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# Contrasting leaf porometer and infra-red gas analyser methodologies: an old paradigm about the stomatal conductance measurement

**Guillermo Toro · Jaume Flexas · José Mariano Escalona**

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**Abstract** Here we compare the stomatal conductance ($g_s$) values obtained with leaf porometer and infra-red gas analyzer (IRGA) in different species subjected to different water availability and evaluated in two different environmental conditions. Sunflower, maize, bean, and grapevine were subjected to two treatments of water availability: well-watered and progressive water stress for 3 days and evaluated in two different times with contrasting environmental conditions. The $g_s$ was determined both on the abaxial and adaxial side of leaves using a leaf porometer and an IRGA. The measured $g_s$ strongly differed between IRGA and porometer, in a way that depended on the species, as well as water availability and environmental conditions. Under maximum water stress, $g_s$ measured with leaf porometer was higher than those measured with IRGA in the four species studied. The present results question the use of usual methodologies for the estimation of $g_s$, suggesting that $g_s$ would not only depend on the environmental conditions and the water status of the plants, but also on the method used to measure it.

**Keywords** Gas exchange · Water stress · Stomatal conductance · Hypostomatic · Amphistomatic

## 1 Introduction

Gas exchange measurements have been used widely in research on plant water relations and responses to water stress. Specifically, the leaf stomatal conductance to water vapor ($g_s$) has been applied to the selection of genotypes with improved performance under different climatic conditions because it is considered a reliable parameter for plant–water relations (Buckley 2017; Medrano et al. 2003). The $g_s$ is a key trait in the regulation of the whole plant carbon and water balances because it controls the $CO_2$ uptake and water loss (transpiration rate) at the leaf level (Farquhar and Sharkey 1982). Variation in $g_s$ has been described in response to many stressful factors such as air temperature, relative humidity, vapor pressure deficit and water stress (Damour et al. 2010).

In general, there are two instruments commercially available to measure $g_s$, infra-red gas analyzers (IRGA), with closed or open system and leaf porometers (closed system). IRGA represents an advanced technique to measure both water and $CO_2$ diffusion

G. Toro (✉)
Centro de Estudios Avanzados en Fruticultura (CEAF), Camino Las Parcelas 882, km 105 Ruta 5 Sur, Sector Los Choapinos, 2940000 Rengo, Chile
e-mail: gtoro@ceaf.cl

J. Flexas · J. M. Escalona
Research Group on Plant Biology Under Mediterranean Conditions, Department of Biology, Universitat de les Illes Balears, cta. de Valldemossa Km 7.5, 07122 Palma de Mallorca, Spain

from the leaf (Lavoie-Lamoureux et al. 2017), via the absorbance characteristic wavelength in the infrared spectrum (Busch 2018). IRGAs are used in several portable photosynthesis systems to measure $CO_2$ assimilation, transpiration rate (E) and $g_s$, the latter being calculated by Ohm's law analogy (Gaastra 1959). Currently, the determination of $g_s$ by IRGA is considered the best technology and accurate for gas exchange measurements in plants, however IRGA is expensive, bulky, require significant training to operate it due to its intricate principles and techniques, and also take a fair amount of time and it is often difficult to match chamber conditions to the outside environment (Pearcy et al. 1989). On the other hand, the porometer represents a good alternative for measuring $g_s$, because it is highly portable, cheap, faster and easy to use than IRGA. In the market, there are available different types of porometer, which may be defined in two main categories: dynamic diffusion (e.g., Delta-T Devices: AP4) and steady-state porometers (e.g., Decagon Devices: SC-1) (Monteith et al. 1988). The dynamic diffusion system measures the rate of relative humidity increase in a chamber attached to leaf, caused by water vapor released through the stomata (Monteith et al. 1988), while steady-state system monitor the relative humidity at two points along the flux path and, once the flux gradient reaches a steady state, it calculates the leaf diffusion conductance (the reciprocal of resistance) (Bell and Squire 1981). In general, screening experiments require a high degree of replication, especially experiments that use $g_s$ measurement as a stress reference. Here, the use of a porometer system becomes important, because it is possible to measure $g_s$ during morning time in less time than using an IRGA.

