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Training system and its influence on iso-anisohydric behavior of cv. Syrah

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Abstract. Water use efficiency is one of the most valued objectives in vine growing in Mediterranean climates (De la Fuente *et al.,* 2015). Due to this, the grape growers provide different adaptation strategies according to their efficient consumption against the presumable water deficit generated under these environmental conditions. The use of non-positioned shoot systems (like sprawl, bush, etc.) can help to achieve this objective.

One of the most common adaptation strategies to face environmental extreme conditions or low water availability (high ETo, increasing vapour pressure deficit, etc.). is the stomata closure. Traditionally, this behaviour has been explained by a genetic or varietal factor. In Vitis vinifera, the varieties have long been differed in isohydric or anisohydric, according to its physiological response opposite to water deficit or even hydric stress (Schultz 2003; Soar et al. 2006).

Nevertheless, some varieties have shown different behaviours according to environmental conditions. For this reason, this varietal classification is nowadays brought into question (Hugalde and Vila, 2014, Levin et al., 2020). Syrah cv. assumes a stomatal opening regulation under high potentials but responds with stomatal closure or regulation at low potentials, depending on the evapotranspiration demand, causing a physiological behavior typically called anisohydric (Soar et al. 2006). Grenache (Schultz 2003) closes stomata even under high water potentials, which allows them to regulate their water use, anticipating hydric stress situations. This behavior is usually called isohydric. The present study reveals that this mentioned behavior can also be affected or regulated by the training-trellising system.

Water use, vine physiology (net photosynthesis, stomata conductance), vine hydric relations (leaf water potential) and soil (soil matric potential, soil water content) have been tracked during the growing season, on three different systems: sprawl with 12 shoots•m-1 of row (S1); sprawl with 18 shoots•m-1 of row (S2) and vertical positioned system or VSP with 12 shoots•m-1 of row (VSP1).

Results on water use show that VSP1 used less available water (0.5-1.8% vol.) than non-positioned and free systems (S1 and S2), between budburst and flowering/setting, in the top 50 cm of soil profile. Leaf water potential reflected higher water stress in the S1 and S2 systems compared to VSP1, between veraison and harvest. Besides, stomatal conductance (gs) measured at noon was as also higher in VSP1 than S1 and S2 systems anytime along the cycle. However, water use efficiency (WUE) and intrinsic efficiency (A/gs) were +20% and +25% respectively higher in S1 and S2 treatments compared to VSP1.

Concluding, although S1 and S2 cause greater water use and stress at the beginning of the cycle (from flowering to veraison, mainly), they show a faster response regarding stomata closure, which makes these systems more efficient (less leaf transpiration, higher photosynthetic rate, better intrinsic and water use efficiency) than the trellis (VSP), differences that could resemble isohydric and anisohydric behavior respectively. All these results confirmed that when the DPV was high and the photosynthetic balance was negative, the vine closed its stomata to avoid dehydration due to the transpiration rate and so reducing meantime its water consumption (Cuevas e*t al.*, 2006), but even within the same cultivar, we can find both behaviors depending on training system, total leaf exposed area, crop load and of course, water availability use.

1. Introduction

Water availability in food production is highlighted in the last IPCC report, as main adverse impact related to losses and damages caused by climate change, which will continue to intensify. Therefore, water is unlikely to be sufficient for food production in 2050, due to the increased agricultural and ecological droughts for keeping the world production, which probably will be affected by water scarcity [1,2].

Water use efficiency such as natural resources is one of the most valued objectives in vine growing in Mediterranean climates [3], where water use efficiency is a key point. During the growing season, total available water is significantly lower than the evaporative demand, being this a limiting factor for quality production, even more under the current situation of water scarcity due to summer drought periods, becoming longer than usual.

Because of this, the plant adopts different physiological adaptation strategies to environmental and soil conditions, with the aim to be most efficient consumption against to the presumable water deficit or scarcity.

One of the most common adaptation strategies to face drought or the increase on deficit vapor pressure (DPV), is the stomata closing. Traditionally, this behavior has been explained by a genetic or varietal factor. In *Vitis vinifera*, the varieties have been usually differed in isohydric or anisohydric, according to its physiological response opposite to water deficit [4,5].

