Universitat
de les Illes Balears

Title: *Effect of high CO_2 and water availability on whole plant carbon and water balance in grapevine*

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Master's Thesis

Master's degree in **Biotecnologia Aplicada**
(With a speciality/Itinerary: **Medi Ambient**)
at the
UNIVERSITAT DE LES ILLES BALEARS

Academic year: 2017/18

Date: 14/09/2018

UIB Master's Thesis Supervisor: José Mariano Escalona Lorenzo

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Abstract

Water scarcity is one of the main challenges in vineyards sustainability in most of the grapevine áreas, now and even more in near future to due climatic change perspectives. For that reason, water use efficiency measurements are one of the highest interest to improve the sustainability of this crop. Since the vast majority of studies relays on measurements of carbon and water fluxes at the leaf scale, less data are available at the whole-plant scale.

For this reason, in this experiment, whole-plant water and carbon fluxes in grapevine exposed to two different water regimes and under high CO_2 concentrations were measured, in order to check its effect at the whole-plant scale.

The results obtained showed that water and carbon fluxes are well coordinated between themselves under both water treatments. Under drought conditions, both fluxes were reduced, but surprisingly the estimated water use efficiency was decreased, contrarily to what is shown at the leaf scale. Finally, high CO_2 measurements indicated an increase of whole-plant NCE as well as increased whole-plant water use efficiency, mainly in non-watered plants.

Introduction

Among other human activities, the combustion of fossil fuels as well as the clearing of forests are increasing the atmospheric CO_2 concentration (Clark, 1982; Houghton *et al.*, 1983; Rotty and Marland, 1986; Houghton, 1988). The predicted increase in atmospheric CO_2 concentration may have direct effects on individual plants, populations, communities and ecosystems (Strain, 1987; Bazzaz, 1990; Woodward *et al.,* 1991). One of its biological effects on plants would be an increase of the net photosynthesis and plant growth, since CO_2 is the first component for plants metabolism (Strain, 1987).

Meanwhile plants fix Carbon, this induce an obligatory loss of water, since CO_2 and water share the same pathway to enter in (for CO_2) and go out (for H_2O) the leaf. Thus, the Water Use Efficiency (WUE) can be basically defined as the ratio of Carbon fixed per unit of water lost. But several definitions of WUE can be found, following the scale at which it is defined: extrinsic WUE is known as the ratio between leaf assimilation rate (A) to leaf transpiration rate (E) (at the leaf scale of at the whole plant scale) and intrinsic WUE correspond to the ratio of leaf assimilation rate to leaf stomatal conductance (g_s). WUE can also be defined as the yield (or total biomass production) to the total water consumed by the plant (for agricultural purpose). The importance of this parameter in agriculture lays on the capacity to increase yield production and decrease transpiration water loss (Douthe *et al.*, 2018, *in press*).

In the literature, it is described that WUE increases with drought (when measured at the leaf scale) or with increased CO_2 (Strain, 1987).

Leaf gas exchange depends on many factors related to crop management and the environment, such as cultural practices and the considered genotype (Escalona *et al.*, 2016). One of the most important environmental factors determining these parameters is the soil water availability (Escalona *et al.*, 2016). The progressive water shortage during summer, typical of Mediterranean climate, results in a decrease of leaf photosynthetic activity, which results in a slowdown and a subsequent shutdown of vegetative growth, which can substantially compromise the production and quality of grapes (Medrano *et al.*, 2002; Chaves *et al*., 2010). Water deficit causes a significant reduction in both

instantaneous values of photosynthesis, daily integral of net carbon uptake and also leaf transpiration (Escalona *et al.*, 2016). Interestingly, it is accepted that water deficit increase the leaf WUE (Medrano *et al.,* 2002; Escalona *et al.*, 2016).

Simultaneous estimation of canopy water use (E) and dry matter production (A) can be done by using portable infrared gas analyzers (IRGA) at the single leaf level and scaled up to the canopy using canopy leaf area (Greco and Baldocchi, 1996) or by enclosing canopies in open system flow-through chambers in which water vapor and CO_2 fluxes are measured using an IRGA (Garcia *et al.*, 1990). The main disadvantage of the first system to estimate canopy gas exchange are errors associated with the non-destructive estimation of canopy leaf area and the overestimation of whole-canopy CO_2 assimilation rates due to the proportion of non-photosynthesizing or respiring leaves in a given canopy (Poni *et al.*, 1997). Furthermore, the heterogeneous architecture of grapevine canopies mean that individual leaves vary in terms of their position on the canopy e.g. interior (shaded) vs. exterior (exposed) leaves (Schultz, 1993), age, temperature and position on the shoot, all of which affect leaf physiological performance such as stomatal conductance and CO_2 assimilation rate (Petrie *et al.*, 2009).

The very large majority of studies focused on plant gas exchange have been conducted at the leaf scale, but very few data are available at the whole plant scale. In grapevine, the use of whole plant chambers has led to novel findings that involve effects of regulated deficit irrigation (Poni *et al*., 2009; Tarara *et al.*, 2011). Furthermore, these systems can also permit to continuously monitor the concentrations of both CO_2 and H_2O inlet and outlet water-vapor pressure, allowing measurements of whole-canopy carbon and water net exchange, and hence, the whole-canopy water-use efficiency (WUE) (Poni *et al.*, 2014). Many researches (Petrie *et al.*, 2009) indicate that WUE can be reliably measured on a whole-canopy basis and compared to other techniques, including single-leaf intrinsic and extrinsic WUE (Schultz and Stoll, 2010), isotopic composition ($^{13}C/^{12}C$), or biomass accumulation per unit of water used (Gibberd *et al.*, 2001).

Considering that, the aim of this experiment was to determine the net carbon exchange (NCE) and transpiration (E) of grapevine plants at whole-plant scale in different

environmental conditions: in irrigated and non-irrigated conditions and under atmospheric CO_2 concentration and higher CO_2 concentrations.

Material and methods

- **Plant material and site of study**

The experiment was conducted during the summer 2018 in an experimental vineyard planted in 2009 at the University of the Balearic Islands. The plant material used during the experiment consisted of Grenache vines submitted to two different water regimes: a moderate water irrigation and non-irrigation. The measurements were carried out in two representative plants of each water regime, which were used along the entire experiment. The measurements were performed during the months of July and August and were developed as it follows: firstly, irrigated plants were measured (beginning with atmospheric CO_2 conditions and ending with high CO_2 conditions) and secondly, non-irrigated plants were measured (also beginning with atmospheric CO_2 conditions and ending with high CO_2 conditions). For each session of measurement, plants remained inside the whole-plant chamber 2 or 3 days before the measurements were taken to avoid the disturbing effect of the chamber installation. Each measuring session last at least 3 consecutive days.

