effects on ‘Bobal’ berry composition under the environmental conditions of the present research. Nonetheless, contrasting responses of grape composition to maximizing light interception during the morning hours cannot be ruled out under different conditions and cultivars to those of the present study due to the complex interactions of grapevine canopies and the environment.

5. Conclusions

This is the first study that assessed the agronomic response of grapevines (cv. Bobal) to a canopy management consisting in leaning shoots towards the West over three consecutive seasons and under different watering regimes. Vines from the WSP treatment maximized light interception in the morning and consequently transpiration increased in these hours in comparison to the most common system, the VSP. These increases in response to West leaning canopies, and coupled with temporary improvements in vine water status had an increasing effect on vegetative growth and yield in deficit-irrigated field-grown vines and on berry weight in potted fully-irrigated plants. Canopy inclination had little effect on grape composition and differences in WUE among training systems were not statistically significant. Further research would be required to study the effects of different light interception patterns on the carbon and water balances and grape biochemical composition.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Monthly rainfall, reference crop evapotranspiration (ET_o) and average atmospheric vapor pressure deficit (VPD) at the sites where the potted vine and field trials were conducted during 2012, 2013 and 2014.

Month	2012			2013			2014		
	Rainfall (mm)	ET_o (mm)	VPD (kPa)	Rainfall (mm)	ET_o (mm)	VPD (kPa)	Rainfall (mm)	ET_o (mm)	VPD (kPa)
Potted vine trial									
April	35.8	123.2	0.95	76.4	104.1	0.80	9.9	130.7	1.12
May	1.7	151.0	1.18	14.4	139.4	1.03	8.9	151.3	1.16
June	4.9	166.0	1.33	7.7	162.1	1.28	10.4	162.8	1.42
July	0.0	167.5	1.23	3.2	178.9	1.48	23.6	178.2	1.49
August	0.0	148.1	1.42	62.3	135.9	1.30	1.5	152.9	1.39
September	37.6	109.5	1.28	0.7	112.1	1.19	32.2	115.7	1.31
Total	*80.0*	*865.2*	*1.23*	*164.7*	*832.5*	*1.18*	*86.5*	*891.7*	*1.32*
Field trial									
April	46.9	97.1	0.67	97.5	99.3	0.81	10.8	138.2	1.27
May	8.2	154.4	1.35	60.2	131.9	0.90	0.8	159.1	1.34
June	21.2	188.2	2.07	11.3	167.5	1.52	14.8	168.7	1.79
July	0.2	198.1	2.29	58.3	192.3	1.96	5.1	195.4	2.21
August	0.2	174.6	2.57	14.9	155.8	1.87	0.0	173.8	2.19
September	37.7	111.1	1.51	6.9	114.2	1.47	64.7	111.2	1.61
Total	*114.4*	*923.5*	*1.74*	*249.1*	*861.1*	*1.42*	*96.2*	*946.4*	*1.73*

Table 2. Irrigation water amounts applied at pre- and post-veraison to the deficit irrigation treatment in the field trial in each year of study. Data shown are means and standard errors of four replicates.

Growing Season	Irrigation water amounts applied (mm)		
	Pre-veraison	Post-veraison	Total
2012	34.4 ± 1.4	56.1 ± 1.6	90.5 ± 1.6
2013	26.6 ± 1.1	37.9 ± 1.5	64.5 ± 1.4
2014	89.0 ± 0.6	37.6 ± 0.5	126.6 ± 0.5

Table 3. Results of the ANOVAs (*p* value) conducted to assess the effects of canopy inclination, year of study and their interaction on the parameters assessed in the trial on

potted vines during 2013 and 2014, and the effects of canopy inclination, watering regime, year of study and their interaction on the parameters assessed in the field trial.