According to different stomatal response, grapevine species have been classified as isohydric or anisohydric. Isohydric plants are those that close their stomata when they feel a relevant fall in soil water potential, or an increase in atmospheric demand, with the objective to not depleting the available water. These varieties (like Grenache) respond to water stress by modifying their growth and physiology: fast stomata regulation and high sensitivity, high stem conductivity, and Ψ_L constant during the day. These factors help to close stomata at high Ψ_L , avoiding damages by hydric stress [4].

On the contrary, anisohydric plants do not close their stomata and keep their transpiration activity, even when soil water content decreases. These varieties (like Syrah) respond using all the available water for growth and physiological processes, hoping that it will be maintained during the whole cycle. This behaviour can compromise the plant's survival. They maximize their stomatal opening and therefore, their CO_2 assimilation, under the risk of embolism. Also, high temperatures and DPV produce low ABA sensibility in the stoma [5]. As a consequence, their leaf water potential (Ψ_L) falls more than the others, and the plant suffers water stress [6].

Training and trellising system are one of the most relevant factors to manage the water plant consumption [7], solar radiation and sunlight interception, because modifying the canopy, clusters' microclimate conditions will change [8]. Canopy management (placing of leaves, brunches and clusters inside the plant) can modulate several processes like transpiration, photosynthesis or conductance among others, through modifying its total leaf area surface exposed, especially in some relevant variables like temperature or light flux (time of sun exposure per surface) [9].

Therefore, the hypothesis was if conductance regulation can be seriously modified by the training system, causing a different isohydric or anysohidric behavior, especially in warm, dry climates or under water scarcity conditions.

The main objective of the present work was to examine the possible effects of three different training systems on the water consumption (relations between soil – plant), leaf water potential, photosynthesis, transpiration, stomata conductance, water use efficiency (WUE) and intrinsic efficiency (A/Gs) and if they cause a different or similar pathways (isohydric behaviours) under Mediterranean warm climate conditions.

2. Materials and methods

This field experiment was conducted (2005-2007) in an experimental trial in Toledo (Spain), on a fine clay-sandy soil (Palexeralf, Soil Survey Staff, 2003) with a 50 cm depth clay superficial horizon (50-55% of clay). The weather conditions were typical for Mediterranean semiarid climate (Papadakis, 1966). The cultivar was Syrah, grafted on 110R and spaced 1.2 m, in the NW-SE (+8.3° to West) orientated rows with 2.7 m between rows. Irrigation system drippers (3•l h^{-1}) were spaced 1.2 m along the planting line and the amount applied during the cycle (231, 248 and 162 mm/year for 2005, 2006 and 2007 respectively) was equal for all treatments. Climatic conditions of this year were extremely warm. Differences can be observed mainly in accumulated growing degree days (2413-2525-2030 GDD; respectively year), low rainfall (199-271-274 mm respectively year) and in evapotranspiration reference (1269, 1211.1-1064.6 mm; Eto respectively year) index too. Trial was designed with three treatments placed into four blocks at random and each experimental plot consisted of 20 control plants, separated by rows and edge plants. The three examined treatments (to assess the impact of training system and crop load) were: i) VSP1, *Espaldera* or vertical positioned system (VSP1) with 12 shoots/m of crop load, ii) S1, Sprawl with 12 shoots/m of crop load and iii) S2, Sprawl with 18 shoots/m of crop load. (50% crop load more than VSP1 and S1). Plants were spur pruned and trained in a bilateral cordon at the height of 1.40 m. The sprawl system had a single couple vegetation wires from 0.4 m to the basal wire and they opened 0.6 m between wires. VSP system had a couple wires from 0.3 m to the basal wire and a higher wire at 1.5 m to basal wire.