- **Whole-plant gas exchange chambers**

During the entire experiment, plants were kept inside two open-top whole-plant gas exchange chambers (Figure 1) and were measured continuously. Firstly, chambers' structures were assembled and then covered with plastic film (RX 140-PropafilmTM). The entering air flow from the atmosphere was pushed into the chambers by a turbine (S & P 500), at a constant air flow of 280 mol min^{-1}.

Figure 1. The two whole-plant gas exchange chambers used during the experiment.

The circuit which was controlling all parameters measured included (Figure 2):

- Gas Analyzer (Li-840, Li-Cor, Inc., Lincoln, NE, USA): which measured in and out CO_2 and H_2O concentrations.
- Flowmeter (Series 641 Air Velocity Transmitter, Dwyer, Indiana, US): to regulate the amount of flow entering into the chambers.
- Thermocouples (type K, RoHS, Model TP-01): which measured the temperatures inside and outside the chambers.
- Electro-valves: to sequentially inject the air from the different chambers to the Li-840 analyzer.
- Data logger (Campbell CR1000): data logger of the rest of devices.

Figure 2. System that controlled and measured all the inputs and outputs of both chambers.

The air flow entering and outgoing the chamber was measured during 5 consecutive minutes each, alternatively. The 4 first minutes of data were eliminated to ensure a complete turn-over of the gas in the measuring circuit. With data collected, net carbon exchange (NCE) and transpiration (E) were calculated through the following equations:

$$NCE = \frac{F(C_e - C_o)}{L_a}$$

$$E = \frac{F(W_o - W_e)}{L_a}$$

where F is the air flow through the chamber, C_e and W_e are the entering $[CO_2]$ and $[H_2O]$ of the chamber, C_o and W_o are the outgoing $[CO_2]$ and $[H_2O]$ of the chamber and L_a is the total leaf area of the plant.

- **Leaf area measurements**

In order to choose the useful method to obtain plant leaf area of the measured plants, a revision of the methods that can be find in the literature was made.

- Lopes and Pinto (2005) proposed an **empirical model** for estimating primary shoot leaf area. Primary leaf area of shoots is estimated by a model using a calculated variable obtained from the average of the largest and smallest primary leaf area multiplied by the number of primary leaves. For lateral leaf area estimation another model is presented which uses the same type of calculated variable plus the area of the largest leaf.

- Carbonneau (1976) method is based on **measurement of MVL** (main vein length) for one of every three leaves on four representative fruiting shoots (4-5 plants per row). This method is simple and accurate but in order to estimate total leaf area one still has to measure all primary and lateral leaf veins, a very laborious procedure.

- In several fruit tree species (citrus, almond, pecan olive, walnut and asian pear) Spann and Heerema (2010) found a **linear relationship between the biggest leaf length multiply by shoot leaf number and shoot leaf area**. This method has some advantages like that it allows the primary and lateral leaf area to be estimated separately. This method is easy, accurate and non-destructive; it also reduces measuring time.

- **Plant canopy analyzers** (LAI-2000, LI-COR Inc., Lincoln, NE, USA). This kind of sensor measures the light extinction through the foliage. However, a general trend towards underestimating LAI due to foliage clumping (Jonckheere *et al.,* 2004; Johnson and Pierce, 2004), and the requirement for an above canopy reference reading in order to get accurate LAI estimations are known weaknesses of the LAI-2000 approach.

- **Ground-based sensors** to get information about the geometry and/or structure of the canopy. Specifically, **laser sensors** have been tested in grapevine, in

which in addition to laser sensors (Llorens *et al.*, 2011), radiometric sensors mounted on tractors were used (Mazzetto *et al.*, 2010).

- **Hemispherical canopy photography** is a technique for studying plant canopies via photographs acquired through a hemispherical (fisheye) lens from beneath the canopy (oriented towards zenith) or placed above the canopy looking downward. It is able to capture the species-, site- and age-related differences in canopy architecture, based on light attenuation and contrast between features within the photo (sky versus canopy). One of the main problems cited in the literature of hemispherical photography for determination of LAI is the selection of the optimal brightness threshold in order to distinguish leaf area from sky area thus producing a binary image, affected by camera angle and resolution, and analysis software, among other factors (O'Neil *et al.*, 2002, Phattaralerphong *et al.*, 2006).

- **Inclined point quadrat**. This method was developed by Wilson (1960) and consists of piercing a vegetation canopy with a long thin needle (point quadrat) under known elevation (i.e. the angle between the needle and the horizontal plane when vertically projected) and azimuth angles (i.e. the bearing of the needle from north when horizontally projected) and counting the number of hits or contacts of the point quadrat with "green" canopy elements. It is the elevation angle that determines the impact of the canopy structure on the number of hits.

Finally, once all different methods were studied, leaf area of the plants used in the experiment was calculated following Sánchez de Miguel *et al.* (2010) methodology. For this, it was previously needed to record data on a representative sample of leaves of all size in order to obtain a calibration equation. Once the calibration equation was calculated, measurements on all shoots of the plants subsequently used in the experiment were taken. In these shoots three different measurements in the main shoot (1) and another three in its lateral shoots (2) were taken: the length of the main nerve of the largest leaf (L) and the smallest leaf (S) of the main shoot, the length of the main nerve of the largest leaf (L) and the smallest leaf (S) of the lateral shoots and the number of leaves. So, 6 parameters per shoot were finally obtained.

Then, for a given shoot whether it was a main or lateral, the data regarding the length of the main nerve (cm) was converted to an area (cm^2) by using the relationship found between both variables.

Results

- **Variation of CO_2 concentration at night**

It was observed that atmospheric CO_2 concentration (so the concentration entering the chamber) varied largely depending on the hour of the day or night. While during the day CO_2 concentration was pretty stable with a variation of 7-8 µmol mol^{-1} at the most, during the night values were much more instable. During the darkest hours, variations in CO_2 concentration reached 12 µmol mol^{-1} between two consecutive hours.
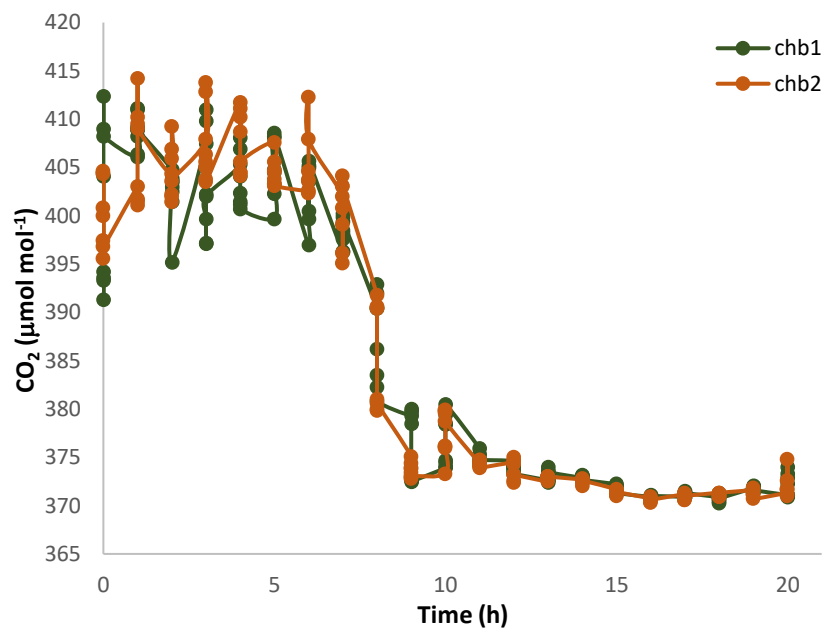