Parameter	Pot trial			Field trial						
	Inclination (I)	Year (Y)	I x Y	Inclination (I)	Water regime (W)	Year (Y)	I x W	I x Y	W x Y	I x W x Y
Leaf area	0.656	**< 0.001**	0.533	**0.017**	**< 0.01**	**< 0.01**	0.469	0.343	**< 0.01**	0.909
Pruning weight	0.206	**< 0.001**	0.076	0.196	**0.046**	**0.013**	0.434	0.143	0.607	0.178
Clusters per vine	0.612	0.760	0.363	0.869	**0.024**	0.077	0.739	0.909	**< 0.01**	0.625
Cluster weight	0.782	0.092	0.110	**< 0.01**	**< 0.01**	**0.002**	0.558	0.068	**< 0.01**	0.503
Yield	0.52	0.394	0.096	**< 0.01**	**< 0.01**	0.156	0.095	0.535	**< 0.01**	0.302
Berries per cluster	0.526	**0.003**	0.553	**< 0.01**	**< 0.01**	0.153	0.987	0.276	**< 0.01**	0.933
Berry weight	**0.034**	**< 0.001**	0.061	0.087	**< 0.01**	**< 0.01**	0.599	0.993	**< 0.01**	0.293
WU daily	0.347	**< 0.001**	0.134							
WU morning	**< 0.001**	**< 0.001**	**0.023**							
WU afternoon	0.069	**< 0.001**	0.519							
WUE	0.808	**< 0.001**	0.357	0.143	0.122	**< 0.01**	0.567	0.708	**< 0.01**	0.878
Total soluble solids	**0.014**	**0.029**	**< 0.001**	0.373	**< 0.01**	**< 0.01**	0.809	0.426	0.575	0.316
pH	0.347	**0.001**	**0.001**	0.124	0.094	**< 0.01**	0.973	0.673	**< 0.01**	0.079
Titratable acidity	**0.003**	**< 0.001**	**0.005**	0.785	**0.020**	**< 0.01**	0.234	0.884	0.219	0.416
Anthocyanins	0.078	**0.106**	0.709	0.993	**< 0.01**	**< 0.01**	0.858	0.261	0.754	0.269
Polyphenols	0.449	**< 0.001**	0.287	**0.035**	**< 0.01**	**< 0.01**	0.155	0.279	0.673	0.834

Bold values indicate statistically significant effects of a given factor on a given parameter. WU = Water use; WUE = Water use efficiency.

Table 4. Average daily, morning and afternoon water use (L) and standard errors in potted vines trained with a West shoot positioned (WSP) and vertical shoot positioned (VSP) system in 2012 (n = 13), 2013 (n = 7) and 2014 (n = 5).

Year	Treatment	Morning	Afternoon	24 h
2012	**VSP**	0.97 ± 0.04 a	1.39 ± 0.11	2.54 ± 0.08 a
	WSP	1.15 ± 0.05 b	1.28 ± 0.09	2.78 ± 0.07 b
2013	**VSP**	2.43 ± 0.15	2.74 ± 0.16	5.27 ± 0.13

	WSP	2.63 ± 0.18	2.50 ± 0.14	5.15 ± 0.12
2014	**VSP**	4.42 ± 0.13 a	4.76 ± 0.17	8.96 ± 0.27
	WSP	5.10 ± 0.13 b	4.60 ± 0.15	9.70 ± 0.29

Different letters indicate significant differences between canopy inclinations for a given period within a year ($p < 0.05$).

Table 5. Average values of leaf area, pruning weight, yield components and water use efficiency (WUE) in grapevines trained with a West shoot positioned (WSP) and vertical shoot positioned (VSP) system both, in potted conditions and field-grown under deficit irrigation (DI) and rain-fed conditions. In both trials, data shown are means and standard errors of four replicates, for the 2013 and 2014 growing seasons in the pot experiment and for the 2012-2014 period in the field experiment.

