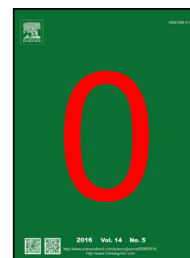




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RESEARCH ARTICLE

## Gas exchange and water relations of young potted loquat cv. Algerie under progressive drought conditions

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### Abstract

Relationships between plant water status and gas exchange parameters at increasing levels of water stress were determined in Algerie loquats grown in 501 pots. Changes in soil water content and stem water potential and their effects on stomatal conductance ( $g_s$ ) and net photosynthesis ( $P_n$ ) rate were followed in control plants and in plants without irrigation until the latter reached near permanent wilting point and some leaf abscission took place. Then, the irrigation was restarted and the comparison repeated. Soil water content and stem water potential gradually diminished in response to drought reaching minimum values of 0.9 mm and  $-5.0$  MPa, respectively, 9 days after watering suspension. Compromised plant water status had drastic effects on  $g_s$  values that dropped by 97% in the last day of the drought period.  $P_n$  was diminished by 80% at the end of the drought period. The increasing levels of water stress did not cause a steady increase in leaf temperature in non-irrigated plants. Non-irrigated plants wilted and lost some leaves due to the severity of the water stress. However, all non-irrigated plants survived and reached similar  $P_n$  than control plants just a week after the irrigation was restarted, confirming drought tolerance of loquat and suggesting that photosynthesis machinery remained intact.

**Keywords:** *Eriobotrya japonica*, deficit irrigation, stem water potential, net photosynthetic rate, stomatal conductance

### 1. Introduction

Plants need large amounts of water to grow and reproduce. When soil water content does not match these plant demands water stress appears. If plant water demand is not satisfied, root water potential and turgor reach very low values, stimulating the synthesis of several plant growth

regulators, including abscisic acid (ABA) (Davies and Zhang 1991). ABA, formed in roots, is then transported to the leaves where induce partial or complete stomatal closure to limit water losses due to transpiration (Davies *et al.* 2005; Lisso *et al.* 2011). The closure of stomata has a drastic effect reducing photosynthetic  $\text{CO}_2$  fixation and, in turn, on plant growth and productivity (Davies and Lakso 1978; Flore *et al.* 1985; Chaves *et al.* 2002; Medrano *et al.* 2002; Díaz-Espejo *et al.* 2006). However, the magnitude of these negative effects depends on the severity and duration of water stress and also on the drought tolerance of the crop and the phenological stage in which the plant is. Some species have developed mechanisms to maintain the turgor of photosynthetic tissues and the stomatal aperture in order to preserve certain levels of photosynthesis (Matthews and Boyer 1984; Nunes *et al.* 1989).

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Among those plants tolerant to severe water deficits, we might cite loquat (*Eriobotrya japonica* Lindl.). Loquat is an evergreen subtropical fruit crop native to southeast China (Lin *et al.* 1999) that belongs to the family Rosaceae subtribe Pyrinae (Potter *et al.* 2007). Despite its origin, loquat seems well-adapted to the Mediterranean climate where drought periods during summer are very frequent. During this period, loquat can withstand prolonged periods of water stress to which responds in terms of early blooming once irrigation (or rain) comes (Cuevas *et al.* 2007). Loquat is, in fact, a crop model for the application of regulated deficit irrigation, since the plant responds to irrigation withholding in summer producing a more profitable crop thanks to the advancement of harvest dates (Hueso and Cuevas 2008, 2010). In the last years, our research team refined the best strategy of regulated deficit irrigation for Algeria loquat as that suspending irrigation starting in early June, and prolong it until plants reach a level of water stress integral ( $S_{\psi}$ ) of around 47 MPa days, usually 8–9 weeks of no irrigation in order to maximize harvest advancement (Fernández *et al.* 2010; Cuevas *et al.* 2012). These levels of  $S_{\psi}$  were measured in commercial plots, where individual trees never exceeded values of stem water potential below  $-2.2$  MPa despite suffering periods of drought of up to three months.