The $g_s$ is principally regulated by water availability (Medrano et al. 2002) and variation in the vapor pressure deficit (VPD) (Aliniaeifard and van Meeteren 2013), as well as air humidity and temperature (Fanourakis et al. 2016; Turner 1991), but also may be regulated by anatomical and morphological characteristics of stomata. Given the fundamental role that the stomata have in the gas exchange, knowing the stomatal distribution allows to improve the accuracy of measurements of $g_s$. The location of stomata on the leaf surface is also likely to impact significantly on leaf $g_s$ (Richardson et al. 2017). There are two main stomatal distributions, hypostomatic (stomata restricted to the lower or abaxial leaf sides) and amphistomatic (stomata on both abaxial and adaxial leaf sides) (Muir 2015; Richardson et al. 2017). Many studies have reported $g_s$ values using different leaf porometers (Figueiredo et al. 2008; Gunes et al. 2008; Rauf and Sadaqat 2008; Rebetzke et al. 2000; Speirs et al. 2013), but only in few of them the researchers reported a previous calibration between IRGA and promoter (Turnbull et al. 2014).

At present, there is no clear information regarding the possible differences in the measurement of $g_s$ between IRGA and porometer. A recent meta-analysis by Lavoie-Lamoureux et al. (2017) showed very obvious differences between measurements techniques (IRGA and porometer) when evaluating grapevine plants under moderate soil water deficit (leaf water potential about -1.2 MPa). In contrast, according to Turnbull et al. (2014) the $g_s$ of *Eucalyptus* species was reliably proportional between IRGA and porometer. In general, the porometry can yield accurate estimates of $g_s$ in many conditions, however, some limitations should be recognized (McDermitt 1990). Early studies have reported some errors and problems in $g_s$ measurements using porometer (Idso et al. 1988; McDermitt 1990; Monteith 1990; Tyree and Wilmot 1990), which has been mostly associated with relative humidities larger than 80% (McDermitt 1990) and/or large differences between leaf and atmospheric temperatures (Tyree and Wilmot 1990). On the other hand, the act of measure $g_s$ may alter the plant water loss rate in such way that, under very high VPD conditions, the measured $g_s$ in the enclosed chamber may be much reduced than the $g_s$ on plants in the free air (Idso et al. 1988). Thereby, the objective of this study was to determine the degree of variation between the $g_s$ measured with a porometer (Model SC-1, Decagon Devices, Pullman, WA, USA) and IRGA Li-6400 (Li-Cor Inc., NE, USA) to sunflower, maize, bean and grapevine, subjected to two different water availability regimes: well-watered and progressive water stress; and evaluated in two different times with contrasting environmental conditions.

## 2 Material and methods

### 2.1 Plant material and growth conditions

The experiment was carried out under field conditions. Plants of sunflower (*Hellianthus annuus* L.), maize

(*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and grapevine (*Vitis vinifera* L. cv. Tempranillo) were used and grown in 15-L (grapevine) and 5-L (sunflower, maize and bean) plastic pots, with a mixture of peat and perlite (3:1 v/v). These species were selected to include variability in stomata ratios (adaxial/abaxial), thus, the three amphistomatous species (maize, sunflower, and bean) had a stomata ratio of 0.86, 0.73, and 0.11, respectively, while the hypostomatous species (grapevine) had 0.0 (no adaxial stomata) (Table 1). Before the onset of experiment, the plants were irrigated three times per week with tap water and fertilized weekly with 30% nutrient solution, and the plants were randomized. Then, the average air temperature and relative humidity corresponding to the experiments performed at 19–21 July and 2–4 August are shown in the Fig. 1. Climatic data were obtained using a weather station 7450 Groweather (DAVIS instruments Corp., Hayward, California, USA), situated in the experimental field at the University of Balearic Islands (Mallorca, Spain).

### 2.2 Treatment of water availability and different environmental conditions

In field conditions, the plants were subjected to two different water availability regimes: well-watered (WW) and progressive water stress (pWS), and to evaluate the plant under different environmental conditions, we profited the naturally-occurring differences in air temperature and relative humidity during 19–21 July (EC-Jul) and 2–4 August (EC-Aug) (Fig. 1). To establish the water availability treatments, the WW plants were irrigated daily until field capacity, while the pWS treatment was imposed by ceasing irrigation for 3 days. These treatments were imposed and evaluated in the EC-Jul and EC-Aug. Therefore, the changes in environmental conditions between EC-Jul and EC-Aug englobes the differences in the air temperature, relative humidity and VPD.