Parameter	**Pot trial**		**Field trial**		
	VSP	**WSP**	**Water regime**	**VSP**	**WSP**
Leaf area (m^2 vine^{-1})	5.5 ± 0.5	5.8 ± 0.5	**Rain-fed**	2.1 ± 0.2 a	2.3 ± 0.2 ab
			DI	2.6 ± 0.1 bc	2.9 ± 0.1 c
Pruning weight (kg vine^{-1})	0.55 ± 0.1	0.59 ± 0.1	**Rain-fed**	0.26 ± 0.1 a	0.30 ± 0.1 b
			DI	0.38 ± 0.1 c	0.38 ± 0.1 c
Clusters per vine	6.6 ± 0.4	6.9 ± 0.4	**Rain-fed**	7.5 ± 0.4 a	7.3 ± 0.3 a
			DI	8.1 ± 0.3 b	8.1 ± 0.3 b
Cluster weight (g)	507 ± 62	521 ± 56	**Rain-fed**	263 ± 8.7 a	261 ± 9.9 a
			DI	329 ± 10.0 b	365 ± 8.6 c
Yield (t ha^{-1})	7.0 ± 0.7	7.5 ± 0.6	**Rain-fed**	3.6 ± 0.6 a	4.0 ± 0.5 a
			DI	5.4 ± 0.6 b	6.3 ± 0.5 c
Berries per cluster	245 ± 41	224 ± 43	**Rain-fed**	129 ± 7 a	167 ± 8 b
			DI	134 ± 7 a	165 ± 8 b
Berry weight (g)	2.6 ± 0.1 a	2.8 ± 0.1 b	**Rain-fed**	2.0 ± 0.1 a	1.9 ± 0.1 a
			DI	2.5 ± 0.1 b	2.4 ± 0.1 b
WUE (g L^{-1})	4.2 ± 0.3	4.3 ± 0.2	**Rain-fed**	3.2 ± 0.3	3.4 ± 0.4
			DI	2.7 ± 0.2	3.2 ± 0.1

Different letters indicate significant differences for a given parameter between canopy inclinations in the pot experiment or between watering regimes and canopy inclinations in the field experiment ($p < 0.05$).

Table 6. Average values of total soluble solids (TSS), pH, titratable acidity, polyphenols and anthocyanins concentration in berries of potted vines trained with a West shoot

positioned (WSP) and vertical shoot positioned (VSP) system in the 2013 and 2014 growing seasons. Data shown are means and standard errors of four replicates.

Year	Treatment	TSS	pH	Titratable acidity	Polyphenols	Anthocyanins
		º Brix		g L^{-1} tartaric acid	mg g^{-1}	
2013	**VSP**	15.5 ± 0.1	3.30 ± 0.02	5.4 ± 0.1	1.94 ± 0.05	0.56 ± 0.04
	WSP	15.2 ± 0.1	3.37 ± 0.02	5.4 ± 0.1	1.97 ± 0.05	0.50 ± 0.04
2014	**VSP**	15.2 ± 0.1 a	3.20 ± 0.01 a	7.0 ± 0.1 b	1.63 ± 0.06	0.63 ± 0.05
	WSP	16.3 ± 0.1 b	3.30 ± 0.01 b	6.0 ± 0.1 a	1.92 ± 0.07	0.81 ± 0.06

Different letters indicate significant differences between canopy inclinations for a given parameter and year ($p < 0.05$).

Table 7. Average values of total soluble solids (TSS), pH and titratable acidity (TA), anthocyanins and polyphenols concentration in berries from vines trained with a West shoot positioned (WSP) and vertical shoot positioned (VSP) system under deficit irrigation (DI) and rain-fed conditions for the 2012-2014 period. Data shown are means and standard errors of four replicates.

