STOMATAL RESTRICTIONS TO PHOTOSYNTHESIS IN GRAPEVINE CULTIVARS GROWN IN A SEMIARID ENVIRONMENT.

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ABSTRACT
Diurnal changes in the leaves of field-grown grapevine (*Vitis vinifera* L.) cultivars Syrah and Tempranillo were followed over summer 2009 with respect to gas exchanges. Net photosynthetic rate (A_N) of both cultivars rapidly increased in the morning, decreasing slowly until the late afternoon, when reached the lowest values. Stomatal conductance (g_s) changed in parallel with A_N, indicating that A_N was greatly affected by g_s. This pattern was repeated every day throughout the summer, with slight modifications according to plant water status. Under severe water stress situations, when as a result of drought g_s decreased below 0.05 mol H_2O m^{-2} s^{-1}, intrinsic water use efficiency (WUE_i) declined sharply in Tempranillo, which did not happen in Syrah, where despite stomatal closure kept increasing WUE_i. Water stress intensified leaf to air vapour pressure deficit (VpdL) response however instantaneous WUE ($WUE_{inst)}$) levels plunged to very low with high VpdL in both cultivars.

KEY-WORDS
leaf to air vapour pressure deficit – leaf water potential – net photosynthetic rate – stomatal conductance – transpiration rate – water use efficiency

INTRODUCTION
The regime of use supported by groundwater during the last four decades has led to overexploitation of aquifers in the Upper Guadiana (La Mancha, Spain). Recovery plans set by the Government include, among other measures, to limit water for agricultural use. In the annual operating regimes of the different aquifer systems the maximum permitted quantities for growing vines have fluctuated between 80 and 150 mm per year, according to the accumulated water resources. This restriction imposed on producers of grapes creates a need to find ways to improve water use efficiency in viticulture. The use of regulated irrigation techniques and better adapted to drought cultivars may be ways to achieve it.

Drought is the main environmental factor limiting plant photosynthetic capacity, even in plants adapted to semiarid conditions such as grapevine (*Vitis vinifera* L.): water deficit in soil, high temperature and radiation, high water vapour pressure deficit between air and leaf are different factors that characterize. Under drought conditions, the stomata have an important role in CO_2 assimilation and transpiration rate: stomatal closure is generally assumed to be the main cause of drought-induced decreases in photosynthesis, since stomatal closure decreases CO_2 availability in the mesophyll (Flexas *et al*., 2002)

In the present study, the daily changes in gas exchange measured a few days during the summer in two field-grown grapevine cultivars, Syrah and Tempranillo, are computed to provide practical information about their photosynthetic behavior and efficiency in water use. Furthermore, effects of environmental factors, such as high leaf to air vapour pressure deficit, VpdL, are discussed.

MATERIALS AND METHODS

Environmental conditions and plant material

Measurements were made in 2009 summer season on an experimental vineyard located in 'La Mancha' (Central Spain region). The plot was grafted in 2002 on 110-R rootstock with many grapevine cultivars, including the two studied: Tempranillo and Syrah. Vines grown on trellises at a distance of 3 m x 1.5 m (row by vine spacing), were trained to a double cordon Royat system, with 3 spurs of 2 buds on each arm and 120° E - 300°W oriented.

The plot is located on the Upper Guadiana river alluvial fan, on soils Calcixerollic-Petrocalcic Xerochrepts with limestone encrusting below 35-40 cm in depth (Montero, Brasa, 1998).

The water conditions in autumn-winter season (2008-09) were according to expectations: cold temperatures (monthly averages between 5 and 10 °C) and 250 mm of accumulated rainfall in late March 2009. However, spring and summer (2009) were extremely dry and hot with only 70 mm of rainfall and an effective temperature above 1800 °day from 1 April to 31 August.

The Vineyard was irrigated by a drip irrigation system with 2 drippers per vine. We followed the S.I.A.R. (Irrigation Advisory Service for Farmers in Castilla-La Mancha) recommendation, which estimates the crop water requirements for 100 mm of irrigation restriction corresponding to deficit irrigation. In our case, we modify slightly the S.I.A.R. criteria, moving the beginning of irrigation until the Ψ_{PD} was below -0,40 Mpa, and continuing with irrigation until harvest. The extremely dry summer required to exceed the limit mentioned above: a total of 125 mm of water were actually added.