Figure 3. Daily cycle of atmospheric CO_2 concentration in both chambers. Green circle dots for "Chamber 1" and orange circle dots for "Chamber 2".

- **Whole-plant net carbon exchange and transpiration in different water regimes**

Whole-plant net carbon exchange and transpiration measurements of watered plants at atmospheric CO_2 concentrations were performed in July (pea size). In those conditions, whole plant net carbon exchange (NCE) reached its maximum peak at 10-11 h. with values of 10 µmol m^{-2} s^{-1} (Figure 1). Whole-plant transpiration (E) values followed almost the same pattern, achieving its maximum peak of 3 mmol m^{-2} s^{-1} at 10-12 h. and decreasing since then.

When measured in August (ripening) in non-irrigated conditions, a decrease in both NCE and E plant was observed (Figure 4). Regarding to whole-plant net carbon

exchange, values diminished near 4 ppm in comparison to those obtained in irrigated conditions. Also, maximum peak of 8 μmol m^{-2} s^{-1} was reached earlier in the morning, around 8 h. Whole-plant transpiration maximum peak was observed at 13 h. approximately, with E values of 1.5-1.7 mmol m^{-2} s^{-1}.

Furthermore, night respiration values obtained in non-irrigated conditions (August) were greater than those observed in irrigated conditions (July) (Figure 1).
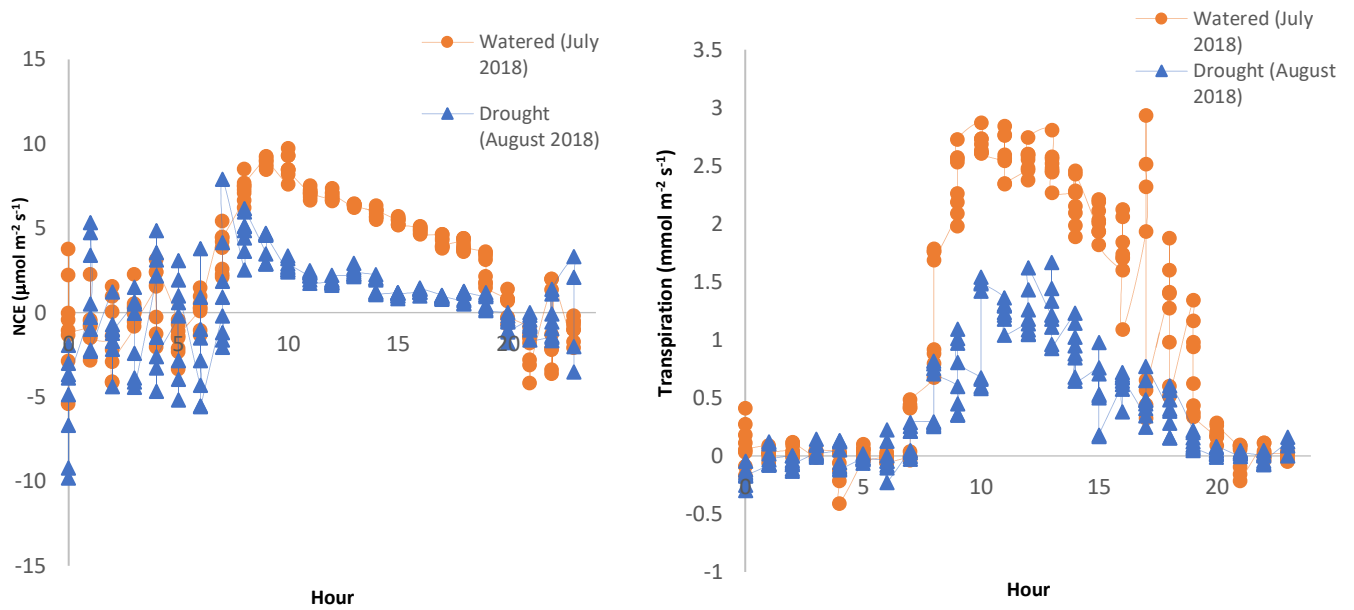

Figure 4. Net carbon exchange (left, μmol m^{-2} s^{-1}) and transpiration (right, mmol m^{-2} s^{-1}) daily cycle for 2 treatments: orange circle dots for "Watered" (measured in July) and blue triangle dots for "Drought" (measured in August).

- **Effect of high CO_2 on net carbon exchange and transpiration**

Once plants were measured at CO_2 environmental conditions, they remained inside the whole-plant chambers where CO_2 concentration was artificially increased. For this, a CO_2 tank was connected directly to the chambers flow, increasing CO_2 concentration from atmospheric to $\cong$ 900 μmol mol^{-1}.

Both irrigated and non-irrigated plants used this CO_2 concentration increase to raise its net carbon exchange. In watered plants, the maximum peak of assimilation was at 10 h., reaching the 22 μmol m^{-2} s^{-1}, so a NCE around 12 μmol m^{-2} s^{-1} higher than under atmospheric conditions (Figure 5). In non-irrigated plants, NCE values were also higher