Parameter	Water regime	VSP	WSP
TSS (ºBrix)	**Rain-fed**	22.5 ± 0.3 b	22.3 ± 0.2 b
	DI	21.1 ± 0.2 a	21.0 ± 0.2 a
pH	**Rain-fed**	3.38 ± 0.02	3.40 ± 0.02
	DI	3.40 ± 0.02	3.42 ± 0.02
TA (g L^{-1} tartaric acid)	**Rain-fed**	5.6 ± 0.1 ab	5.8 ± 0.1 b
	DI	5.4 ± 0.2 a	5.3 ± 0.2 a
Anthocyanins (mg g^{-1})	**Rain-fed**	1.03 ± 0.3 b	1.04 ± 0.3 b
	DI	0.81 ± 0.4 a	0.80 ± 0.4 a
Polyphenols (mg g^{-1})	**Rain-fed**	4.00 ± 0.5 b	4.25 ± 0.5 b
	DI	3.26 ± 0.7 a	3.32 ± 0.7 a

Different letters indicate significant differences between watering regimes and canopy inclinations for a given parameter according to the Tukey's test ($p < 0.05$).

Figure captions

Figure 1. Diurnal patterns of stem water potential (Ψ_{stem}) in potted grapevines (cv. Bobal) from the Vertical Shoot Positioned (VSP) and West Shoot Positioned (WSP) treatments on 26 June 2013 and 24 July 2014. Values are averages and standard errors of four determinations per treatment. Asterisks indicate statistically significant differences between canopy inclination systems for that particular date at $p < 0.05$. Vertical bars indicate the standard error.

Figure 2. Daily trends of hourly mean whole-canopy (a) transpiration, (b) net CO_2 exchange (NCE) and (c) instantaneous water use efficiency (WUE_i) calculated as the ratio NCE to transpiration for potted cv. Bobal grapevines in Moncada, Valencia, Spain. Values are hourly averages of three vines per treatment and two days of measurements (7th and 8th August 2013). Vertical bars indicate the standard error. Asterisks mean significant difference between canopy management systems at $p < 0.05$. VSP = Vertical Shoot Positioning; WSP = West Shoot Positioning.

Figure 3. Seasonal pattern of midday stem water potential in the four combinations of treatments assessed in the cv. Bobal field trial located in Requena, Valencia, Spain during 2012, 2013 and 2014. Values are averages and standard errors of eight determinations per treatment and date. Vertical bars indicate the standard error. DI = Deficit Irrigation; VSP = Vertical Shoot Positioning; WSP = West Shoot Positioning.

Figure 4. Diurnal pattern of stem water potential (Ψ_{stem}) in the four combinations of treatments applied in the cv. Bobal field trial located in Requena, Valencia, Spain.