### 2.3 Leaf gas-exchange measurements

Two measurements of gas exchange were made every day during 3 days of experiment both EC-Jul and EC-Aug on fully expanded leaves between 10:00–13:00 and 15:00–18:00 h. Instantaneous determinations of leaf $g_s$ were done using a portable IRGA photosynthesis system (LI-6400, Li-Cor Inc., NE, USA) and a steady-state leaf porometer (SC-1, Decagon Devices, WA, USA). The IRGA was equipped with a transparent leaf chamber (6 $cm^2$). The $g_s$ measured with IRGA (IRGA-$g_s$) were performed with natural radiation and the cuvette was always oriented to the sun (1223 ± 8 μmol $m^{-2}$ $s^{-1}$ during experimental time), $CO_2$ concentration inside the IRGA cuvette was fixed at 400 μmol $mol^{-1}$, temperature and relative humidity tracked values from external ambient. All data from IRGA measurements were recorded with different configuration of stomatal ratio (according to manufacturing´s recommendation) depending on the plant species: 0 to grapevine, 1 to maize and sunflower, and 4 to bean. The IRGA was calibrated every day according to manufacturer´s recommendations. The $g_s$ with leaf porometer (LP-$g_s$) were performed in Auto Mode configuration and contained a desiccant under the diffusion plate. The LP-$g_s$ was calculated as the sum of the $g_s$ measured both in the abaxial and adaxial sides of the same leaves measured with the IRGA (Dumont et al. 2014; Rebetzke et al. 2000). The leaf porometer was calibrated every 30 min during the complete experimental period. Each selected leaf was

**Table 1** Initial characterization of stomatal density, ratio and distribution in four species under well-watered conditions

| | Stomatal density (stomata $mm^{-2}$) | | Stomatal ratio (adaxial/abaxial) | Stomatal distribution (%) | |
|---|---|---|---|---|---|
| | Adaxial | Abaxial | | Adaxial | Abaxial |
| Sunflower | 187 ± 4.0 e | 256 ± 9.9 g | 0.73 ± 0.04 a | 42 | 58 |
| Maize | 68 ± 2.1 c | 80 ± 5.2 c | 0.86 ± 0.02 a | 46 | 54 |
| Bean | 53 ± 3.3 a | 221 ± 8.8 f | 0.24 ± 0.02 b | 19 | 81 |
| Grapevine | 0 b | 142 ± 12.2 d | 0 c | 0 | 100 |

For stomatal density, different letters mean significant differences between species. For stomatal ratio, different letters mean significant differences between species

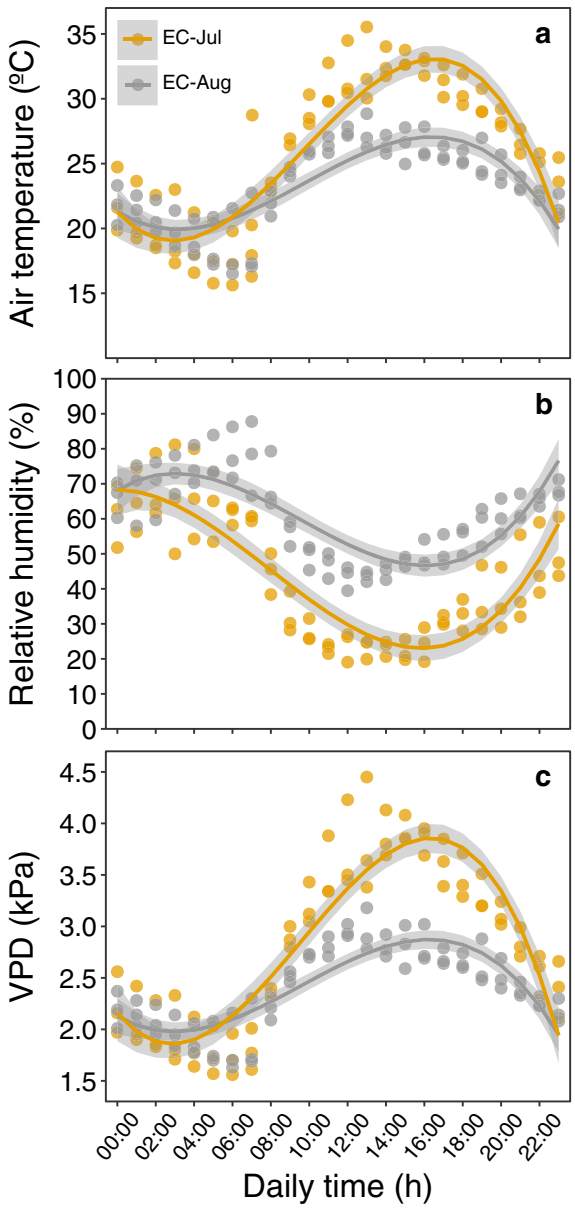


**Fig. 1** Daily time values of (**a**) air temperature, (**b**) ambient relative humidity and (**c**) air vapor pressure deficit (VPD) in the experimental field. Dataset were collected on the days of measurements and represent the average of the stomatal conductance at EC-Jul (yellow) and EC-Aug (grey). Solid line (yellow and grey) represent the model fitted from points raw. Shaded area shows the 95% confidence interval. (Color figure online)

firstly measured with porometer, and after 3 min (without the leaf in chamber) the leaf from the same position was enclosed in the IRGA chamber and the value was recorded after 3–4 min to steady state.