With the aim of determining the maximum levels of water stress a loquat plant may survive, we suspended irrigation in young Algeria loquats grown in pots of reduced volume as long as possible. On these plants, we carefully monitored plant water status and gas exchange parameters in response to the increasing levels of water stress in order to check its effect on  $CO_2$  assimilation rates and see which levels of water stress may provoke complete stomatal closure and cancel photosynthesis. We also pretend to determine the relationships among soil and plant water status and gas exchange parameters by means of correlation and regression analyses to guide irrigation practice linking photosynthesis rate to soil and plant water status.

## 2. Materials and methods

### 2.1. Plant material and experimental conditions

Measurements of leaf gas exchange and water relations were performed during summer 2012 in an irrigation experiment carried out on 5-year-old potted loquat trees (Fig. 1), located at the Cajamar Experimental Station in Almeria, Southeast Spain ( $36^{\circ}48'N$ ,  $2^{\circ}43'W$ ). This area presents a semi-arid subtropical climate with an average rainfall of 241 mm and no rain in summer. The annual evaporation, measured with a Class A pan placed on bare soil at the vicinity of the experimental orchard, averages



**Fig. 1** Irrigated (I, A and C) and non-irrigated (NI, B and D) plants and details of leaves at the end of the experiment.

1940 mm per year, with extreme values of 8.7 mm per day on July, 2012.

The loquat trees were grafted on Provence quince and were grown in pots with a volume of 50 l (outside diameter 0.5-m, and height of 0.41-m) filled with a mixture of sandy-loam soil typical of the area (2/3) and peat (1/3). On these plants we established two treatments, a control treatment in which the trees were maintained near field capacity (I) and a treatment in which the trees were non-irrigated (NI) until they were near wilting point. The effects of the treatments were analyzed following a randomized complete design with 5 trees (replicates) per treatment. To keep control irrigation treatment close to field capacity, drip irrigation was daily performed providing 6 l per pot and day. There was no rain at all during the experimental period. The experiment was designed to last two weeks. However, the harsh conditions suffered during that summer (see results) forced us to shorten irrigation withholding period, and restart the irrigation 10 days after the beginning of the experiment. The time course of ambient temperature and relative humidity and therefore vapor pressure deficit is provided as supplementary material (Appendix A).

### 2.2. Soil and plant water status and gas exchange measurements

To determine the relationships of gas exchange with the conditions suffered by the trees, we carefully monitored during the experimental period (from June 17 to July 6) the

environmental conditions, soil water content, plant water status, gas exchange parameters and leaf temperature. Measurements were performed just before irrigation suspension (day 0), and 2, 4, 7, and 9 days after irrigation withholding (daiw). A new measurement was performed one week after irrigation was restarted.

Weather (air temperature, relative humidity and vapor pressure deficit) parameters were recorded every 5 min using a data logger HOBO (model U23-001, Onset Computer Corp., Bourne, MA, USA). The averages during the period of measurement are provided as supplementary data in Appendix A. Changes in soil moisture were followed recording pot weight changes due to evapotranspiration and for changes in volumetric soil water content during the experiment. The changes in the weight of the pots were recorded using a weighing scale of 0.1 kg precision (Cobos Serie K-Rekord, Barcelona, Spain). No drainage or runoff took place in the pots of I or NI plants. The volumetric soil water content was determined with a time domain reflectometry (TDR) System (Trase 6050X1, Soil Moisture Equipment Corp, Santa Barbara, CA, USA). Readings were taken on five trees per treatment during the water deficit period and after re-irrigation using 15 cm long waveguides.

Plant water status was monitored by measuring midday stem water potential ( $\Psi_{st}$ ) with a pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Measurements were performed each time in five fully expanded leaves (one per tree and treatment) located in branches near tree trunk and randomly selected. Leaves were bagged and covered with aluminum foil early in the morning, more than 2 h before leaf detachment (McCutchan and Shackel 1992).