Gas exchange measurements

Stomatal conductance (g_s), transpiration rate (E), net photosynthesis (A_N) and leaf vapour pressure deficit (VpdL) were measured using a Li6400 portable photosynthesis system (Li-cor Inc, Lincoln, NE, USA). We use a clear-bottom chamber suitable for clamping surfaces of 6 cm^2. Measurements were made six time points along the day (with a 2 hours interval) in different days between **cluster closure (CC)** and harvest: the first one was approximately in the summer solstice, the second one in **veraison (V)** and the last one coinciding with the **harvest (H)**. On the first measure **(CC)**, vines still remained without irrigation, while the second **(V+1, V+3)** and third measure **(H+1, H+3)** were made 1 and 3 days after rewatering (0,2 x ETo).

All measurements were made on the sun-exposed leaves, inserted in the shoot axes and located in the upper third of the trellis: photon flux density (PPDF) incident on the selected leaves usually was higher than 1400 $\mu mol.m^{-2}.s^{-1}$, except in some of the first measurements made in the early hours, with the sun still low on the horizon.

For each measure, The Li6400 was clamped sequentially onto 8 leaves, randomly selected from 4 adjacent vines of each cultivar. The system was run in constant flow mode (500 $\mu mol.s^{-1}$) and sample chamber CO_2 concentration was always kept at 400 ppm at all times using a 6400-01 CO_2 mixer.

Leaf water potential

In every leaf, leaf water potential (N_{leaf}) was determinated immediately after gas exchange using a Scholander chamber (SKPM-1400, Skye Inst. Lim., U.K.). Also, the days of monitoring, N_{PD} was calculated in each cultivar, as the average of N_{leaf} measurements made on 12 leaves before down.

RESULTS AND DISCUSSION

The seasonal course of maximum daily photosynthesis (A_{max}) and maximum daily stomatal conductance (g_{max}) was similar for field-grown Syrah and Tempranillo cultivars throughout the summer: the high values reached in late spring were decreasing as the summer was settled and never were recovered, despite irrigation. Many authors attribute this to the progressive soil drying (Schultz, 1996, Soar *et al.*, 2006b). The stomatal aperture control is the path used by plants to avoid water loss against drought, a strategy that is due, largely, to the availability of water by roots. With the irrigation regime applied, we kept growing with a moderate water stress ('I'$_{PD}$ between 0.25 and 0.55 Mpa), so A_{max} ^{and} g_{max} values remained relatively high: between 13-14 µmol CO_2 m^{-2} s^{-1} and between 0.33 and 0.20 mol H_2O m^{-2} s^{-1}, respectively.

In our observations, g_s daily dinamics began with a rapid increase from dawn to mid-morning, when it reached maximum values (g_{max}), then declined until after midday and finally remained at low values along the afternoon (Fig. 1).