### 2.4 Stomatal density, ratio and distribution

The stomatal density, ratio and distribution were determined in leaves from plants under WW conditions. The stomatal density was quantified in peel impressions through the brushing of nail-polish both in adaxial and abaxial side (avoiding mid-vein) (Fisher 1985) of four full exposed mature leaves per each species and was expressed as number of stomata per $mm^2$. From each leaf, three different vision fields of separate impressions of the lamina were obtained and the numbers of stomata were counted with a microscope at × 400 magnification. The stomatal ratio was calculated from the ratio between adaxial and abaxial stomatal densities. The stomatal distribution between leaf surfaces was determined as the percentage of stomata in both leaf sides.

### 2.5 Data analysis

The experiment was designed in randomized blocks with four replicates per treatment to each plant species. Two-way ANOVA was applied to assess the differences between plant species and the presence of stomata in the adaxial and/or abaxial side of the leaves. Differences between means were established using a Tukey test. Regression coefficients and correlations between pairs of variables were assessed by linear regressions using determination coefficients ($r^2$) and P values ($P < 0.01$). To evaluate the differences between slopes of the regression lines between IRGA-gs and LP-gs, ANCOVA was used from 'car' package. To minimize the effects of homoscedastic data, we performed a Tukey's ladder of powers approach to transform $g_s$ data set using 'rcompanion' package. All analyses were conducted using the R software (Team 2014) and the graphs were made with 'ggplot2' package.

## 3 Results

Figure 1 shows the differences in air temperature, relative humidity and VPD founded to the evaluation times EC-Jul and EC-Aug. No differences were observed to air temperature, relative humidity and VPD between the two daily measurement times, i.e. morning (10:00 to 13:00 h) and noon (15:00 to 18:00 h) (Fig. 1). During the measurements in the

EC-Jul, the range of air temperature, relative humidity, and VPD (Fig. 1a–c, respectively) were 30–32 °C, 25–30% and 3.2–3.8 kPa, respectively, while to EC-Aug were 25–27 °C, 55–65% and 2.7–2.9 kPa, respectively. Therefore, the differences between EC-Jul and EC-Aug were profited to generate the two contrasting environmental conditions, in which the four species under different water irrigation regimes were studied.

Both sunflower and maize showed similar stomatal distribution in the adaxial and abaxial side of leaves showing no significant differences in stomatal ratio to both species, while bean showed 19 and 81% of the stomata in the adaxial and abaxial side, respectively, and bean had different ($P < 0.05$) stomatal ratio to grapevine (Table 1). The stomatal densities in both the adaxial and abaxial sides were different ($P < 0.05$) among species (Table 1).

In our study, the $g_s$ range was highly heterogeneous and dependent on the technique used in the four species (Fig. 2). Under WW condition, the average of LP-$g_s$ observed in the four species evaluated under both EC-Jul and EC-Aug (Fig. 2a–d and i–l, respectively) ranged between 2 and 3.5-fold IRGA-$g_s$ ($P < 0.05$). The pWS increased, even more, the differences between LP-$g_s$ and IRGA-$g_s$ under both EC-Jul and EC-Aug (Fig. 2e–h and m–p, respectively), LP-$g_s$ reaching 3 to 5-fold IRGA-$g_s$.

In all species, regardless of the treatment, the LP-$g_s$ was significantly larger than the IRGA-$g_s$ at the day of maximum stress (Fig. 3). The results showed that in the EC-Jul under WW condition, the $g_s$ of sunflower, maize, and bean (Fig. 3a–c, respectively) using both LP-$g_s$ and IRGA-$g_s$ were higher than in the EC-Aug under WW condition (Fig. 3i–k, respectively). Marked differences were observed in IRGA-$g_s$ and LP-$g_s$ in sunflower, maize, and bean (amphistomatic species) between EC-Jul (Fig. 3a–c, respectively) and EC-Aug under WW condition (Fig. 3a–c and i–k, respectively). Thus, the reduction of IRGA-$g_s$ and LP-$g_s$ of sunflower, maize, and bean in average were 45, 46 and 72% lower under WW condition in EC-Aug (Fig. 3i–k) than EC-Jul (Fig. 3a–c). In contrast, the $g_s$ of grapevine measured with any method was not affected by the EC-Jul or EC-Aug under WW condition (Fig. 3d, l, respectively). On the other hand, both the IRGA-$g_s$ and LP-$g_s$ of sunflower, maize and grapevine under pWS was larger in EC-Aug (Fig. 3m, n, p, respectively) as compared to EC-Jul (Fig. 3e, f, h, respectively), but reduced in bean (Fig. 3g, o, respectively). No differences were observed in pWS for bean in LP-$g_s$ between EC-Jul and EC-Aug (Fig. 3g, o, respectively). However, in our study, the $g_s$ of the amphistomatic species under WW conditions and EC-Aug (Fig. 3i–k) was lower than that in the EC-Jul (Fig. 3a–c). Grapevine is a hypostomatic species, i.e., the water losses are controlled absolutely by the abaxial side of leaves, and under WW condition, no differences were found between EC-Jul (Fig. 3d) and EC-Aug (Fig. 3l) both IRGA-gs and LP-gs.