$P_n$  and stomatal conductance ( $g_s$ ) were measured using a Portable Open Infrared Gas Analyzer (CIRAS-2; PP System, Hitchin, Herts, UK) on fully expanded leaves. Readings were taken after steady-state conditions in gas exchange were achieved (around 1 min). The  $CO_2$  concentration inside the chamber was automatically controlled by the CIRAS-2 porometer at 400 ppm (ambient  $CO_2$  concentration, approximately), whereas the radiation, the temperature and the evaporative demand were those of the environment too.  $CO_2$  and water vapor concentration differences, as well as leaf temperature obtained from energy balance equations, were used to calculate leaf area based  $P_n$ , transpiration rate ( $E$ ) and  $g_s$  using von Caemmerer and Farquhar's equation (von Caemmerer and Farquhar 1981). For gas exchange measurements, three fully expanded sunlit leaves in five trees per treatment (15 leaves per treatment) were randomly selected and tagged for continuous measurements. The measurements of  $P_n$  and  $g_s$  were made during the period of water stress and after re-irrigation. Irrigation was restarted 10 days after the

beginning of the experiment. Daily variations of leaf gas exchange were measured between 10:00 and 13:00 h GTM.

In order to establish the relationships among soil and plant water status and gas exchange parameters, correlation and regression analyses were performed. Statistical analyses were done using Statgraphic and Sigmaplot software.

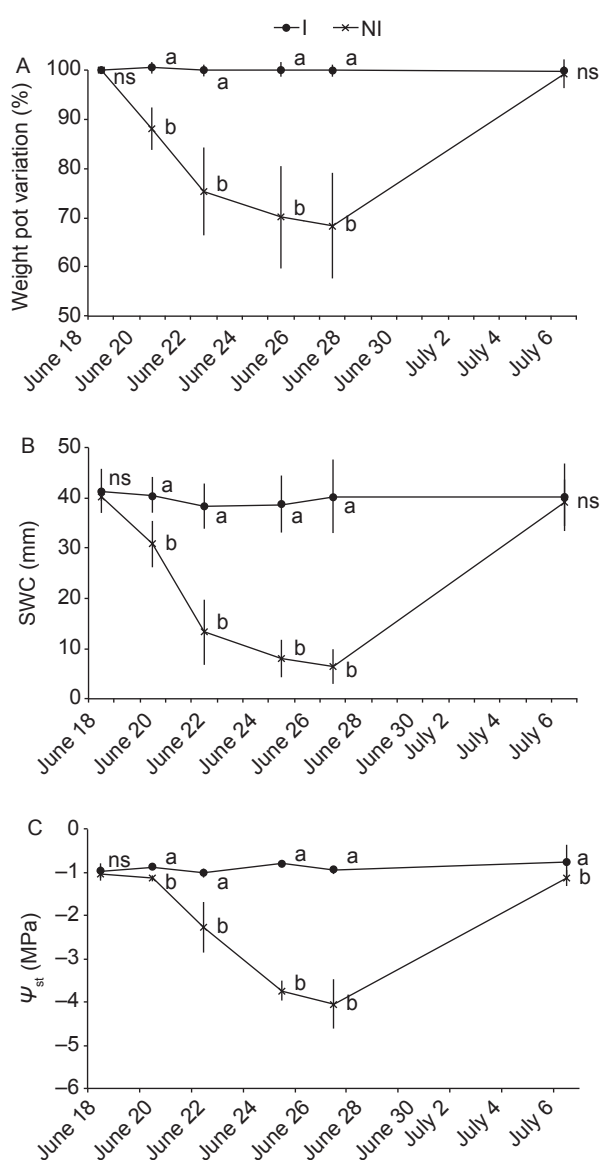
### 3. Results

#### 3.1. Soil and plant water status

The suspension of irrigation caused a rapid and significant loss in pot weight, more acute in the first seven days of the experiment when a daily average of 0.88 kg of water was lost in the pots of non-irrigated loquats (Fig. 2-A). Pot weight losses continued at a lower rate from day 7 to the last day of the irrigation withholding (9 daiw). Once irrigation was restored, potted loquats regained original weight (Fig. 2-A). The weight of the pots in irrigated plants remained constant along the whole period of the experiment. Initial measurements of I and NI plants showed that the treatments did not differ in this regard, as they did not do it at the beginning of the experiment for any other parameter under control (Fig. 2).

Volumetric soil water content measured using TDR also diminished as the dry period advanced, showing significant differences between I and NI plants 2 daiw ( $I=40.59$  vs  $NI=30.79$  mm), coinciding with the first significant loss of pot weight. The differences became higher 4 daiw ( $I=38.31$  vs  $NI=13.36$  mm) and after (Fig. 2-B). Soil water content in I plants did not show large variations during the period of the experimentation, oscillating around field capacity value (Fig. 2-B). Since runoff and drainage were not observed, lack of changes in pot weight in I plants confirm the adequacy of the irrigation program.