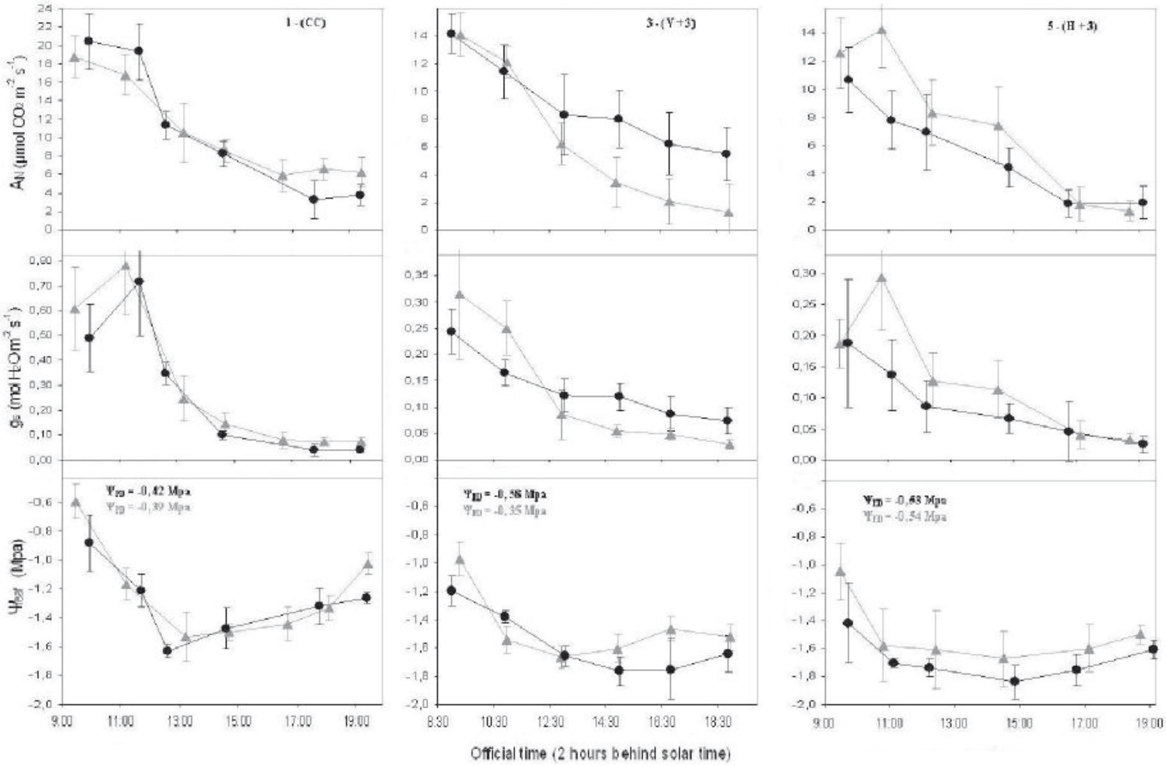

Fig. 1. Diurnal time course of net photosynthesis (A_N), stomatal conductance (g_s) and leaf water potential ('I'$_{leaf}$) for Tempranillo (–A–) and Syrah (—s—) grapevines for three summer days (1-cluster closure, **CC** - without irrigation; 3-veraison, **V+3** - three days after rewatering and 5-harvest, **H+3** - three days after rewatering too). Each data point shows averages ± sd of measurements made in eight leaves. For details see materials and methods.

This pattern was repeated every day throughout the summer, with slight modifications according to plant water status (within 1 to 3 days after rewatering) and conditioned the daily course of net photosynthesis, which closely followed the same behavior. Fig. 2 shows the response curves of A_N to partial stomatal closure in both cultivars, Syrah and Tempranillo, illustrating how small reductions in net assimilation have significant water savings for the vines.

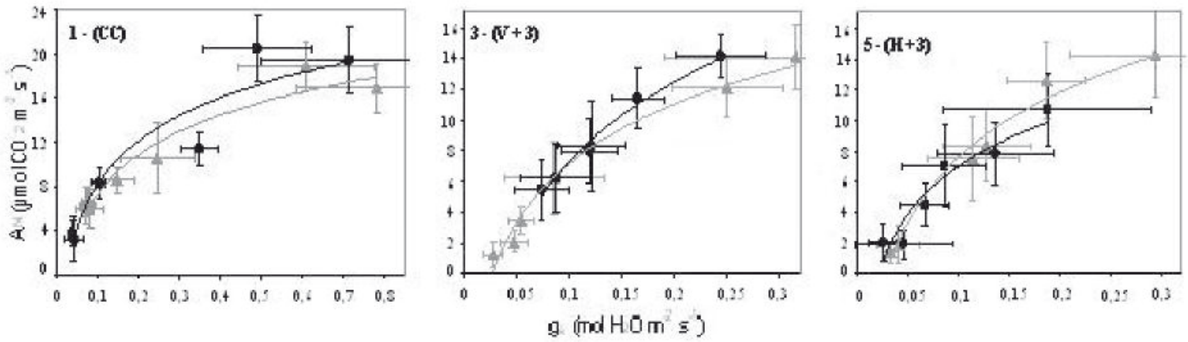

Fig. 2. A_N plotted as a function of g_s for Tempranillo (–A–) and Syrah (—s—) grapevines mesured at cluster closure (CC), veraison (V+3, three days after rewatering) and harvest (H+3, three days after rewatering too). Each data point shows averages ± sd of measurements made in eight leaves.

Due to this close correlation between A_N and g_s, it is assumed that the drought induced decrease of photosynthesis is mediated by stomatal closure, as a down-regulation which seems to depend more on the availability of CO_2 in the chloroplast than on leaf water potential (Flexas *et al.*, 1998) (see the daily course of Ψ_{leaf} in Fig.1). In any case, under mild water stress, it is likely that grapevine photosynthesis is governed almost exclusively by stomatal closure.