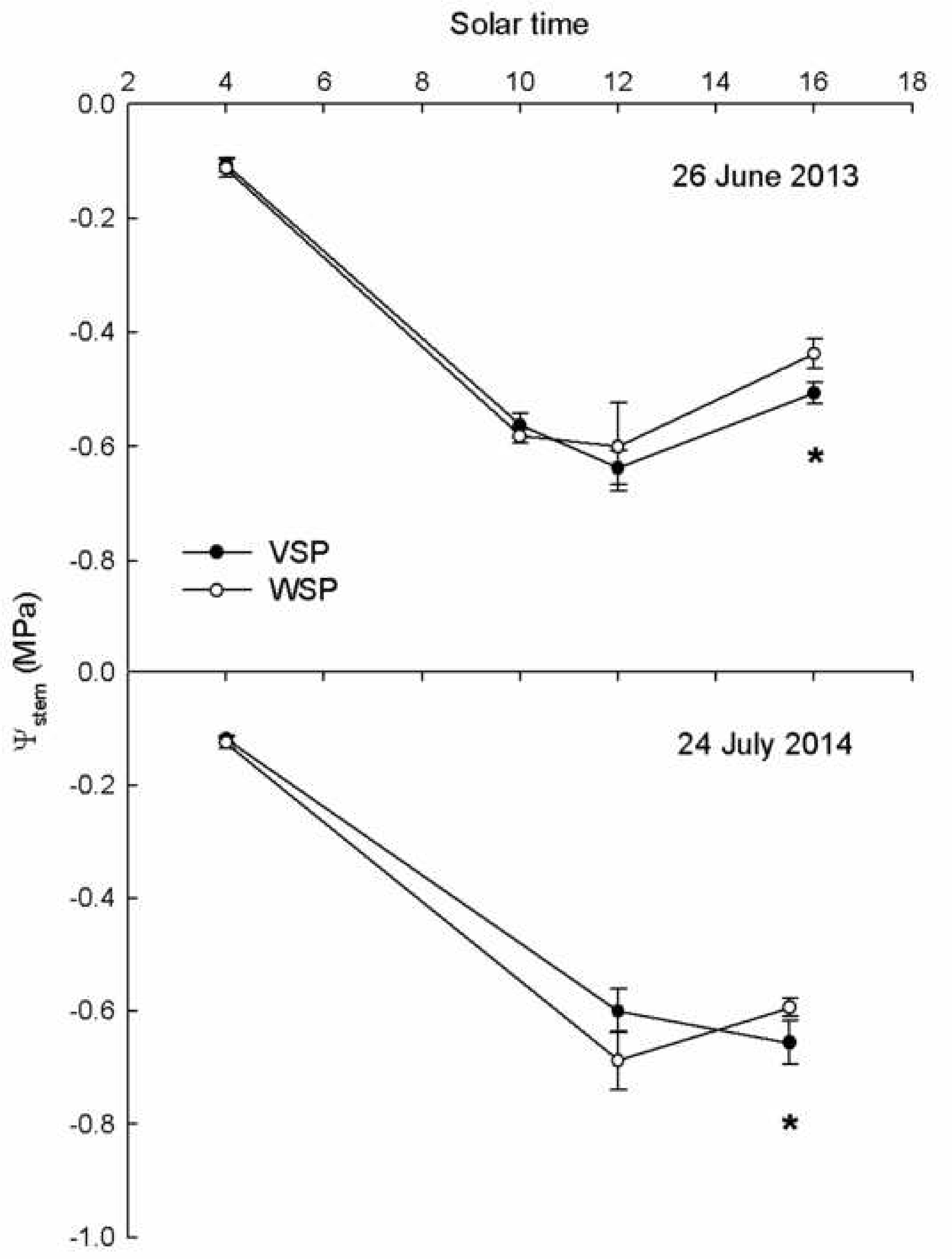
Measurements were conducted on 17 July and 22 August, 2012. Values are averages and standard errors of eight determinations per treatment. Asterisks indicate statistically significant differences between canopy management systems within each watering regime for that particular date at $p < 0.05$. DI = Deficit Irrigation; VSP = Vertical Shoot Positioning; WSP = West Shoot Positioning.

Supplementary Figures

Supplementary Figure 1. Detail of the modifications made in the common vertical training system (VSP) to lean the canopies towards West in the WSP treatment. Pictures were taken facing South.

Supplementary Figure 2. Detail of the whole-canopy gas exchange chambers used in potted vines trained vertical (VSP) and in a West shoot positioned (WSP) system to measure transpiration, net CO_2 and instantaneous water use efficiency (WUE_i).

Figure 1


Solar time
2
4
6
8
10
12
14
16
18
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-0.2
-0.4
-0.6
-0.8
26 June 2013
VSP
WSP
*
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
24 July 2014
*
Ψstem (MPa)