The LP-$g_s$ was consistently overestimated and greater (above 1:1 line) than IRGA-$g_s$ in the four species (Fig. 4). In the EC-Jul, a strong relationship between LP-$g_s$ and IRGA-$g_s$ was observed in sunflower ($r^2 = 0.922$), maize ($r^2 = 0.782$), bean ($r^2 = 0.881$) and grapevine ($r^2 = 0.861$) under pWS condition (Fig. 4a–d). In WW, the relationship between LP-$g_s$ and IRGA-$g_s$ was also strong in bean ($r^2 = 0.689$) and grapevine ($r^2 = 0.735$) (Fig. 4a, d, respectively), however, there was no correlation in sunflower ($r^2 = 0.063$) and maize ($r^2 = 0.095$) (Fig. 4a, b). Overall, all species in the EC-Aug showed a weak relationship (i.e., below $r^2 = 0.6$) between LP-$g_s$ and IRGA-$g_s$ (Fig. 4e–h). Comparing the fitting lines for each species, our results showed that no differences were observed in WW condition between EC-Jul and EC-Aug for both sunflower (Fig. 4a, e, respectively) and maize (Fig. 4b, f, respectively), but differences were found for bean (Fig. 4c, g) and grapevine (Fig. 4d, h). Under pWS condition, the fitting lines between LP-$g_s$ and IRGA-$g_s$ of maize in the EC-Jul and EC-Aug were similar (Fig. 4b, f, respectively), but differences were observed in sunflower (Fig. 4a, e), bean (Fig. 4c, g) and grapevine (Fig. 4d, h).

## 4 Discussion

The measurement of leaf $g_s$ has been reported widely in different species as a good approximation of the dynamic of water loss and the plant water status (Lavoie-Lamoureux et al. 2017; Miner and Bauerle 2017; Poormohammad Kiani et al. 2007; Rosales et al. 2012). The use of porometer makes it possible to quickly determine the leaf $g_s$. However, environmental factors such as water availability and relative humidity can affect the accuracy of the measurements

