

## Quantifying the photosynthesis limitation imposed by deficit irrigation in olive trees with a novel methodology based on measurements of sap flux density

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### ABSTRACT

With the ultimate goal of achieving more resilient agricultural systems through deficit irrigation practices managed with a reliable and sensitive indicator of water stress, we conducted an experiment in olive trees with the following specific objectives: (1) to calibrate and validate a method to estimate stomatal conductance ( $g_s$ ) and photosynthesis ( $A_N$ ) automatically based on sap flux density measurements ( $J_s$ ) measurements and (2) to quantify the  $A_N$  limitations produced by soil water content and vapor pressure deficit in a super-intensive olive orchard. Results show that  $g_s$  can be determined from  $J_s$  measurements directly and that the approach is sensitive enough to allow us inferring how stomata respond to soil water content controlled by irrigation and vapor pressure deficit and quantify their limitation on  $A_N$ . The main advantages of this method are that it provides absolute values of  $g_s$ , with physiological meaning to interpret plant water stress and that  $A_N$  limitation imposed by deficit irrigation can easily quantified from the calculated values.

### INTRODUCTION

There is an increasing need of developing more *resilient agricultural systems* to achieve the goals of the 2030 Agenda for Sustainable Development of the UN. More resilient systems include the use of deficit irrigation practices as they make crops less dependent on varying rainfall, using a reasonable amount of water. The correct use of deficit irrigation strategies requires both a good understanding of physiological mechanisms involved in the response of plants to water stress and the use of reliable and sensitive indicators of water stress (Fernández, 2014). Stomatal conductance is a good plant-based indicator for irrigation purposes:  $g_s$  reflects finely the level of water stress and how much  $A_N$  is limited (Flexas et al. 2013) which determines biomass production and, thus, yield. However, its use for irrigation scheduling purposes faces a major limitation from the difficulty of being automatically and continuously monitored. We demonstrated recently (Hernandez-Santana et al. 2016) that  $g_s$  can be estimated continuously from  $J_s$  values normalized by vapor pressure deficit ( $D_a$ ). The automated estimation of  $g_s$  opens the possibility of using it both as a reliable water stress

indicator and in photosynthesis models, which applied to fruit tree orchards can help to estimate yield in advance. Thus, our aims were to (1) to calibrate and validate a method to estimate  $g_s$  and  $A_N$  automatically based on  $J_s$  measurements and (2) to quantify the  $A_N$  limitations produced by soil water content and vapor pressure deficit in a super-intensive olive orchard.

## MATERIAL AND METHODS

The experiment was conducted in summer of 2016 in an orchard nearby Seville (Spain) (37° 15' N, -5° 48' 102). The area has a Mediterranean climate with hot and dry weather from May to September and mild and wet for the rest of the year. The studied trees were central individuals located in plots with four irrigation treatments: two control treatments where irrigation fulfilled tree water demand, using one (C1L) or two (C2L) dripper lines, and two deficit irrigation treatments in which only 45% of the water added to control was applied along the whole irrigation season using one (WS1L) or two (WS2L) dripper lines. Four trees were instrumented per irrigation treatment with one probe set installed into the stem at the east facing side. The Compensation Heat Pulse (CHP) method (Green et al. 2003) was used to obtain point  $J_s$  measurements within the sapwood of the sample trees (Tranzflo NZ Ltd., Palmerston North, New Zealand) at 5 mm below the cambium. Heat pulses (60 J; 60 W over 1 s) were applied once every 30 min and data was stored by a CR1000 datalogger connected to AM25T multiplexer (Campbell, Campbell Scientific Ltd., Shepshed, UK).

Measurements of  $g_s$  and  $A_N$  to calibrate the method were conducted on clear days from May to July every 30-60 mins from dawn to midday in three sun-exposed current-year leaves per instrumented tree of the canopy facing SE. In addition, two measurements of maximum  $g_s$  and  $A_N$  ( $g_{s,max}$  and  $A_{N,max}$ ) were conducted every other week in every instrumented tree from mid-July to the beginning of September (8:00-9:00 GMT) to validate the method. We used a Licor LI-6400 portable photosynthesis system (LI-COR, Lincoln NE, USA), with a 2 cm × 3 cm standard chamber, at ambient light and CO<sub>2</sub> conditions.