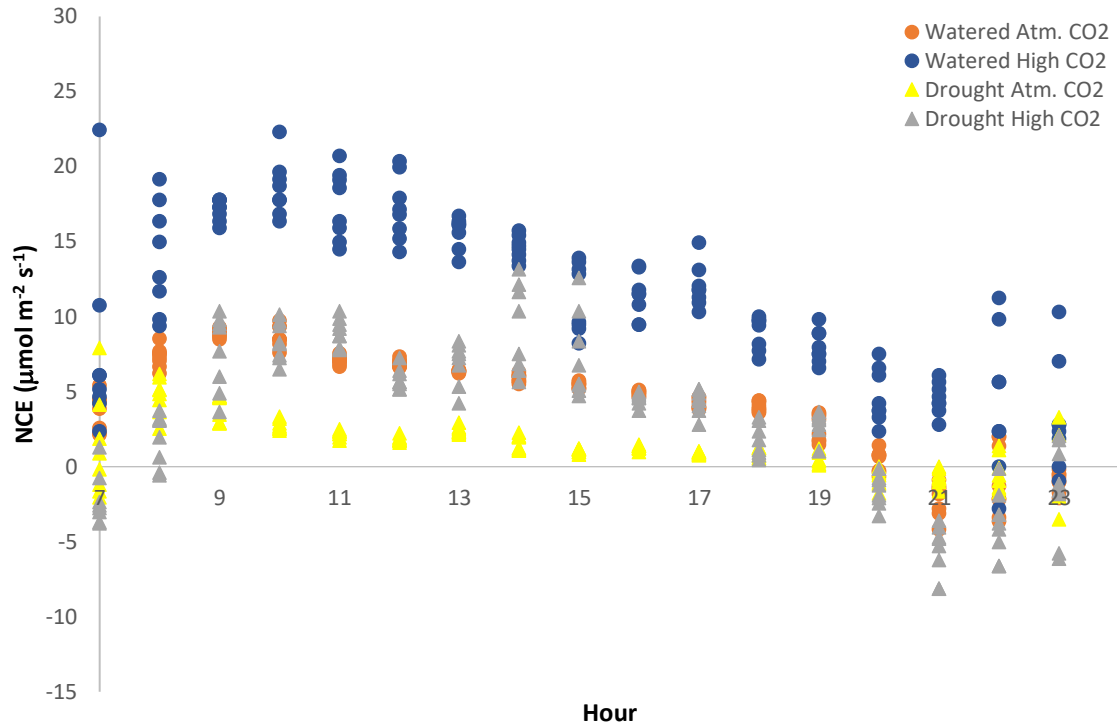reaching 10 μmol m^{-2} s^{-1}, so close to the values observed in irrigated plants at atmospheric conditions.

Figure 5. Net carbon exchange (NCE, μmol m^{-2} s^{-1}) values of a daily cycle for 4 different treatments: orange circle dots for "Watered plants at atmospheric CO_2 concentration", blue circle dots for "Watered plants at high CO_2 concentration", yellow triangular dots for "Drought plants at atmospheric CO_2 concentration" and grey triangular dots for "Drought plants at high CO_2 concentration". The "High CO_2" corresponds to ~900 μmol mol^{-1}.

Regarding to transpiration values, surprisingly there wasn't a big difference between the two CO_2 treatments in irrigated plants. However, this difference did exist in non-irrigated plants, where those exposed to higher CO_2 concentrations decreased their E values until 0.7-0.8 mmol m^{-2} s^{-1} at their maximum peak (Figure 6).
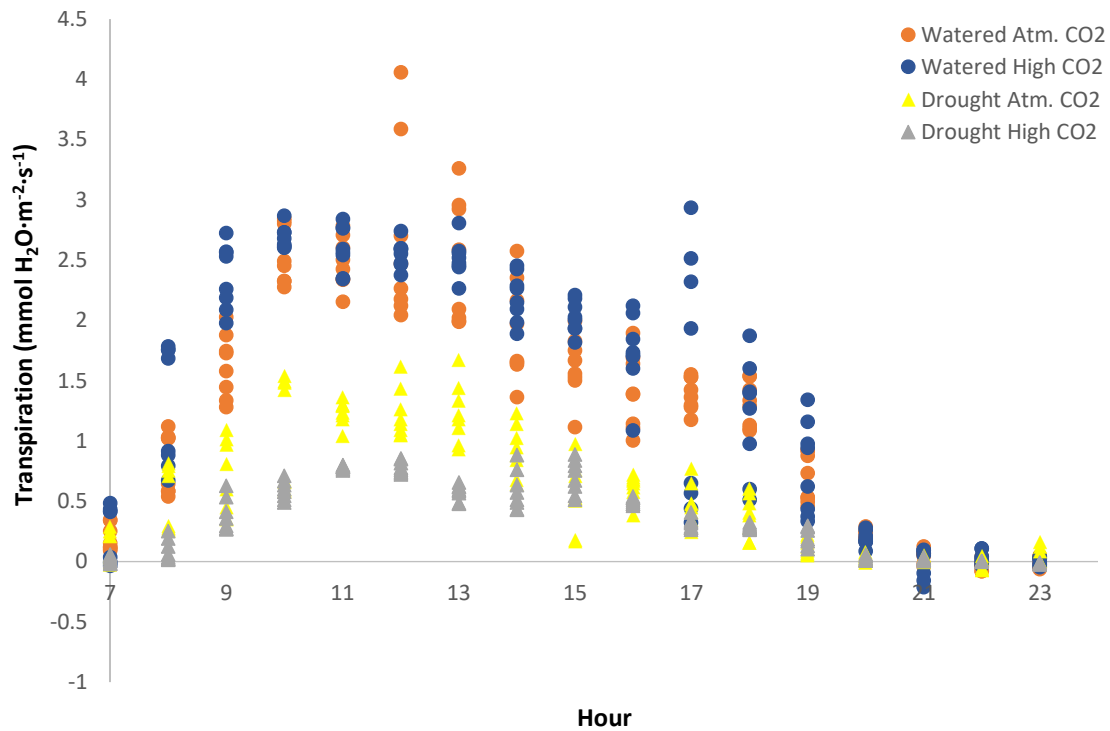
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Figure 6. Transpiration (E, mmol m^{-2} s^{-1}) values of a daily cycle for 4 different treatments: orange circle dots for "Watered plants at atmospheric CO_2 concentration", blue circle dots for "Watered plants at high CO_2 concentration", yellow triangular dots for "Drought plants at atmospheric CO_2 concentration" and grey triangular dots for "Drought plants at high CO_2 concentration". The "High CO_2" corresponds to ~900 µmol mol^{-1}.

- **Light responses of carbon and water balance under different water regimes and CO_2 conditions**

With NCE and E data obtained in all different conditions, it was possible to establish the whole plants NCE and E responses to light (Figure 7). Under atmospheric conditions it was observed that surprisingly NCE values of watered plants reached its maximum peak at ≈700 µmol m^{-2} s^{-1} and after it began to decrease as light increased. On the other hand, E values seemed to follow a positive relationship with the light until ≈1000 µmol m^{-2} s^{-1} when they began to decrease. When the same plants were exposed to higher CO_2 concentration, their NCE values increased while their E values slightly decreased. The carbon assimilation increased together with the light until approximately 1300 µmol m^{-2} s^{-1} where they began to saturate. Instead, E values followed a positive relationship with PAR values and didn't show saturation.