Figure 2

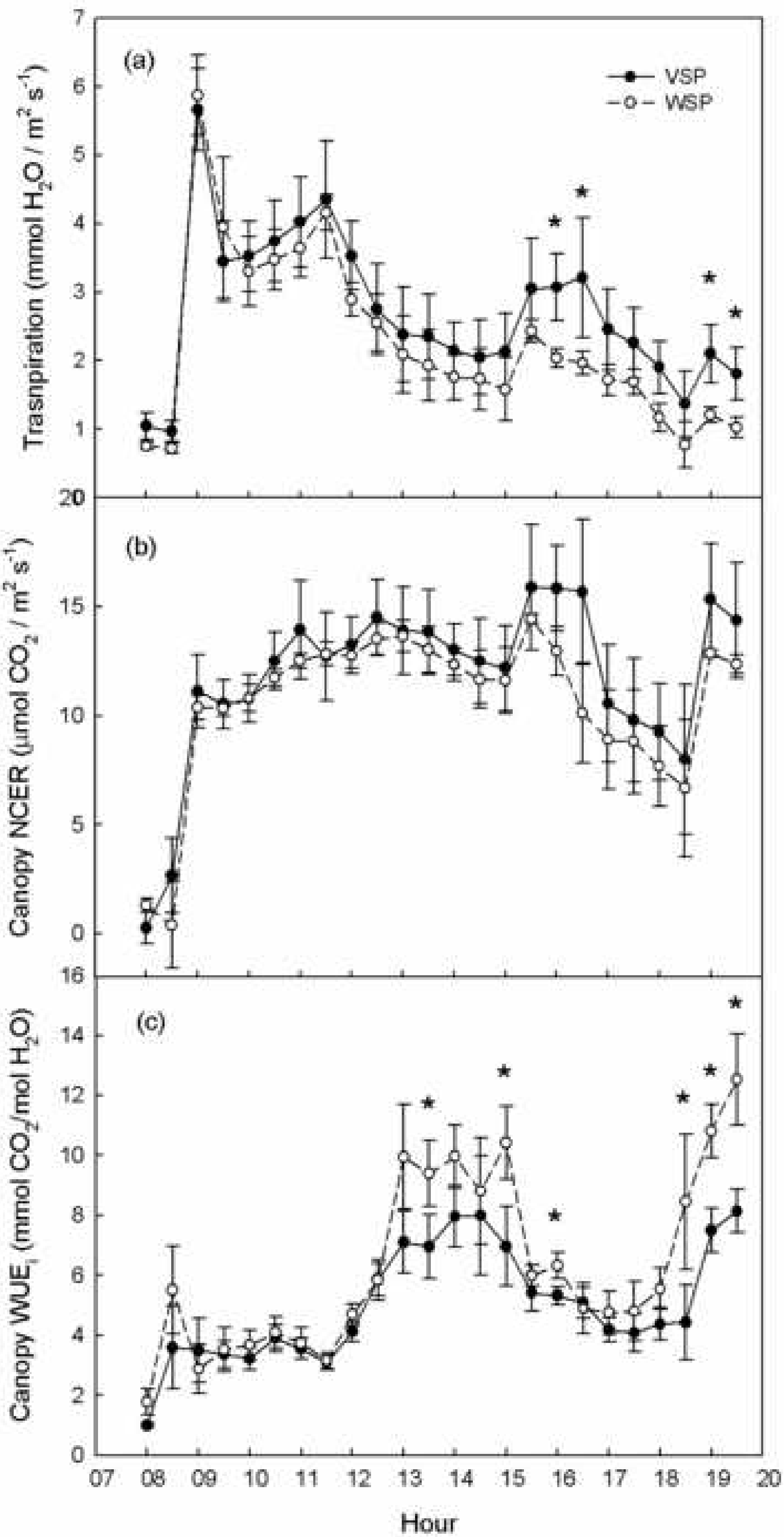

(a)
VSP
WSP
Trasnpiration (mmol H_2O / m^2 s^{-1})
(b)
Canopy NCER (µmol CO_2 / m^2 s^{-1})
(c)
Canopy WUE_i (mmol CO_2/mol H_2O)
Hour