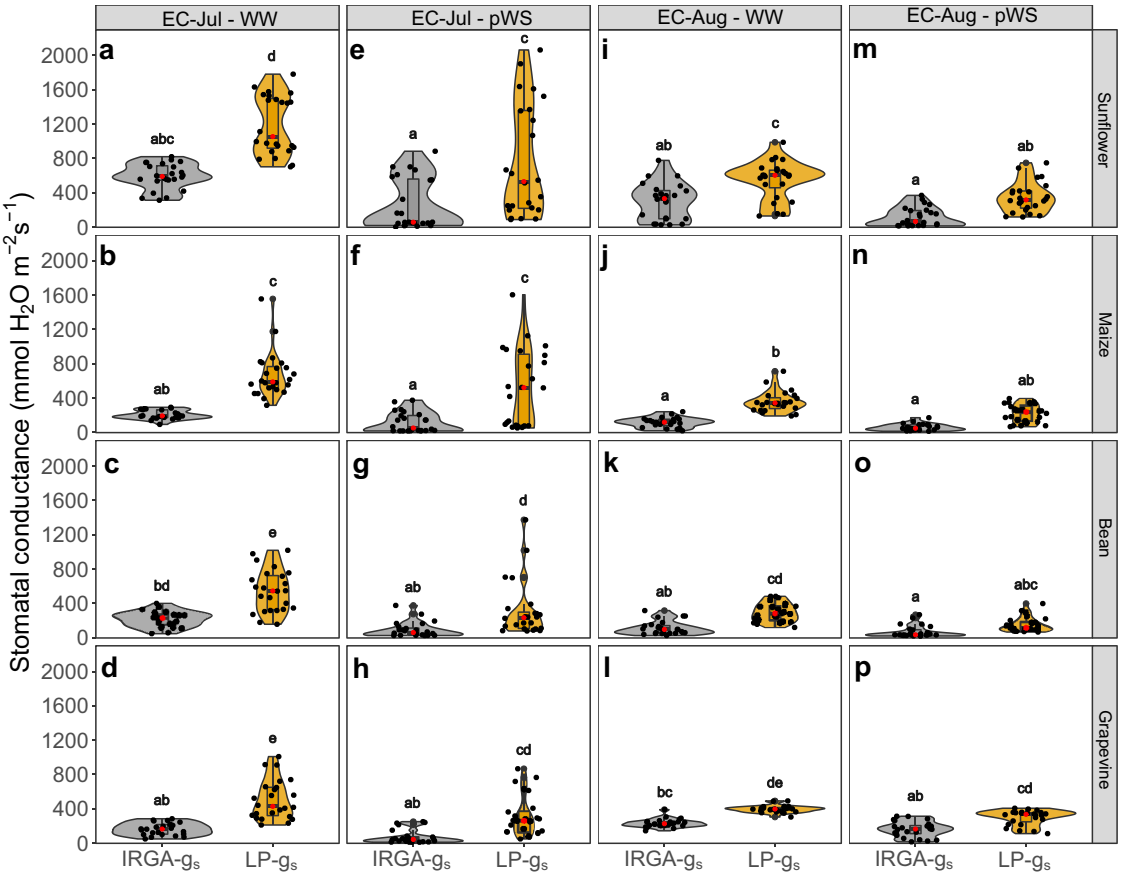


**Fig. 2** Distribution of the IRGA-$g_s$ and LP-$g_s$ (grey and yellow violin chart, respectively) to four-plant species under **a**–**h** EC-Jul and **i**–**p** EC-Aug both well-watered (WW) and progressive water stress (pWS). The width and length of violin chart indicates the density and range of data, respectively. The means are represented by red points. Different letters indicate significant differences between water availability and environmental condition (July and August) for each species ($P < 0.05$). Each violin plot has n = 24. (Color figure online)

(Fanourakis et al. 2016; McDermitt 1990; Turner 1991). Calibrations based on the relationship between IRGA-$g_s$ and LP-$g_s$ in different species and environmental conditions could be a helpful tool to improve the gas exchange measurements. In this study, we determine the variation between the IRGA-$g_s$ and LP-$g_s$, in four species under two regimens of water availability and two environmental conditions.

The stomatal density observed in this study were consistent with those shown in previous works for the same species (Bray and Reid 2002; Ehlers and Goss 2003). The stomatal density was used to calculate the stomatal ratio, which was helpful to adjust the IRGA configuration to measure $g_s$. In this concern, no difference in stomatal ratio was observed between sunflower and maize (0.73 and 0.86, respectively), then the same stomatal ratio in IRGA was configured (STOMRT = 1). To bean and grapevine, the configurations were different ($P < 0.05$). The stomatal ratio to bean and grapevine of 0.11 and 0 (Table 1) were represented in the IRGA configuration as STOMRT = 4 and 0, respectively. Few studies had reported the use of stomatal ratio configuration of the IRGA (Jayasekara et al. 1996), but the importance of stomatal ratio is due to its effects on the estimation of the boundary layer conductance and, thus, the $g_s$.

It is known that $g_s$ depends on the species and environmental conditions but, also, it has been suggested that the obtained value also depends on the type of technique used to measure it (Lavoie-Lamoureux et al. 2017). The high variability of IRGA- and LP-$g_s$ in each species showed clearly an