$\Psi_{st}$  clearly reproduced the changes in water availability showing significant differences between treatments also beginning 2 daiw (Fig. 2-C). The differences remained significant for the entire dry period, but diminished in a great extent a week after irrigation was restarted (Fig. 2-C). In the last day of the dry period, the average  $\Psi_{st}$  value in NI plants was  $-4.05$  MPa. At that time, the measurements made with the pressure chamber became very difficult, since the emergence of the water drop through the petiole of the leaf was largely delayed, even at very high pressures, causing in occasions the tearing of the leaf blade inside the chamber. In these circumstances, the movement of the petiole out of the pressure chamber made dangerous the measurements given the high pressures applied. A single plant reached a negative value of  $\Psi_{st}=-5.00$  MPa, before we decided cancelling the dry period. I plants showed values of  $\Psi_{st}$  between  $-0.70$  and  $-1.15$  MPa all the time.



**Fig. 2** Changes in pot weight (A), volumetric soil water content (SWC, B) and stem water potential ( $\Psi_{st}$ ) (C) during the experiment (from June 18 to July 6, 2012) in irrigated (I) and non-irrigated (NI) plants. The different letters in each measurement date indicate significant differences at 0.05 level. ns, not significantly different.

### 3.2. Gas exchange parameters

Water stress did indeed affect gas exchange parameters, but did it 4 daiw, that is, two days later than the effects on soil and plant water status were observed. From equivalent values of stomatal conductance before irrigation withholding ( $I=150.40$  vs  $NI=148.60$   $\text{mmol m}^{-2} \text{s}^{-1}$ ),  $g_s$  records dropped in NI plants, while remained more or less stable in I plants (Fig. 3-A). The differences became significant 4 daiw and increased afterwards, since  $g_s$  values plummeted in NI loquats as the dry period progressed. At the end of the dry

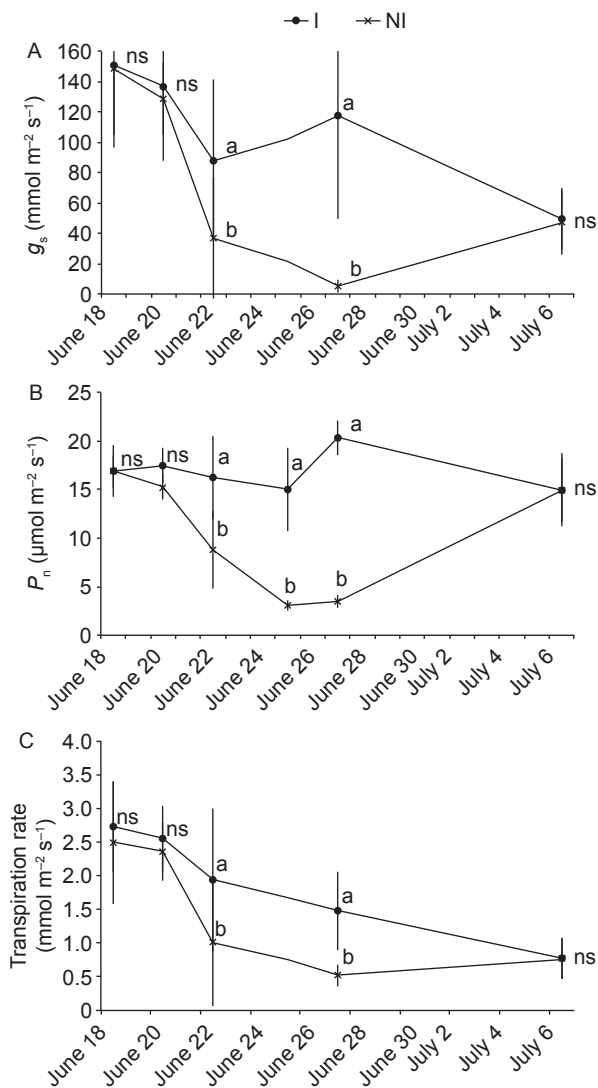
period,  $g_s$  records in NI plants averaged only  $5.37$   $\text{mmol m}^{-2} \text{s}^{-1}$ .  $g_s$  was occasionally reduced in I plants, probably due to the harsh environmental conditions suffered during the experiment (high temperatures and low relative humidity leading to high vapor pressure deficits; see supplementary data). In any case,  $g_s$  differences between I and NI plants remained significant until the end of the dry period. Once irrigation was restarted, these differences disappeared.