Stomatal conductance was estimated using the procedure described in Hernandez-Santana et al. (2016) and  $A_N$  was estimated using the model of Farquhar et al. (Farquhar et al. 1980) and the previously estimated  $g_s$ . Specific temperature response for olive were taken from Diaz-Espejo et al. (2006) and the maximum rate of ribulose biphosphate (RuBP) carboxylation ( $V_{cmax}$ ) and maximum rate of electron transport ( $J_{max}$ ) were determined from five  $A_N-C_i$  response curves measured with a Licor LI-6400 in the instrumented trees of the control and water-stress plots.

Air temperature, air humidity and PAR were recorded every 30 min at canopy height by a standard weather station (Campbell Scientific Ltd., Shepshed, UK) located in the center of the experimental area .

## RESULTS AND DISCUSSION

All relationships established between  $J_s/D_a$  and  $g_s$  were strong (coefficients of determination between 0.44 and 0.84) and significant ( $P < 0.05$ ) for all the trees, extending the results observed in Hernandez-Santana et al. (2016). Validation measurements confirmed the uniqueness of the equations established for each tree, regardless the environmental conditions or the time of the day when the measurements were conducted (Fig. 1). The robustness of the  $J_s/D_a - g_s$  relationships found for several trees and different environmental conditions are explained by the fact that although empirical, the relationships are physiologically based on the control that stomata can exert on transpiration flux (estimated here from sap flow related measurements in the tree trunk) under conditions of high coupling to the atmosphere. The method is simple, relatively inexpensive, it does not require any upscaling and it does not assume any parameter or the measurement of total leaf area or more environmental variables than  $D_a$ . The major limitation of our approach is the empirical nature of the calibration factor relating  $J_s/D_a$  with  $g_s$  which makes necessary to establish a relationship for every sap flow sensor. The modelled  $A_N$  also showed good accordance with measured  $A_N$  for every instrumented tree (Fig. 2).

We also calculated the time course of  $g_{s,max}$  and  $A_{N,max}$  for almost 100 days (Fig. 3) for every instrumented tree. Maximum stomatal conductance was estimated using the previously established relationship of  $g_s - J_s/D_a$  and  $A_{N,max}$  using the Farquhar et al. model. The seasonal course of the calculated  $g_{s,max}$  and  $A_{N,max}$  was mainly driven by  $D_a$  and the irrigation treatment:  $g_{s,max}$  and  $A_{N,max}$  decreased as  $D_a$  increased and they decreased with soil water content, produced by the deficit irrigation. We also quantified  $A_{N,max}$  limitation imposed by deficit irrigation as the difference between the average  $A_{N,max}$  reduction in the control plots ( $67.14\% \pm 0.99$ ) and in the water-stress plots ( $45.13\% \pm 0.95$ ). We calculated this reduction considering the absolute maximum  $A_{N,max}$  found in control plots and thus, we assumed that this reduction was mainly produced by  $D_a$ . The difference between the reduction of control and water-stress plots allowed us to quantify the reduction imposed by soil water deficit, which was on average  $21.27\% \pm 0.52$  for the whole dataset. The reduction by soil water deficit was slightly lower (17.28%) for the lowest  $D_a$  (0.5-0.7 kPa) than for the highest  $D_a$  (24.39% for 2.4-2.6 kPa). As demonstrated here, this approach is sensitive enough to allow us inferring how stomata respond to soil water content and  $D_a$  and quantify their limitation effect on  $A_N$ . This approach has two potential advantages compared to other indices and methods used for irrigation scheduling: it could be based on absolute values of  $g_{s,max}$ , which has a solid physiological meaning of plant water stress and allows to quantify the  $A_N$  limitation imposed by deficit irrigation, which is a direct indicator of yield.

## ACKNOWLEDGMENTS

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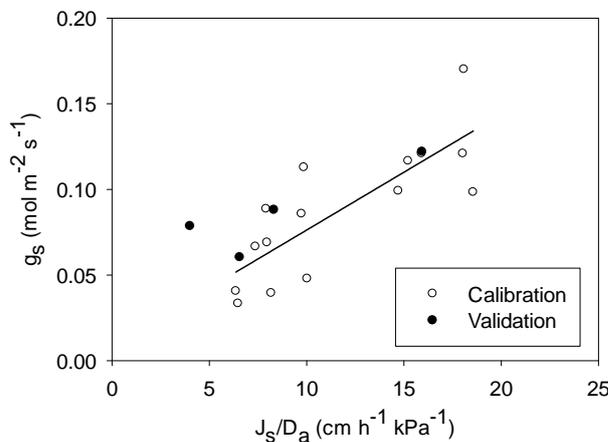