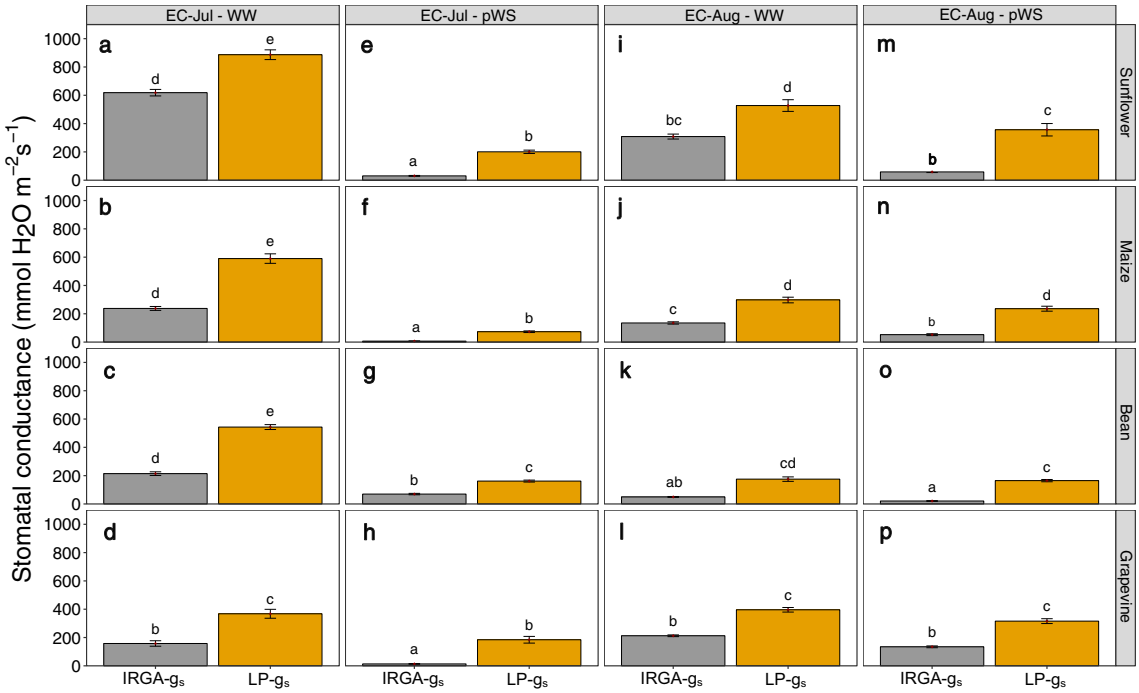


**Fig. 3** Comparison of the mean IRGA-$g_s$ and LP-$g_s$ (grey and yellow bar chart, respectively) in four-plant species under **a**–**h** EC-Jul and **i**–**p** EC-Aug both well-watered (WW) and progressive water stress (pWS) at the day of maximum stress. Different letters indicate significant differences between water availability and environmental condition (July and August) for each species ($P < 0.05$). Error bars represent the standard errors of the mean. (Color figure online)

association with the environmental conditions (Fig. 2). The interaction between environmental factors such as air temperature and relative humidity can be one of the major sources of variation for $g_s$ (Ball et al. 1987; Bunce 2000). In our study, LP-gs showed greater dependence on the combination of air temperature and relative humidity and, in turn, was higher than observed using IRGA-gs (Fig. 2). In general, plants grown under high relative air humidity develop malfunctioning stomata, resulting in high $g_s$ (Arve et al. 2011; Fanourakis et al. 2016), but we showed that $g_s$, for example, measured in sunflower and maize under well-watered conditions in the EC-Aug (Fig. 3i–k) was lower than under EC-Jul (Fig. 3a–c). Amphistomatic species have a differential stomatal response, that may allow regulating the stomatal closure in the surface with higher evaporative demand, assuming there would be differences in the evaporative demand between both leaf surfaces (Richardson et al. 2017). In hypostomatic species as grapevine, i.e., the water losses are controlled absolutely by the abaxial side of leaves, however, the humidity did not affect the $g_s$, because no differences were observed between IRGA-$g_s$ and LP-$g_s$ in well-watered conditions in the EC-Jul (Fig. 3d) and EC-Aug (Fig. 3l).

Water deficit represents one of the most studied disturbances, and its effects on plants have been studied by different techniques such as water potential (Medrano et al. 2003; Miner and Bauerle 2017) and relative water content (Rosales et al. 2012). In this study, our progressive water stress treatment was monitored only by measurements of $g_s$, which could be a limitation of our work to compare with other studies. However, our gs results were consistent with the literature. Recently, a meta-analysis addressing the factors influence $g_s$ found much larger values of $g_s$ when measured with leaf porometer as compared with IRGA (Lavoie-Lamoureux et al. 2017). According to Pearcy et al. (1989) this could be associated to a difference between leaf and atmospheric temperature, however, a minor parallel experiment showed us that no differences were found (data not shown) between leaf and ambient temperature using leaf porometer and IRGA. Therefore, we must conclude that the