The effects of irrigation withholding on photosynthesis were also noted 4 daiw, when  $P_n$  values in NI plants were barely a 50% of those measured in I plants ( $I=16.23$  vs  $NI=8.74$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The last day of irrigation withholding NI plant averaged  $3.49$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  a value much lower than that measured in control plants ( $20.35$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). After watering was reassumed, NI reached an average  $P_n$  value of  $14.98$  versus an average of  $14.93$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  measured in I plants, informing of the fast recovery of NI loquats. Minor oscillations in  $P_n$  linked to changes in the environment were also observed in I plants (Fig. 3-B).

In line with previous argument, the transpiration ( $E$ ) in NI plants was largely reduced due to the severe water stress. The differences between I and NI plants became significant 4 daiw too ( $I=1.94$  vs  $NI=1.01$   $\text{mmol m}^{-2} \text{s}^{-1}$ ) (Fig. 3-C). The differences were even larger at the end of the dry period ( $I=1.48$  vs  $NI=0.52$   $\text{mmol m}^{-2} \text{s}^{-1}$ ), but once again disappeared one week after reirrigation ( $I=0.77$  vs  $NI=0.76$   $\text{mmol m}^{-2} \text{s}^{-1}$ ). Stomatal closure has been often linked to an increase in leaf temperature since the lack of transpiration through the closed stomata makes difficult the refrigeration of the leaf tissues. Daily morning temperature during the experiment was between  $28.7$  and  $32.9^\circ\text{C}$ . At solar noon, when measurements were taken, temperatures rose to between  $30.7$  and  $36.7^\circ\text{C}$  (Appendix A). Leaf temperature did not show a clear trend during the experiment. In occasions, leaf temperature was above ambient (approximately  $2^\circ\text{C}$  above in both treatments), while in some other moments readings taken in the leaves were below ambient temperatures. The differences between treatments in leaf temperature were not significant and scarcely reached a few tenths of a degree (Appendix A).

Multiple linear regressions were performed to detect the main environmental and physiological determinants of photosynthetic rates in Algeria loquats. The analyses show significant relationships between  $P_n$  and  $g_s$  ( $P=0.007$ ), and between  $P_n$  and  $\Psi_{st}$  ( $P=0.008$ ). The highest coefficient of determination of  $P_n$  rate was obtained for  $g_s$  ( $r^2=0.759$ ). Non-linear regressions confirmed the significant relationship between these two parameters ( $P<0.0001$ ). The best fitting ( $r^2=0.794$ ) was observed for a logistic model (Fig. 4), in which three parts can be identified. For  $g_s$  values close to zero,  $P_n$  was minimal. In the second part of the curve, a linear relationship between  $g_s$  and  $P_n$  can be deduced. Finally, for





**Fig. 3** Changes in stomatal conductance ( $g_s$ , A), net photosynthetic rate ( $P_n$ , B) and transpiration rate (C) during the experiment (from June 17 to July 6, 2012) in irrigated (I) and non-irrigated (NI) plants. The different letters mean significant different at 0,05 level. ns, no significant different.

$g_s$  values higher than  $80 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $P_n$  did not increase much, but showed instead a plateau around values of  $20 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The highest single measurement of  $P_n$  was  $25.6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  in control fully irrigated plants measured at the beginning of the experiment in leaves with a value of  $g_s$  of  $253 \text{ mmol m}^{-2} \text{ s}^{-1}$ . Photosynthesis was never zero. The minimum value of  $P_n$  measured was  $1.8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  in NI plants at the end of the dry period, and when stomata seemed almost close ( $g_s = 1.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). The comparison of the slopes of the regression equations explaining the relationships between  $P_n$  and  $g_s$  in different sections of the range showed significant differences ( $P < 0.001$ ) (Fig. 4). The slope of central section was ten times higher ( $m = 0.1777$ )