In non-irrigated plants, NCE and E values were lower than in irrigated plants. In atmospheric conditions, NCE values were the lowest among all treatments, reaching its maximum peak before 500 μmol m^{-2} s^{-1} and then decreasing with increasing light. When submitted to higher CO_2 concentration, drought NCE values followed an irregular shape with values that increase and decrease at highest lights. E values, instead, followed almost the very same pattern in both CO_2 concentrations, being a bit lower at highest CO_2 conditions.
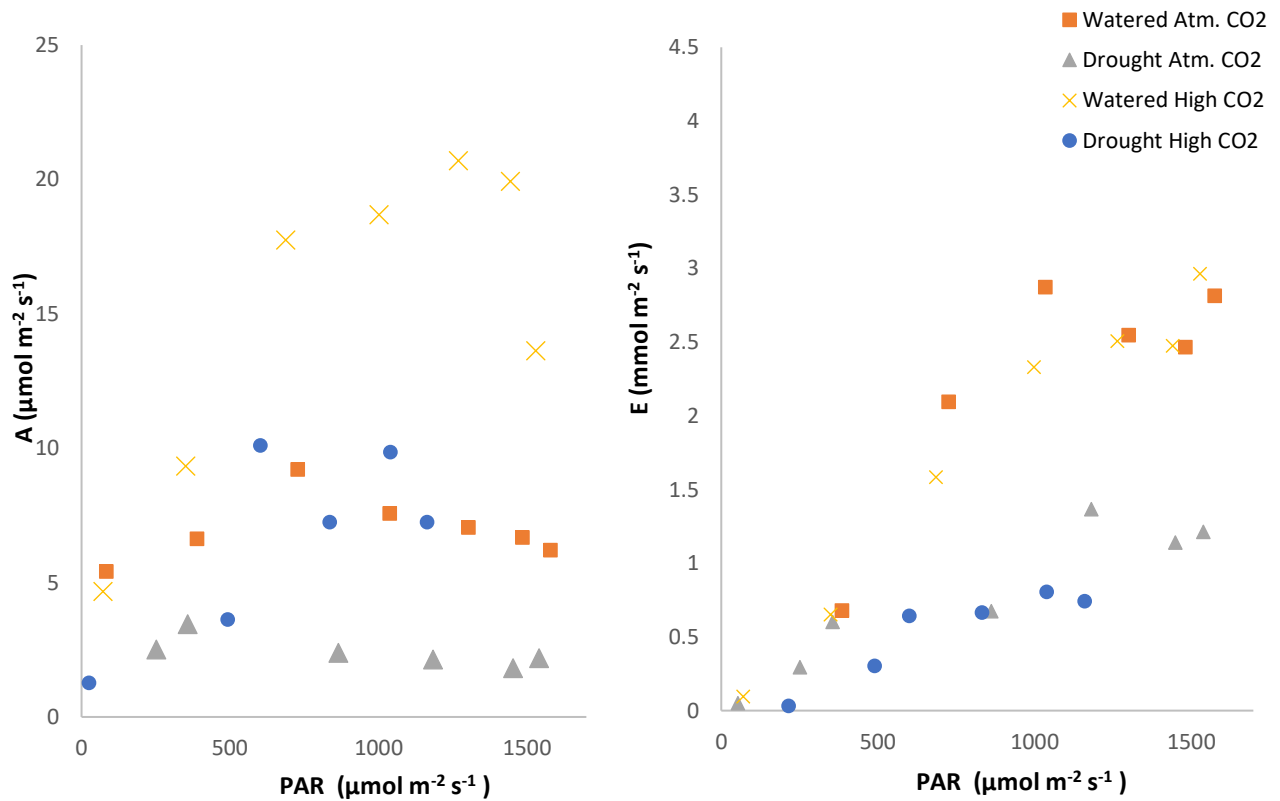

Figure 7. PAR response curves for whole plant net carbon exchange (NCE, μmol m^{-2} s^{-1}, left) and transpiration (E, mmol m^{-2} s^{-1}, right) for each treatment: orange dots for "Watered plants at atmospheric CO_2 concentration", grey dots for "Drought plants at atmospheric CO_2 concentration", yellow dots for "Watered plants at high CO_2 concentration" and blue dots for "Drought plants at high CO_2 concentration". The "High CO_2" corresponds to ~900 μmol mol^{-1}.

- **WUE of watered and drought plants**

Once net carbon exchange and transpiration values were obtained, we were able to calculate the whole-plant water use efficiency (WUE) of plants subjected to each treatment. Under atmospheric conditions, plants subjected to both water regimes described basically the same pattern. We can observe that drought plants had a lower WUE than the watered plants, both under ambient and high CO_2 (Figure 8). Under high

CO_2, WUE always increased respect to the values obtained at atmospheric conditions. Irrigated plants increased their WUE at higher CO_2 concentration, reaching values from 5-10 μmol mmol^{-1}. But it was in drought plants where the increase was even more evident. Non-irrigated plants increased their WUE hugely, reaching values from 10 to 20 μmol mmol^{-1} and even achieving 27 μmol mmol^{-1} of WUE during the first hour in the morning.