Plant water status was estimated measuring leaf water potential at pre-dawn (Ψ_{aa}), maximum photosynthesis rate (Ψ_{max}), mid-day (Ψ_{12h}) and stem ($\Psi_{stem)}$ using a Scholander type pressure chamber (PMS, Portland, Oregon). At the same time, some leaves were covered with a plastic bag before severing the petiole, the gas flow was limited to 0.2 bar s^{-1}and the measurement was performed within the 1–

1.5 min after detaching the leaf from the plant. About 90-120 min before midday, other leaves were covered with an aluminum foil for measuring the stem water potential (Ψ_{stem}). All leaves chosen were of similar age and type but for Ψ_{12h} leaves were well sun exposed and non-sun exposed for Ψ_{stem}. Measurements were carried out on 6 (Ψ_{aa}, Ψ_{max} and Ψ_{12h} and Ψ_{stem}) leaves per treatment at 3 phenological stages (fruitset, veraison, and end of ripening).

Total Available Water was calculated (96 mm) using the Saxton-Rawls model [10], considering the texture properties of the two soil horizons observed in the root-explored horizons. Soil water tension at three depths (20, 50 and 80 cm) was monitored weekly using Granular Matrix Sensors (GMS). Three sensors were placed (one for each depth level) in three replicates of each treatment. On the other hand, soil moisture content was monthly monitored by an encapsulated capacitance sensor (Diviner 2000, Sentek©) inserted in a continuous probe. Tubes were placed in three replicates of each treatment during the growth season.

Photosynthesis or net CO_2 assimilation rate (A), transpiration rate (E), stomata conductance (gs), water use efficiency (WUE) and intrinsic efficiency (A/gs) measures were measured on healthy, mature leaves. To evaluate the effect of soil water availability on leaf gas exchange, measurements were taken at maximum photosynthetic activity (08:00 s.t.) and midday (12:00 s.t.) at flowering-berry set; veraison and harvest, on fully exposed leaves from main shoots at cluster area. Measurements were replicated at six different vines for each date and treatment using a portable IRGA equipment (Li-6400, LI-COR Inc.).

Finally, all data were analyzed by ANOVA with the statistical software SPSS v.15.0. Duncan's multiple range tests at 5% significance level were used to compare means among treatments.

3. Results and discussion

3.1. Leaf water potential, water consumption and soil hydric content

Leaf water potential was also measured at pre-dawn and maximum photosynthesis activity hour. However, no differences were found during the three years, which showed that all treatments had the same rehydration recovery during the night and the same conditions at the beginning of the photosynthesis activity. Nevertheless, during the morning some differences were found (Table 1), at fruit set and verasion, the values for midday (Ψ_{12h}) tended to show small differences between treatments. This is in line with previous works in which the Ψ_{12h} was the best indicator for vine water status [11;12]. Under the same water availability conditions, the biggest difference appeared between fruitset and veraison on stem water potential (Ψ_{stem}), causing to S1 and S2 the highest stress to the plant. For both water availabilities between veraison and harvest, the potential values reached during most of the ripening period correspond to severe stress according to Van Leeuwen *et al.* (2009), due to the severe environmental conditions [13]. Even when there are no differences among treatments, S1 and S2 tended to have a lower value related with VSP1, being closer to -1.5 MPa, which could be a limiting factor for physiological processes [13, 14]. These results are according to Dufourcq *et al.* (2005) trial, where the higher crop load caused lower potentials [15] in the plants and could explain different iso or anisohydric behaviors.

These effects can be related to the soil content water availability (Table 2). Main differences appear between veraison and harvest, where sprawl systems (S1 and S2) scored higher values of tension instead of VSP. It seems that sprawl systems could explore more extensively the soil profile (50 cm or deeper) during the hardest period concerning drought (veraison-harvest), and specially, S2 due to its high crop load. On the other hand, there were no differences at fruitset.

Table 3 shows the evolution of soil average content water measured by the capacity probe for all treatments. At fruitset, VSP1 and S1 presented higher moisture content (1.8-0.5% vol.; respectively) compared with S2. No differences were found between veraison and harvest, even if a tendency of decreasing the soil water content (VSP1>S1>S2) among treatments was found (mainly in 20-30 cm soil level and in 2007, at 70 cm too), giving sense. At levels deeper than 0.7 m, the soil kept its original structure and revealed no differences between treatments, so no statistical differences were found (data not shown).

Sprawl system (and of course, the crop load), makes that S1 (less) and S2 (more) seem to explore deeper than VSP1 the soil profile to obtain more water, causing a real improvement regarding the root system exploitation at fruitset. This produces some differences (leaf water potential) during the rest of vine cycle.