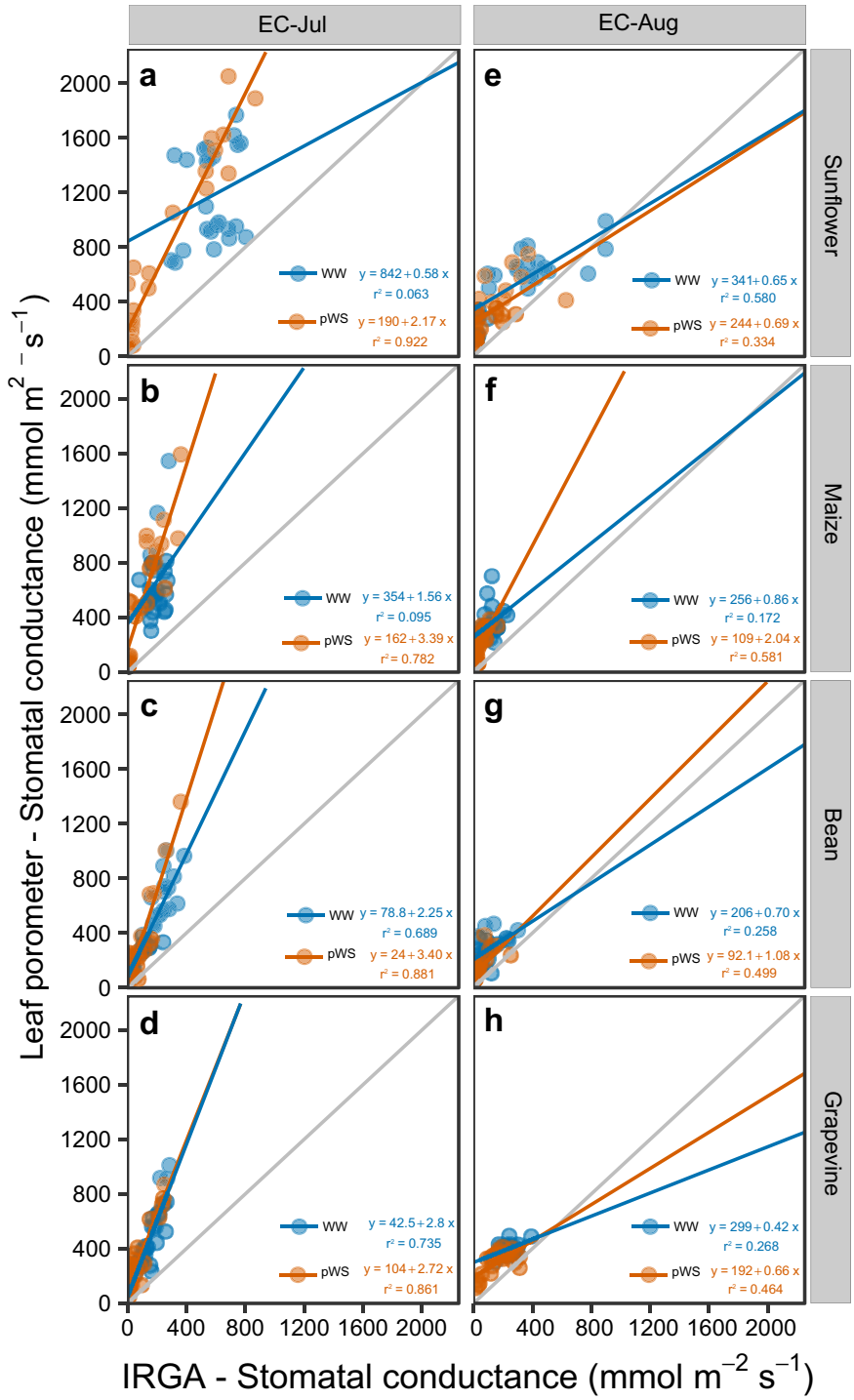


**Fig. 4** Relationships between LP-$g_s$ and IRGA-$g_s$ on four species in the EC-Jul (**a**–**d**) and EC-Aug (**e**–**h**) under WW and pWS (red and blue circles, respectively). Grey solid line is the 1:1 line

relationship between LP-$g_s$ and IRGA-gs is highly variable irrespective of differences between leaf and ambient temperature.

## 5 Conclusion

The evidence presented in this study suggests that the stomatal conductance measurements will be highly

dependent on the combination of environmental conditions and the technique used to measure it, which may be a problem at the moment to make a plant screening using stomatal conductance as a stress marker in a high number of repetitions. The present findings show that the best fitting between IRGA-$g_s$ and LP-$g_s$ was obtained under environmental conditions with elevated air temperature and low relative humidity and especially under progressive water stress for the four species studied. Under well-watered condition, there was a large loss of fitting in sunflower and maize. On the other hand, reduced air temperature and high relative humidity cause weak fitting between IRGA-$g_s$ and LP-$g_s$ in the four species studied, limiting the use of this technique under this environment condition. According to our results, the employment of leaf porometer to compare well-watered and water stressed plants could be used accurately in bean and grapevine, however, in species with high stomatal conductance as sunflower and maize the obtained values may be unreliable.

Globally, the evidence showed in this study strongly suggests the need to make a calibration of leaf porometer in respect to IRGA for each species and environmental condition. Otherwise, the values reported in different studies will remain not comparable, and the conclusions drawn from each individual study challenged.

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**Author contributions** GT and JE designed the experiment. GT performed the measurements. GT, JE and JF analyzed and discussed the data. All authors approved the final manuscript.

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