Water stress events characterised by g_s values above 0.05-0.10 mol H_2O m^{-2} s^{-1}, A_N is mainly limited by stomatal closure, and a complete recovery of the maximum (A_{max}) occurs just one night after irrigation. Under more severe water stress situations, non-stomatal inhibition of A_N are dominant and photosynthesis does not recover rapidly after irrigation (Flexas *et al.*, 2004). In our study (Fig. 3), before reaching severe stress situations (CC), stomatal closure about 0.05 mol H_2O m^{-2} s^{-1} only maximize intrinsic water use efficiency (WUE_i), defined as the ratio of A_N to g_s (and decrease intercellular CO_2, C_i), indicating an exclusively photosynthetic stomatal limitation. In later phases, (V) and (H), and only after 2-3 days after irrigation, when a result of drought g_s cited below limits are exceeded, WUE_i drops sharply (and C_i steeply increases) in Tempranillo, indicating that non-stomatal limitations to photosynthesis become dominant. However, that did not happen in Syrah, where stomatal closure below 0.05 mol H_2O m^{-2} s^{-1} continued to increase WUE_i (and decreased C_i). This was one of the clear differences between these two varieties and it seems a handicap for Tempranillo cultivar where the balance of photosynthesis sometimes was even negative.

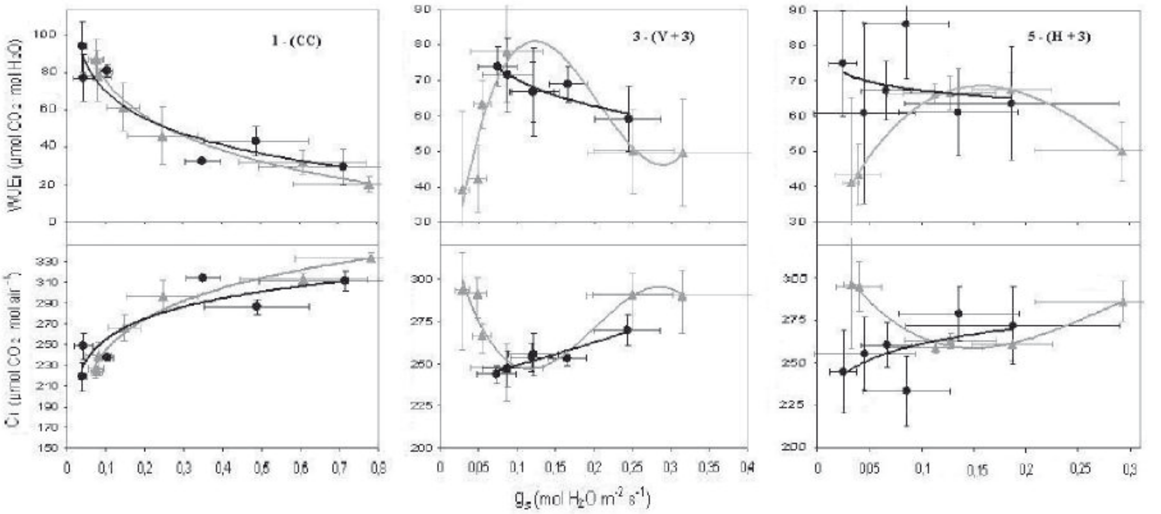

Fig. 3. Intrinsic water use efficiency, WUE_i, and intercellular CO_2, C_i, plotted as a function of g_s for Tempranillo (–A–) and Syrah (—s—) grapevines mesured at cluster closure (CC), veraison (V+3, three days after rewatering) and harvest (H+3, three days after rewatering too). Each data point shows averages ± sd of measurements made in eight leaves.

Stomata respond dynamically to daily fluctuations in the vapour pressure deficit (VpdL) (Soar *et al.*, 2006a), apparently because of the effect of VpdL on the demand for water flowing through the epidermis and the stomatal complex (Schultz, Stoll, 2010). Every day, VpdL usually increased throughout the day to reach very high values, causing an increase in transpiration rate: vines responded by stomatal closure which stopped and even reduced transpiration rate. Thus, water stress intensified VpdL response, increased stomatal closure, allowing better control of transpiration rate by the plant (data not shown). However, the instantaneous water use efficiency (WUE_{inst}), defined as the ratio of A_N to leaf transpiration rate (E), was clearly reduced. The Fig. 4 shows the curvilinear relationship in decrease WUE_{inst} whit increasing VpdL.