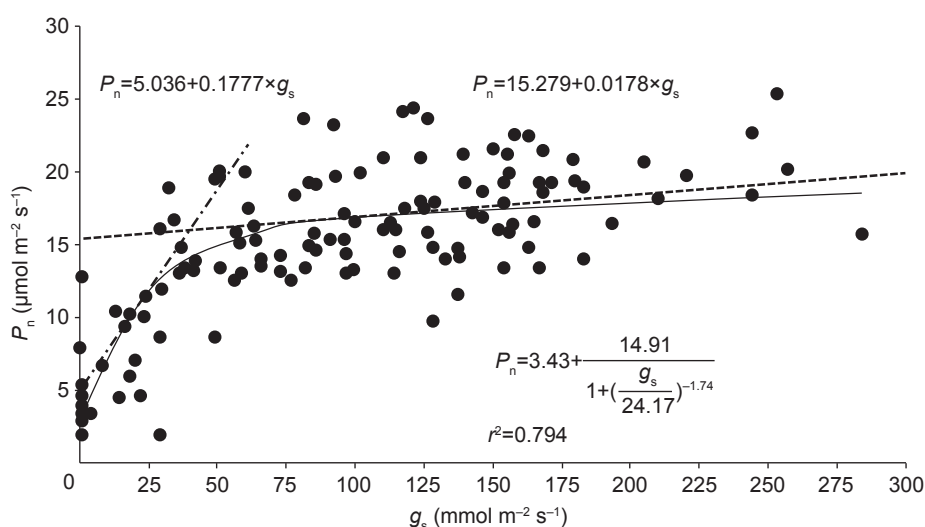
than the increase rate observed in the region of the plateau ( $m = 0.0178$ ), meaning a much higher increase of  $P_n$  rates in the central section of the curve.

On the contrary, no significant relationships were found between  $P_n$  and pot weight, or soil water content, E and leaf temperature (data not shown). The significant relationship between  $g_s$  and  $\Psi_{st}$  was not lineal either, but exponential, meaning in this case that higher (less negative) values of  $\Psi_{st}$  allowed a much higher stomatal aperture (Fig. 5). In other words, a better water status of Algeria loquat plants allows higher stomatal aperture which in turn favors a higher photosynthesis rate. On the contrary, when  $\Psi_{st}$  fell below  $-1.5 \text{ MPa}$ ,  $g_s$  diminished very rapidly precluding then  $\text{CO}_2$  fixation as  $P_n$  drops acutely. If  $\Psi_{st}$  dropped to values lower than  $-3.0 \text{ MPa}$ ,  $g_s$  became almost zero (Fig. 5).

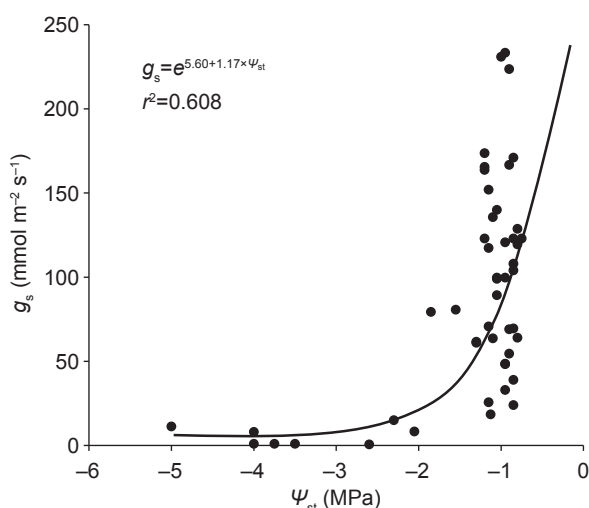
#### 4. Discussion

Irrigation withholding caused a rapid reduction in water availability in young Algeria trees that were planted in small pots with a limited soil volume. This reduction was reflected in a drastic diminution of the weight of the pots, consequence of the water losses that in turn affected plant water status. Soil water content as well as  $\Psi_{st}$  values were extremely low at the end of the irrigation withholding period forcing us to cancel the dry period before the two-week duration scheduled. The strong water losses and the negative effects observed on plant water status must be the result of the difference between the reduced availability of water in the small pots and the evaporative demand (Boyer 1995), quite high during summer in Almeria, Spain (Appendix A). Detrimental effects on plant water status caused by days with high evaporative demand are often reported in the Mediterranean climate, even for fully irrigated trees of many crops (Moriana et al. 2003; Tognetti et al. 2009; Martín-Vertedor et al. 2011). Nonetheless, the main force reducing  $\Psi_{st}$  in NI loquats seems the complete suspension of irrigation. If rapid negative effects were noted on NI plants when irrigation was suspended, equally rapid was the recovery of them when irrigation was restarted, demonstrating thus the extraordinary capacity of loquat to endure severe water restrictions.