Table 1. Leaf water potential at fruitset, veraison and harvest.

Cycle	Treatment	2005 (MPa)		2006 (MPa)		2007 (MPa)	
		ψ_{12h}	ψ_{stem}	ψ_{12h}	ψ_{stem}	ψ_{12h}	ψ_{stem}
Fruitset	VSP1	-1.06	-0.72	-0.86 b	-0.60	-0.74 ab	-0.57 a
	S1	-1.13	-0.75	-0.94 a	-0.65	-0.72 b	-0.55 ab
	S2	-1.04	-0.8	-0.95 a	-0.65	-0.79 a	-0.50 b
	EEM[1](n_1=6)	0.09	0.05	0.01	0.05	0.02	0.05
	Sig[2]	NS	NS	*	NS	*	*
Veraison	VSP1	-1.48	-0.98	-1.32	-0.99 b	-1.43 ab	-1.03
	S1	-1.57	-1.02	-1.37	-1.06 b	-1.49 a	-1.10
	S2	-1.48	-0.87	-1.38	-1.14 a	-1.39 b	-0.99
	EEM[1](n_2=6)	0.05	0.09	0.04	0.03	0.09	0.08
	Sig[2]	NS	NS	NS	**	*	NS
Harvest	VSP1	-1.31	-0.81	-1.34	-1.03	-1.18	-0.75 b
	S1	-1.34	-0.68	-1.48	-1.03	-1.21	-0.75 b
	S2	-1.37	-0.85	-1.58	-1.14	-1.28	-0.89 a
	EEM[1](n_2=6)	0.03	0.09	0.20	0.10	0.08	0.02
	Sig[2]	NS	NS	NS	NS	NS	*

[1] EEM: standard average error for n= 6 samples per treatment; [2] Sig: significant differences; ns and ** means to there is no significant differences and P<0.01 respectively. The values with the same letter are equal (T. Duncan). P-values were determined by analysis of variance.

Table 2. Soil water tension at fruitset. veraison and harvest in 2006 and 2007.

2006	**20 cm**			**50 cm**			**80 cm**		
Trat	Fruitset	Veraison	Harvest	Fruitset	Veraison	Harvest	Fruitset	Veraison	Harvest
VSP1	162,0	17,0	10,3	154,7	16,3 b	64,7	26,0	139,0	67,0
S1	199,0	57,0	96,7	141,0	198,3 a	196,7	20,3	187,3	62,7
S2	171,7	19,7	10,3	109,7	199,0 a	72,0	48,7	163,0	5,0
EEM n= 4	24,9	14,6	31,3	50,2	2,0	48,5	16,0	35,3	50,4
Sig	ns	ns	ns	ns	**	ns	ns	ns	ns

2007	**20 cm**			**50 cm**			**80 cm**		
Trat	Fruitset	Veraison	Harvest	Fruitset	Veraison	Harvest	Fruitset	Veraison	Harvest
VSP1	140.0	150.3	144.3	66.667	137.00	140.33	66	49.67 b	55.00 b
S1	23.7	74.7	71.7	66.333	158.67	157.33	0	40.67 b	36.00 b
S2	14.3	39.7	96.0	135	199.00	164.33	0	175.00 a	166.67 a
EEM n= 4	24.9	14.6	31.3	50.2	2.0	48.5	16.0	35.3	50.4
Sig	ns	ns	ns	ns	**	ns	ns	*	*

[1] EEM: standard average error for n= 4 samples per treatment; [2] Sig: significant differences; ns and *** means to there is no significant differences and P<0.001 respectively. The values with the same letter are equal (T. Duncan). P-values were determined by analysis of variance.

Table 3. Percentage of soil water content at fruitset. veraison and harvest in 2006 and 2007 for average soil profile (10-80 cm).