Figure 3

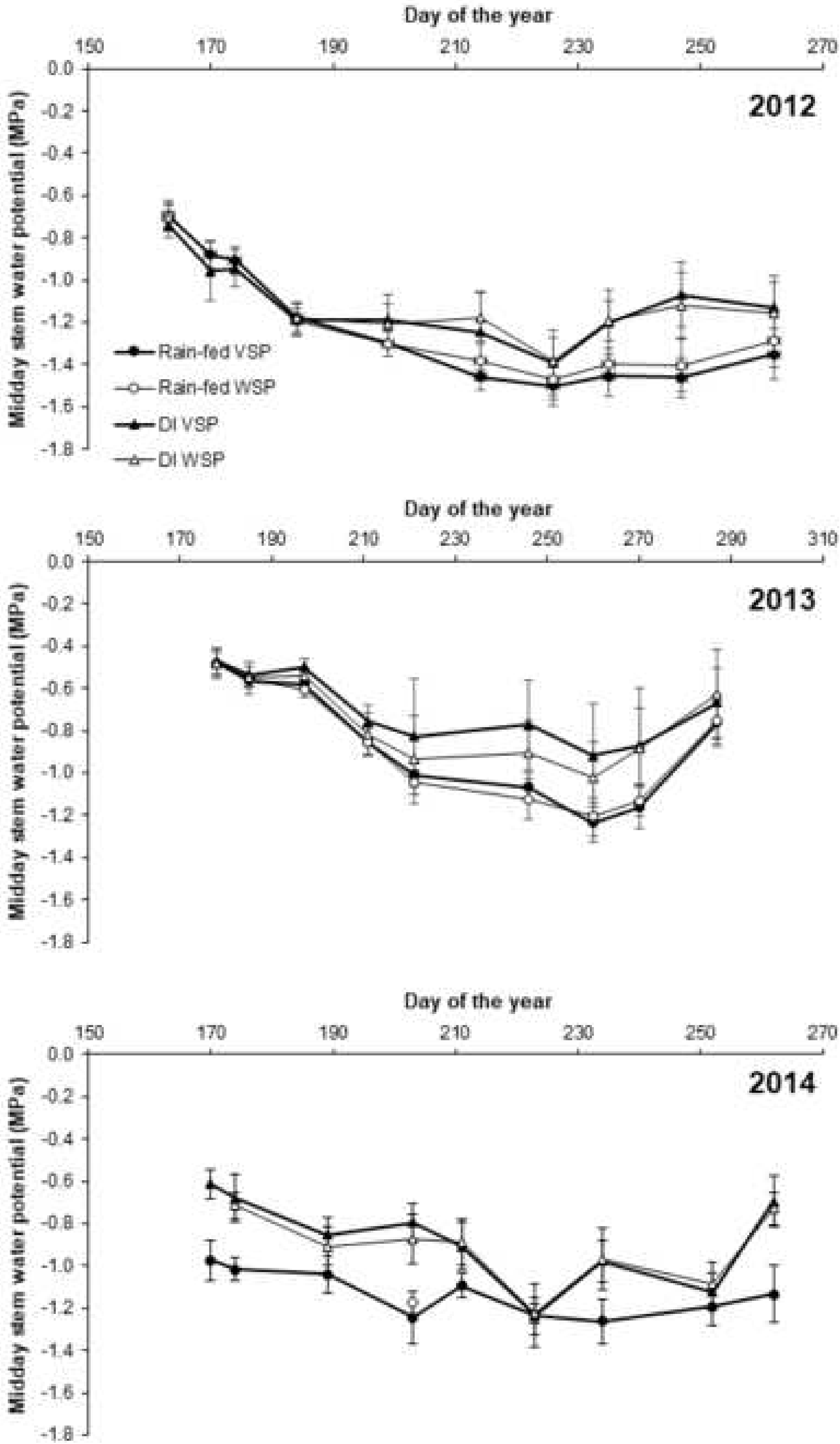
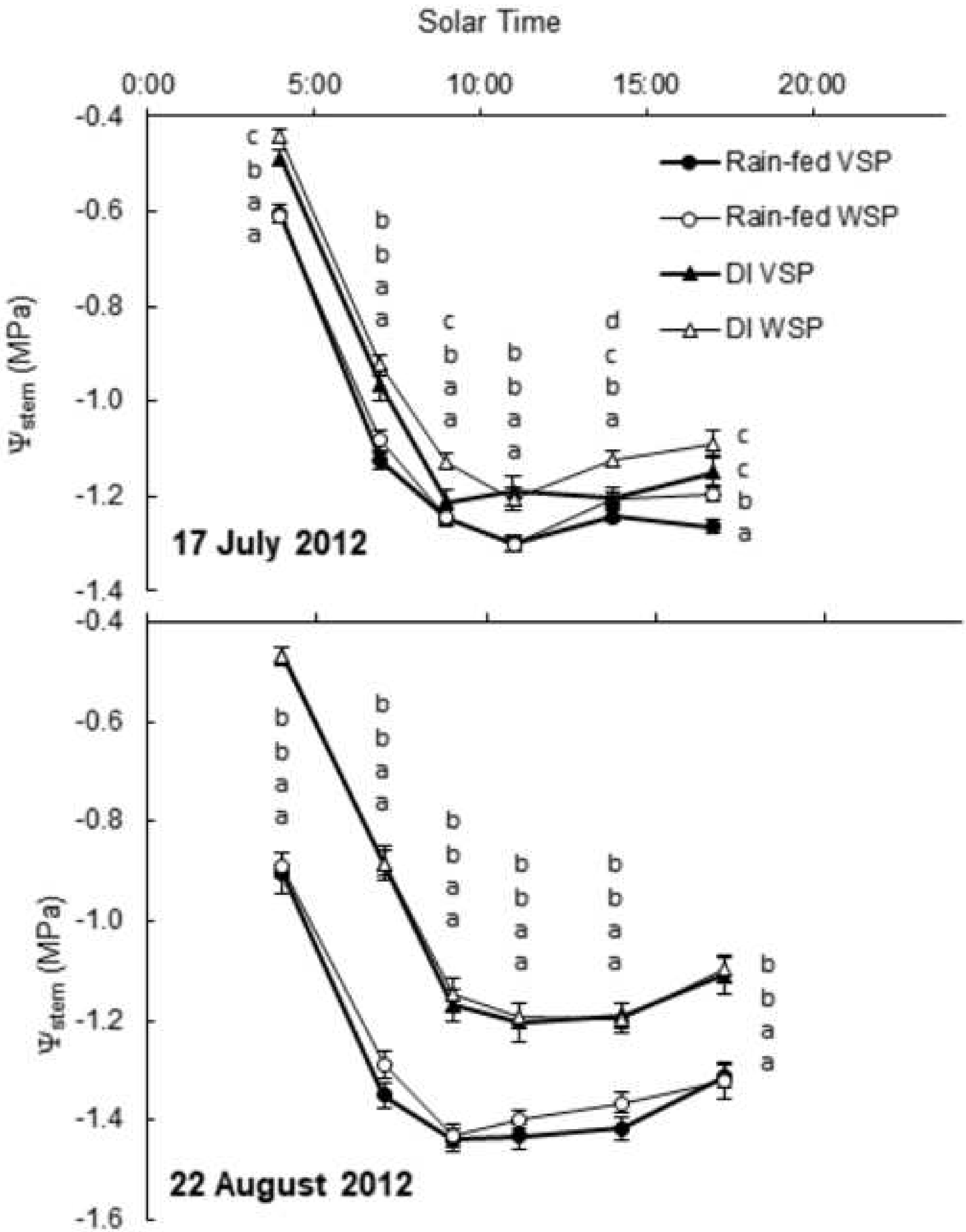

Day of the year
150
170
190
210
230
250
270
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
Midday stem water potential (MPa)
2012
Rain-fed VSP
Rain-fed WSP
DI VSP
DI WSP
Day of the year
150
170
190
210
230
250
270
290
310
2013
Day of the year
2014

Figure 4



Solar Time
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5:00
10:00
15:00
20:00
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
Ψstem (MPa)
Rain-fed VSP
Rain-fed WSP
DI VSP
DI WSP
17 July 2012
22 August 2012