Progressive stomatal closure was the response of NI plants to adapt to the drastic water losses in the soil and to the compromised plant water status. In accordance to this response,  $g_s$  was progressively diminished in NI plants reaching the lowest values at the end of the dry period. The significant relationships between  $\Psi_{st}$  values and  $g_s$  records is shown in Fig. 5, from which can be deduced that when  $\Psi_{st}$  falls below  $-1.5 \text{ MPa}$ ,  $g_s$  diminishes rapidly. If  $\Psi_{st}$  drops to values lower than  $-3.0 \text{ MPa}$ ,  $g_s$  becomes almost zero. This indicates notable effects of moderate levels of



**Fig. 4** Relationship between net photosynthetic ( $P_n$ ) and stomatal conductance ( $g_s$ ). The whole set of data significantly fitted a logistic curve and a linear increase of  $P_n$  was found for values of  $g_s < 80 \text{ mmol m}^{-2} \text{ s}^{-1}$ . For values above this level, a plateau region was observed with the slopes of these regions being significantly different ( $P < 0.001$ ).



**Fig. 5** Relationship between stomatal conductance ( $g_s$ ) and stem water potential ( $\psi_{st}$ ).

water stress on gas exchange parameters, and extreme if water stress is severe. This response is compatible with the remarkable capacity of water-stressed loquats to fully recover once irrigation was restarted.

Stomatal closure in response to abscisic acid (ABA) accumulation in the leaves is a common response to water deficits (Lisso *et al.* 2011), since one of the most important functions of ABA is to avoid water losses and regulates the tolerance to osmotic stress (Boudsocq and Lauriere 2005). In this sense, it seems that ABA accomplishes a double function in response to drought: it diminishes guard cells turgor and, in addition, induces the expression of genes

involved in the tolerance to severe water deficits (Zhu 2002). Low water availability is the main environmental factor limiting photosynthesis and growth in flowering plants (Boyer 1982). This effect often causes severe yield reductions in crops growing in arid and semi-arid zones such as in the Mediterranean climate (Chaves *et al.* 2003). When water availability is limited after drought episodes, the negative effects on plant water status might lead to progressive stomatal closure and to lower rates of photosynthesis (Lawlor 1995). In this regard a lineal relationship between stomatal closure and photosynthetic rates has often been reported. In our case,  $g_s$  reduction in potted loquats caused a rapid fall of  $P_n$  rates (Fig. 4) and a diminution of  $E$ . However, in some species the relationship between these parameters is more complex. In this regard, Farquhar and Sharkey (1982) conclude that although  $g_s$  substantially reduces transpiration, rarely limits significantly photosynthesis, because this process is more often limited for other factors contributing to stomatal closure. For instance, under high  $\text{CO}_2$  levels, the limiting factor for photosynthesis is the regeneration of Rubisco.

In our study,  $P_n$  rate showed a significant relationship with  $g_s$ . The best fitting was observed for a logistic model. Logistic model suggests a maximum level of  $P_n$  established around an asymptote. That asymptote was around  $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$  when stomata were fully open. In the logistic curve, three parts were identified. For  $g_s$  values zero,  $P_n$  was minimal, although not zero, but  $2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . In the second part of the curve, a linear relationship between  $g_s$  and  $P_n$  was found. Finally, for  $g_s$  values higher than  $80 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $P_n$  did not increase much, but instead it showed a