In atmospheric conditions, plants showed maximum value in the early morning and late afternoon. Then, they showed a very constant WUE along the day, in both water regimes. When subjected to high CO_2 conditions, plants WUE described the same shape with peaks in the morning and late afternoon. However, we observed an increase of WUE around 14h, and then a decrease.
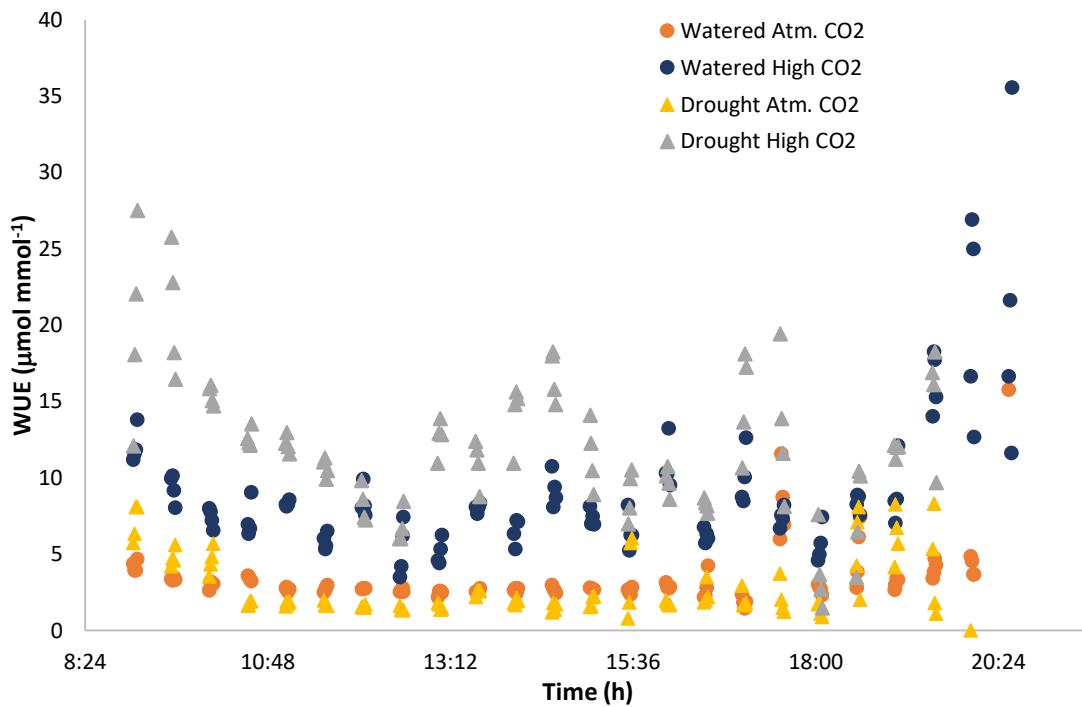
Figure 8. Water use efficiency as NCE/E (WUE, μmol mmol^{-1}) during day hours (from 9-20:30 h) for all different treatments tested: blue circle dots for "Watered plants at atmospheric CO_2 concentration", orange circle dots for "Watered plants at high CO_2 concentration", yellow triangular dots for "Drought plants at atmospheric CO_2 concentration" and grey triangular dots for "Drought plants at high CO_2 concentration". The "High CO_2" corresponds to ~900 μmol mol^{-1}.

- **Rain's effect on carbon assimilation and water transpiration in drought plants**

Unexpectedly, during non-irrigated conditions measurements rain fell allowing us to compare net carbon exchange and transpiration values before and after the rain. This was useful to observe how drought plants responded to a rapid increase of water availability and also how they could readjust gas exchange once drought was restored after the rain.

Table 1. Values of rain accumulated (mm) and mean values of wind speed (m s⁻¹), air temperature (ºC), relative humidity (%), accumulated evapotranspiration (ETO, mm) and maximum radiation PAR (µmol m⁻² s⁻¹).

Period	Rain acc. (mm)	Wind speed (m s⁻¹)	T air (ºC)	HR (%)	ETO acc. (mm)	Rad. PAR (µmol m⁻² s⁻¹)
Before rain	0.000	0.485	26.413	77.738	0.180	1490.514
After rain	15.300	0.679	25.714	71.258	0.159	1303.678
5 days after rain	0.000	0.630	26.116	64.563	0.195	1496.439

Two days before rain, drought plants transpiration was 1.5-2 mmol m⁻² s⁻¹ and NCE about 4-5 µmol m⁻² s⁻¹ (Figure 9). Later, two days after the rain fell, transpiration and net carbon exchange of non-watered plants increased. At that time, plants transpiration increased to 2-2.5 mmol m⁻² s⁻¹ meanwhile NCE 5-7 µmol m⁻² s⁻¹. So, it can be observed that when water availability increased, plants tended to photosynthesize and transpire more but at the same time, their WUE tended to decrease. Five days later, drought conditions were not only restored, but strengthened.
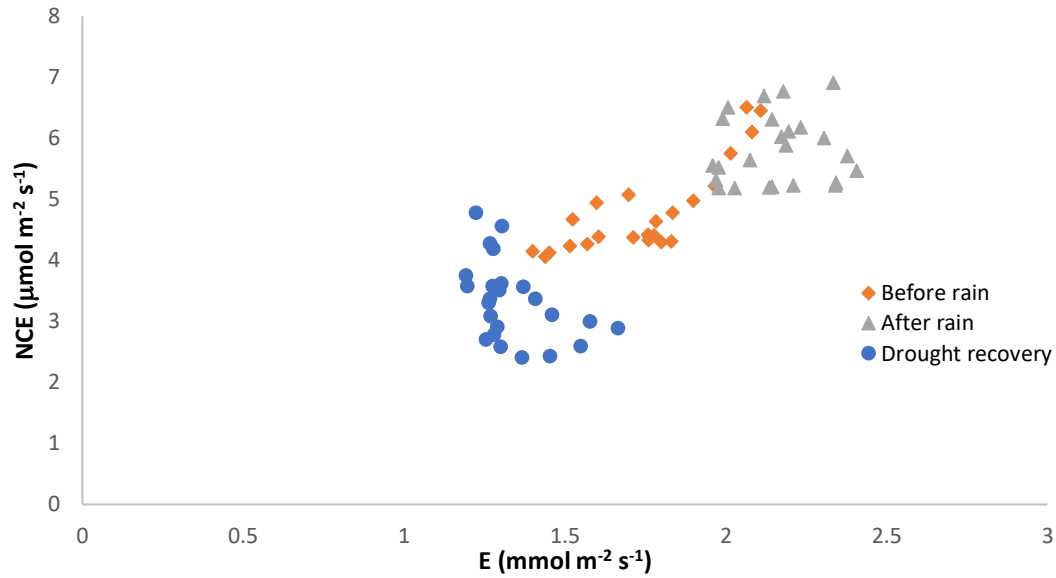
Figure 9. Net carbon exchange (NCE, µmol m-2 s-1) vs transpiration (E, mmol H2O m-2 s-1) values of drought plants submitted to 3 water conditions at photosynthetic peak hours (11-14 h.): orange triangular dots for "Two days before rain", grey triangular dots for "2 days after the rain" and blue circle dots for "Drought recovery (5 days after the rain)".