Cycle	**Treatment**	**% Soil Water avalaible Content**		
		Fruitset	Veraison	Harvest
2006	VSP1	16.34 a	16.69	16.86
	S1	16.94 a	16.24	16.64
	S2	15.10 b	16.35	16.42
	EEM[1] (n_l=32)	0.31	0.16	0.16
	Sig[2]	***	NS	NS

Cycle	**Treatment**	**% Soil Water avalaible Content**		
		Fruitset	Veraison	Harvest
2007	VSP1	24.01	17.02	16.25
	S1	23.89	16.77	16.38
	S2	21.74	16.46	16.50
	EEM[1] (n_l=32)	0.79	0.37	0.32
	Sig[2]	*0.077*	NS	NS

[2] Sig: significant differences; ns. * and ** means to there is no significant differences or P<0.05 and P<0.01 respectively. The values with the same letter are equal (T. Duncan). P-values were determined by analysis of variance.

3.2. Water use efficiency (WUE) and intrinsic efficiency (A/gs)

Differences in solar interception could explain a better use of natural resources [16]. Regarding the water use efficiency, which is the relationship between net photosynthesis and transpiration rate (Table 4), we can see that S1 and S2 treatments had in general, high values (between 8-17%) compared to VSP1 at 8 s.t. It means that the plants in non-positioned systems were more efficient that VSP plants. These differences increased during the morning until 23% at midday.

The intrinsic efficiency (A/gs) is a ratio that could explain better the stomate opening. No differences were found at 8.s.t., when the plant started the maximum photosynthesis net assimilation, but with the increase of temperature during the morning the plants' transpiration rates increased until a moment in which the plant could decide to stop (embolism) or not. It should be noted that VSP1 had lower values of A/gs than S1 and S2 at 12 s.t. (three years). It means that their stomata conductance slowed down and/or net photosynthesis rate too, as we can see in Figure 1, where no matter the net photosynthesis rate, the VSP1 conductance is always higher than S1 and S2.

According to several authors [4;.5; 16;17;18], the main variables in discriminating the iso or anisohydric behaviour are the stomata conductance, DPV and leaf water potential (Figure 2). In our study, we can see that for high leaf water potentials (> 15 MPa), all treatments are equal, close to embolism and plants suffered high stress, but at low potentials, S1 and S2 had a low conductance value than VSP1 (<10 MPa). In Figure 2, S1 and S2 close their stomata before the VSP1, showing a faster response to stomata closure, which makes these systems more efficient (less leaf transpiration, higher photosynthetic rate, better intrinsic and water use efficiency) than the trellis (VSP) during the whole morning, keeping their gs values as lower as possible, but with similar net photosynthesis (A) at low leaf water potentials (more efficient). These differences could resemble anisohydric and isohydric behaviour and, in our case, the behaviour is not dependent on the cultivar but seems to be more related to total leaf area exposure and crop load, which means, the training system.

Table 4. Water Use efficiency (WUE) and Intrinsic efficiency (A/gs) for the three treatments.

		WUE (A/T)		A/gs	
		8 s.t.	12 s.t.	8 s.t.	12 s.t.
2005	VSP1	3,9 b	1,7 b	0,10	0,08 b
	S1	4,2 a	2,2 a	0,11	0,10 a
	S2	4,0 ab	2,1 a	0,10	0,10 a
	Sig[1]	*	**	NS	***
2006	VSP1	4,8 b	2,6 b	0,12	0,09 b
	S1	5,2 a	3,0 a	0,13	0,12 a
	S2	4,8 b	2,7 b	0,13	0,11 a
	Sig	*	**	ns	*
2007	VSP1	3,4 b	2,2	0,10	0,08 b
	S1	4,1 a	2,5	0,09	0,08 b
	S2	4,2 a	3,1	0,09	1,03 a
	Sig	**	NS	NS	*

[1] Sig: significant differences; ** means to there is significant differences with P<0.01. The values with the same letter are equal (T. Duncan). P-values were determined by analysis of variance. n= 40 samples per treatment.