plateau, meaning that higher aperture of stomata scarcely increases  $P_n$  in young potted Algeria loquats (Fig. 4). Low  $\text{CO}_2$  levels within the leaves often limit photosynthesis in cases of severe water stress. In this regard, Flexas *et al.* (2004) concluded after a detailed study that water stress mostly affects  $\text{CO}_2$  diffusion in leaves. This decreasing in  $\text{CO}_2$  diffusion could be a consequence of the reduction in  $g_s$ , but in any case, water deficit would affect the biochemical capacity of the leaves for  $\text{CO}_2$  assimilation. Our experiment shows that despite severe water stress strongly reduced photosynthesis,  $\text{CO}_2$  assimilation never dropped to zero, indicating that the closure of stomata largely reduced, but did not suspend completely photosynthesis in 'Algerie' loquats. This suggests that some  $\text{CO}_2$  diffusion is taking place perhaps through the cuticle despite stomata are almost closed. This  $\text{CO}_2$  entry would allow a slight photosynthetic rate, even at severe water stress. Boyer *et al.* (1997) assure that leaf cuticle protects efficiently leaves from dehydration, but that even when stomata are sealed,  $\text{CO}_2$  and water vapor diffuse slowly through the cuticle.

After restoring irrigation,  $P_n$  levels were rapidly recovered reinforcing the hypothesis that photosynthetic machinery remained intact, after such a severe water stress. Similarly, olive trees subjected to severe water stress had photosynthesis values between 0 and  $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ , but also recovered rapidly after irrigation (Moriani *et al.* 2002). Young apricot trees subjected to two periods of water deficit only diminished photosynthetic rate at the end of the dry periods, showing also a fast recovery afterwards. These results suggest that stomata behavior is not a passive response to water deficit. On the contrary, it seems that the aperture and closure of stomata is under a more complex regulation (Ruiz-Sánchez *et al.* 1997), at least in species well-adapted to the Mediterranean climate such as olive, apricot and loquat. On the contrary, in avocado, photosynthesis is inhibited under mild water stress due to a reduction in  $\text{CO}_2$  diffusion linked to stomata closure and to changes in the structure of the mesophyll of the leaf (Chartzoulakis *et al.* 2002).

In this study, we did not find an effect of irrigation withholding on leaf temperature. In this sense, leaf temperature differences between treatments were not significant and scarcely reached a few tenths of a degree. Leaf temperature is directly determined by leaf energy and water balance, but radiation, air temperature, humidity and wind speed modify leaf temperature (Fuchs 1990). High values of  $P_n$  were observed in control plants even when leaf temperature was very high. On the contrary, in a previous study conducted on adult trees in a loquat orchard, very low  $P_n$  values were observed at midday for fully irrigated plants (Stellfeldt *et al.* 2011). It is widely known that in

Mediterranean climate,  $P_n$  at midday strongly decreases during summer due to high temperature, high vapor pressure deficit and high irradiance (Tehnnunen *et al.* 1984; Ogaya and Peñuelas 2003; Llusia *et al.* 2016). This behavior is a consequence of the strong stomatal control exerted by the plant to avoid large water losses during summer. In this experiment, control plants were fully irrigated in the morning allowing the maintenance of a high enough stomatal conductance and high photosynthetic rates at midday.

Finally, it is worth mentioning that all NI plants bloomed at the same period than I plants did. In previous field experiments, water-stressed loquats during summer bloomed well in advance to control fully irrigated trees (Cuevas *et al.* 2007, 2008). The lack of effects of the dry period on phenology seems because NI plants did not reach the threshold level of  $S_{\psi}$  needed to modify flowering dates ( $S_{\psi} > 29 \text{ MPa days}$ ; Fernández *et al.* 2010). In this experiment,  $S_{\psi}$  in NI plants scarcely reached 20 MPa days, because despite the intensity of the water stress, the dry period was too short to successfully advance blooming dates.

In summary, even though deficit irrigated plants wilted and dropped some leaves, all water stressed plants survived. Furthermore, one week after irrigation was restored, water stressed plants reached  $P_n$  values very like those of non-stressed plants confirming the remarkable water stress tolerance of loquat and suggesting that photosynthesis machinery remain intact even after severe levels of water stress in 'Algerie' loquat. These results also confirm the good adaptation of loquat to the summer dry periods typical of the Mediterranean climate. No unexpectedly, Spain is the second largest producer of loquat in the world after China.

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**Appendix** associated with this paper can be available on <http://www.ChinaAgriSci.com/V2/En/appendix.htm>

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