- **CO_2 concentration effect on carbon assimilation of irrigated and non-irrigated plants**

When CO_2 concentration was artificially increased, the range of concentration allowed us to observe how net carbon exchange varied from atmospheric CO_2 concentration ($\cong$ 375 µmol mol^{-1}) to $\cong$ 1000 µmol mol^{-1} (Figure 10).
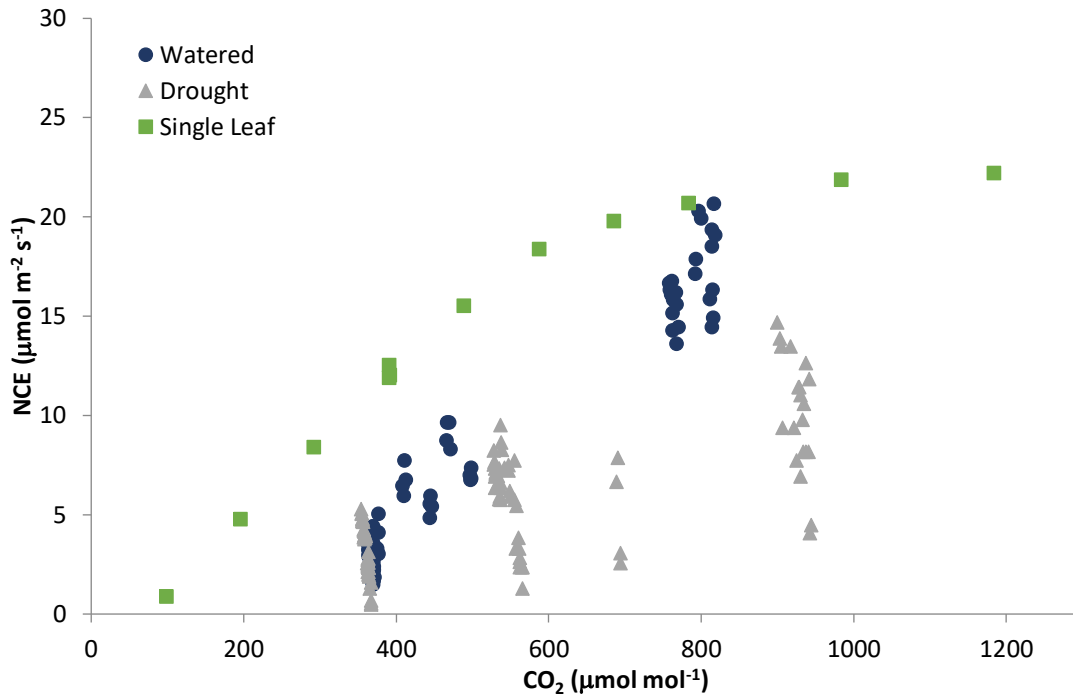
Figure 10. Net carbon exchange (μmol m^{-2} s^{-1}) of watered and non-watered plants measured during the day (from 11-13 h.) in both chambers at different CO_2 concentration (μmol mol^{-1}): blue circle dots are for "Watered plants" and triangular grey dots for "Drought plants". Green square dots are for values of NCE of a single leaf.

In both irrigated and non-irrigated plants, it can be observed how assimilation values during the day increased as CO_2 reference concentration rose. In irrigated plants, values followed a curve slightly higher than in non-irrigated plants. In non-irrigated plants, under atmospheric concentration (≈ 370 μmol mol^{-1}), net carbon exchange reached a maximum peak of 5.3 μmol m^{-2} s^{-1}. When [CO_2] increased to ≈ 600 μmol mol^{-1}, NCE values increased almost to 9.5 mol m^{-2} s^{-1}. Few values were observed near 700-750 μmol mol^{-1} and were irregular and not as higher as expected. Finally, at ≈ 1000 μmol mol^{-1}, net carbon exchange increased more than in the other concentrations, to reach a maximum peak of near 15 μmol m^{-2} s^{-1}. Also, it can be observed the curve expected for a single leaf which followed a logarithmic shape that saturated at $\cong 1000$ μmol mol^{-1}.
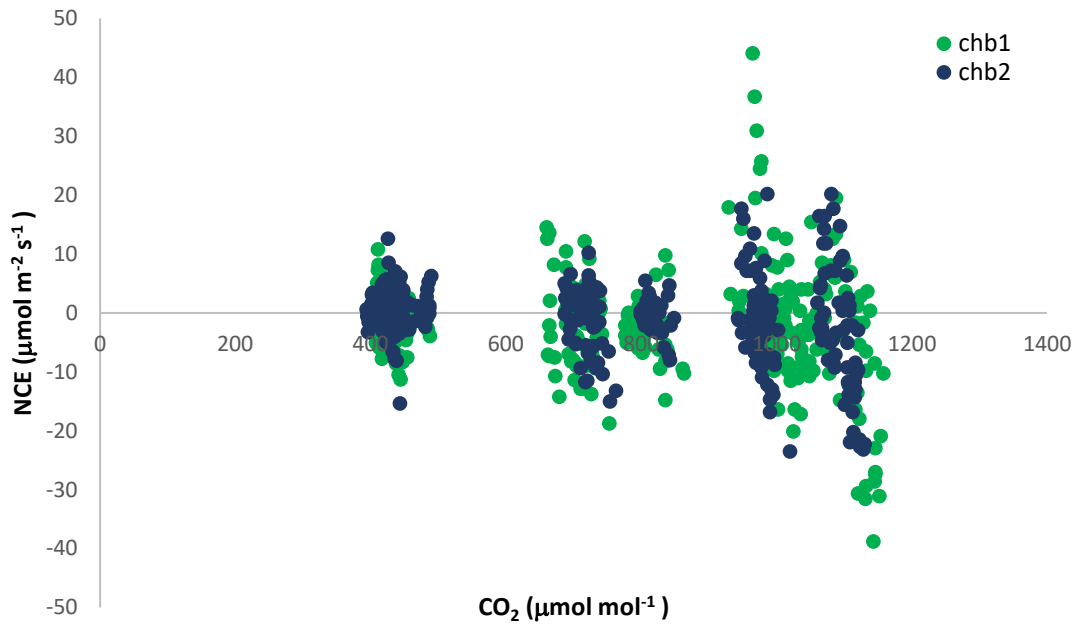
Figure 11. Net carbon exchange (NCE, μmol m^{-2} s^{-1}) of non-watered plants measured during the night (from 22-5 h.) in both chambers at different CO_2 concentration (μmol mol^{-1}). Green circle dots are for "Chamber 1" and blue circle dots for "Chamber 2".

In order to correct for leaks the day values of NCE at different CO_2 concentration, we measured the NCE during the night (ignoring the night respiration). Surprisingly, when measured at night, the apparent NCE did not increase along with the CO_2 concentration, suggesting no leaks of the chambers (Figure 11). We nevertheless observed an increase of the noise on measurement along with CO_2 concentration.

Discussion

- **CO_2 concentration stability along the daily cycle**

We observed two things about daily cycle of the atmospheric CO_2 concentration: it was clearly higher during the night and also very instable when compare to during the day. This phenomenon has been already observed and described (Douthe *et al.*, 2018, *in press*). The strong decrease at sunrise can be explained by the surrounding vegetation that starts to fix CO_2 when light is sufficiently available to induce photosynthesis. On the other hand, the huge instability during the night is harder to explain (not related to wind speed, data not shown). The main problem of night instability is that it prevented to correctly estimate night respiration.