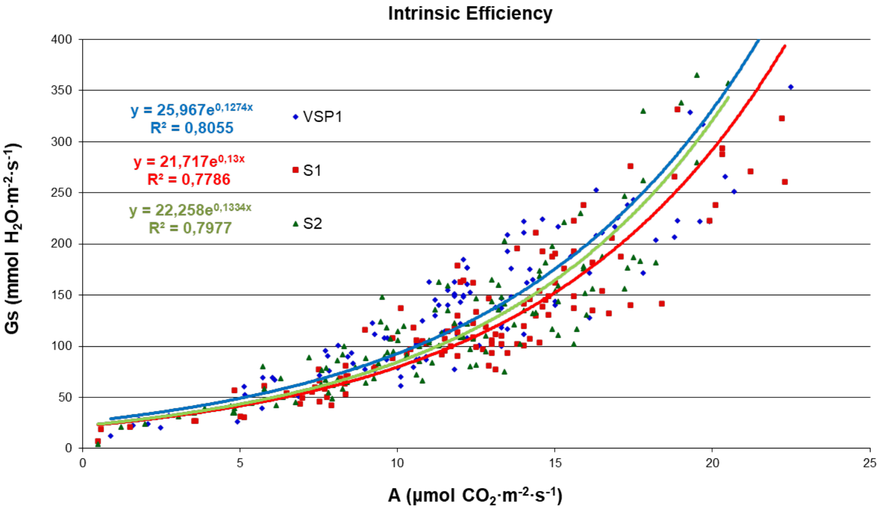

Figure.1. Relationship between conductance (Gs; mmol H_20 m^{-2} s^{-1}) and Net Photosynthesis (A; µmol H_20 m^{-2} s^{-1}).

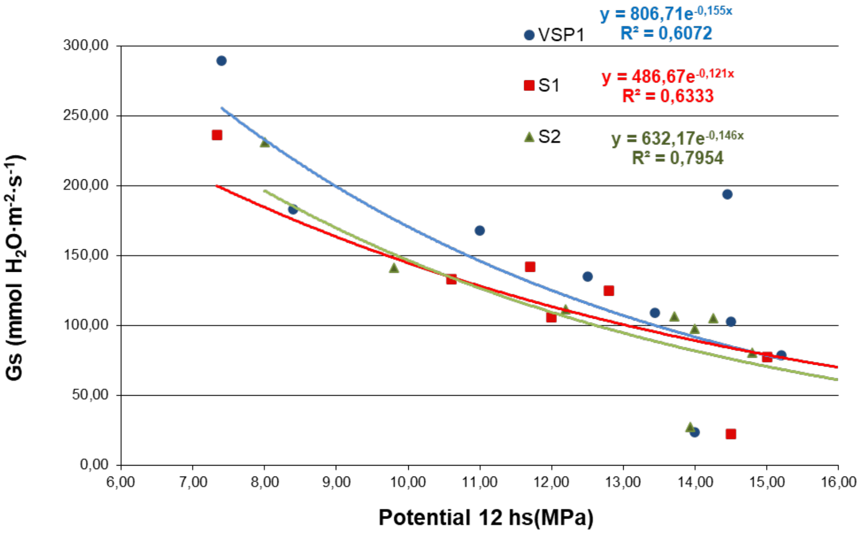

Figure. 2. Relationship between conductance (Gs; mmol H_20 m^{-2} s^{-1}) and leaf water potential at midday ($\Psi_{12h;}$ MPa).

4. Conclusion

Leaf water potential, water consumption and soil hydric content are key factors to know the vine behaviour according to environmental conditions. The leaf water potential at midday (ψ_{12h}) and stem water potential should be enough to assess the grapevine water status, being well correlated with ambient temperature and vapor pressure deficit [17].

Photosynthesis, Transpiration, conductance, etc. decrease from morning to afternoon. This was partly due to a reduction in A and gs, as well as an increase in E (due to DPV and T conditions), with these phenomena increasing as did water stress [18].

Although S1 and S2 seem to suffer stress at the beginning of the cycle (from flowering to veraison, mainly), they show a faster response regarding stomata closure, which makes these systems more efficient (less leaf transpiration, higher photosynthetic rate, better intrinsic and water use efficiency) than the trellis (VSP), differences that could resemble isohydric and anisohydric behavior respectively. All these results confirmed that when the DPV was high and the photosynthetic balance was negative, the vine closed its stomata to avoid dehydration due to the transpiration rate and so reduce meantime its water consumption [18; 19; 20].

Preliminary results show that even within the same cultivar, we can find both behaviours depending on several factors (DPV, T, water availability and irrigation…), but also on the training system: total leaf exposed area and crop load.

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