Figure 1. Example of the relationship between sap flux density ( $J_s$ ) measured at 5 mm below the cambium divided by the air vapor pressure deficit ( $D_a$ ) and the stomatal conductance ( $g_s$ ) measured in sun exposed leaves. White circles are calibration measurements conducted in four days and black circles are validation measurements conducted in four different days. The fitted curve was statistically significant ( $P < 0.001$ ).

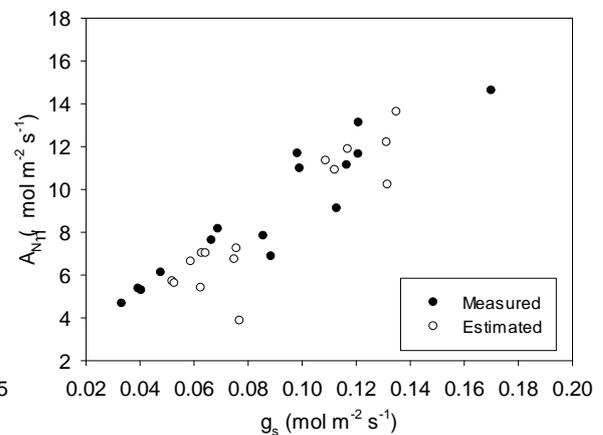


Figure 2. Example of the relationship between stomatal conductance ( $g_s$ ) and photosynthesis ( $A_N$ ) measured (black circles) and modelled (white circles). Modelled  $g_s$  was estimated using the relationship established for sap flux density divided by vapor pressure deficit and  $g_s$  and  $A_N$  was modelled using the modelled  $g_s$  and Farquhar model.

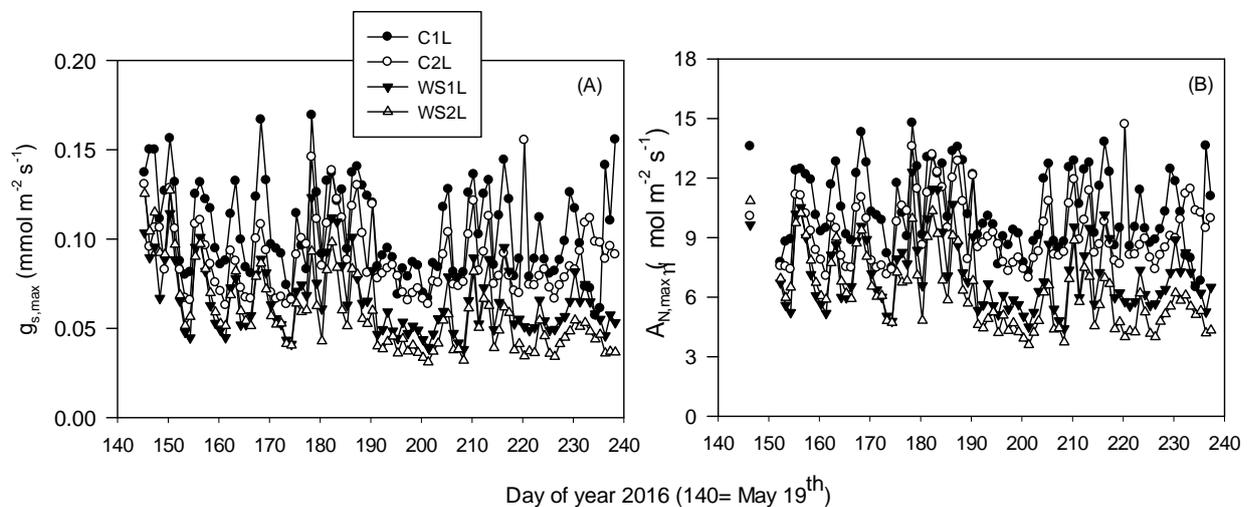


Figure 3. Temporal course of (a) maximum stomatal conductance ( $g_{s,max}$ ) and (b) maximum photosynthesis ( $A_{N,max}$ ), in the four irrigation treatments at Sanabria experimental orchard. Each point is the average of the data of four trees.