- **Whole-plant net carbon exchange and transpiration in different water regimes**

When plants grow in irrigated conditions, their net carbon exchange and transpiration values are thought to be greater than plants grown in non-irrigated conditions. That is based on the fact that transpiration is reduced by stomatal closure (Medrano *et al.*, 2002). Stomatal closure limits CO_2 entry inside the leaf, and decrease at the same the rate of photosynthesis (Chaves, 1991; Nam *et al.*, 1998; Cornic, 2000; Flexas *et al.*, 2004). Results observed during the experiment followed those expectations, since watered plants photosynthesized 5 $\mu mol\ mol^{-1}$ more than plants subjected to water stress (Figure 4, left). Stomatal closure deprives the leaves of CO_2 and photosynthetic carbon assimilation but also favor of photorespiration (Anjum *et al.*, 2011), reducing the observed net photosynthesis as shown in Figure 4, left.

It is well-documented that water stress in grapevines decreases both leaf and canopy transpiration (Medrano *et al.*, 2002; Merli *et al.*, 2015; Tarara and Pena, 2015; Escalona *et al.*, 2016). Furthermore, as regard to net carbon exchange, it can be observed that in watered plants the maximum peak of photosynthesis is achieved near 10-11 h, whereas in drought plants its maximum peak is reached earlier, 3 hours before (at 7-8 h.). A possible explanation for this effect could be that stressed plants open their stomas very

early in the morning and then they closed them rapidly in order to reduce water lost during the peak solar hours. Because of that, transpiration of drought plants was also lower than in irrigated plants, near 1 mmol m^{-2} s^{-1} less than the watered plants.

One of the major drivers of transpiration in grapevines is light interception; at a given VPD (vapor pressure deficit) and water status, whole-plant transpiration is strongly determined by canopy light interception (Jones, 2014). Because of that, during the hours of higher light, transpiration reached their maximum values, also being higher in watered plants and lower in non-irrigated ones (Figure 7, right). Our results reproduced the same typical photosynthesis versus light curves, as usually obtained for a single-leaf. The whole-plant measurements are noisier that using classical gas exchange systems, but the saturating shape of the light response curves is conserved.

- **Effect of high CO_2 on net carbon exchange and transpiration**

As Aston (1984) reported, increasing CO_2 leads to stomatal closure, causing transpiration to decline while net photosynthesis increases. The values obtained during the experiment followed this statement. In irrigated plants, NCE values were 12 μmol m^{-2} s-1 higher than NCE values under atmospheric conditions. At that time of measurements, E values didn't diminish as expected in higher CO_2 conditions, and in contrast values remain at the same level as those observed in atmospheric conditions. However, it did exist differences in E values in non-irrigated plants case. In comparison of drought plants under atmospheric conditions, a slightly decrease of E values at high CO_2 conditions was observed during the day. It seems that when plants are subjected to a double stress (water stress and high CO_2), they close their stoma in a more evident way, while when they are well watered and under high CO_2 conditions, that closure is not so notable. This is consistent with the findings of Gerardin *et al.* (2018), who find that the stomata of leaves under drought stress were more sensitive to changes in light that under well-watered conditions. It is probable that the stomata are also more sensitive to change of CO_2 concentration when they are subjected to water limitation.

- **WUE of watered and drought plants**

Water use efficiency (WUE) values obtained help us to determine which water regime and under which conditions, plants are more capable of using the water available in a more efficient way. Under atmospheric conditions, watered plants have better WUE than non-irrigated plants. This result is inversed to the classical observation based on single-leaf (WUE usually increase with drought). It is hard to explain why the whole-plant observations do not respect this pattern. Probably, the fact that the whole canopy is the sum of sun and shaded leaves, that act in different ways in terms of photosynthesis and transpiration, could explain this pattern. Nevertheless, the same thing was observed by Douthe *et al.* (2018, *in press*), confirming the validity of our results. Under high CO_2, drought plants are the ones that increased more their WUE values when compared to atmospheric CO_2, practically doubling the WUE values under atmospheric conditions. In watered plants, the increase of WUE under high CO_2 conditions is also notable, but in a less extended way. These differences in the increase in WUE under high CO_2 can be explained by the fact that transpiration decreased much more for the drought plants under high CO_2 than for the watered ones. This increased much more the NCE/E ratio, so the WUE.

- **Rain effect**

When rain fell during the measurement of water-stressed plants, plants were able to use that water in to improve both their photosynthetic rate and transpiration rate. Curiously, 5 days after the rain, when plants returned to non-irrigated conditions, it seemed that drought stress was even harder than the initial one, since their A and E values were lower than those reached before the rain (Figure 10). When soil-available water content was reduced, the stomata opened less in order to prevent dehydration (Taiz and Zeiger, 2002; Anjum *et al.*, 2011; Hoshika *et al.*, 2013), this also resulted in a decrease of the photosynthesis rate (Pirasteh-Anosheh *et al.*, 2016).

- **CO_2 concentration effect on carbon assimilation**

Those values could be useful to predict at canopy level and at different CO_2 concentration which net carbon exchange the plant is going to achieve. At canopy level, it must be considered that all leaves are accounted, even those that aren't exposed

completely to the sun. When we compare these values with values measured for one leaf with a LICOR, the latter is clearly higher, because the measured leaf is exposed to a saturating light. Even with this, we observed the classical saturating shape at high CO_2, confirming that the whole-plant chambers are able to measure correctly the CO_2 response of the whole canopy.

When measured at night, NCE values observed were useful to notice CO_2 leaks of the chamber. We curiously noticed that they were no detectable leaks from the chambers. This result is different from what observed with the same chamber in a previous experiment (Douthe *et al.*, 2018, *in press*), probably for an improved sealing of the chambers use in the present study.

Conclusions

One of the aims of this experiment was to use whole-plant chambers in order to determine net carbon exchange and transpiration values of a whole vineyard plant. With the results obtained, and agreeing with several authors that conducted similar experiments, it seems that whole-plant chambers are a good alternative to scale up from leaf level to canopy level.

Taking into account the values obtained, it seems that in vineyard plants, water stress leads to a decrease of both net carbon exchange and transpiration, proving that water availability is a crucial factor to perform both processes. As well as water availability, light is also important to achieve high photosynthetic and transpiration values since NCE and E values increase along with light. Again, in drought plants, NCE and E values are always lower than non-stressed well-watered plants.

As regard to CO_2, in general, when its concentration rises, plants tend to increase its net carbon exchange and decrease its transpiration. This was observed both in irrigated and non-irrigated plants although in well-watered plants, it seemed that plants didn't close their stomas as much as drought plants did and so their transpiration remained mainly the same as in atmospheric conditions. However, in both water regimes (irrigated and non-irrigated) higher CO_2 increased their WUE, especially in drought plants.

We propose that next studies should focus on the reason of why drought decreases the whole canopy WUE, while it is the inverse for the single-leaf WUE.

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