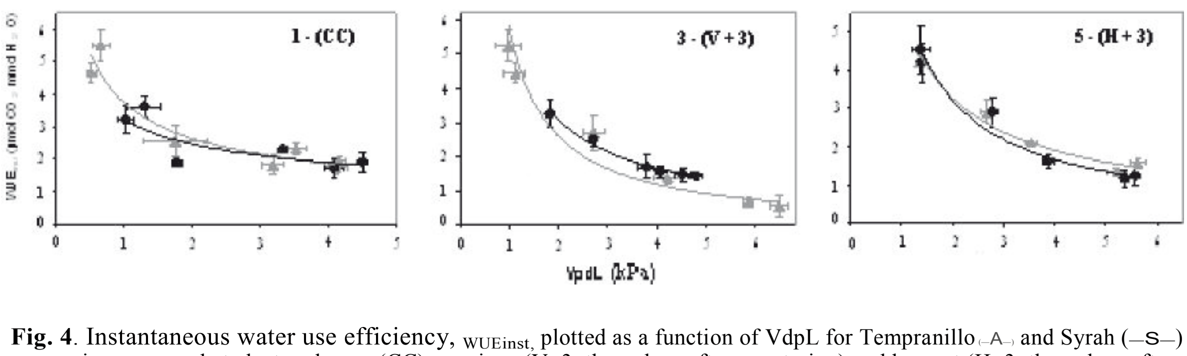

Fig. 4. Instantaneous water use efficiency, WUE_{inst}, plotted as a function of VdpL for Tempranillo (–A–) and Syrah (—S—) grapevines mesured at cluster closure (CC), veraison (V+3, three days after rewatering) and harvest (H+3, three days after rewatering too). Each data point shows averages ± sd of measurements made in eight leaves.

A horizontal line for WUE_{inst} as VpdL increases supports the hypothesis that stomata are behaving optimally with respect to water and carbon dioxide fluxes (Cowan, 1977). However, the negative response of WUE_{inst} to VpdL means that the marginal unit cost (i.e. water transpired) was increased for unit of carbon fixed. The two cultivars seemed unable to maintain high levels of WUE_{inst} to high VpdL. This is not consistent with the optimization theory which assumes that the leaf stomatal behavior is to maximize A_N for a given amount of water to spend.

CONCLUSIONS

In the semi-arid regions, where quantity and quality of production is questioned by the drought, the profitability of growing grapes clearly depends on the use of irrigation. A rational use of this water savings can be made using physiological tools to keep the plants in the boundary between water stress and excessive water consumption. Regulated deficit irrigation (RDI) programs, based on knowledge of the physiological responses of grapevines to water stress and the recovery of different photosynthetic parameters upon re-watering could be used to improve water use efficiency and crop productivity and quality.

BIBLIOGRAPHY

Cowan I.R., 1977. Stomatal behaviour and environment. *Adv. Bot. Res*. 4: 117–228

Flexas J., Escalona J.M., Medrano H., 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. *Australian Journal of Plant Physiology*, 25: 893-900.

Flexas J., Bota J., Escalona J.M., Sampol B., Medrano H., 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol.*, 29: 461-471.

Flexas J., Bota J., Cifre J., Escalona J.M., Galmés J., Gulías J., Lefi E-K, Martínez-Cañellas S.F., Moreno M.T., Ribas-Carbó M., Riera D., Sampol B., Medrano H., 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Ann. Appl. Biol.*, 144: 273-283.

Montero F. J., Brasa A., 1998. El viñedo en Castilla-La Mancha ante el siglo XXI. Ediciones de la Universidad de Castilla-La Mancha. Colección Ciencia y Técnica, nº 23: 15-36.

Schultz H. R., 1996.Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Acta Horticulturae,* 427: 251-266.

Schultz H.R., Stoll M., 2010. Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Australian Journal of Grape and Wine Research*, 16: 4-24.

Soar C.J., Speirs J., Maffei S.M., Penrose A.B. McCarthy M.G., Loveys B.R., 2006a. Grapevine varieties Shiraz and Grenache differ in their stomatal response to VPD:apparent links with ABA physiology and gene expression in leaf tissue. *Australian Journal of Grape and Wine Research*, 12: 2-12.

Soar C.J., Dry P.R., Loveys B.R., 2006b. Scion photosynthesis and leaf gas exchange in *Vitis vinifera* L. Cv. Shiraz: mediation of rootstock effects via xylem sap ABA. *Australian Journal of Grape and Wine Research*, 12: 82-96.