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THERMOGRAPHIC MEASUREMENT OF CANOPY TEMPERATURE IS A USEFUL TOOL FOR PREDICTING WATER DEFICIT EFFECTS ON FRUIT WEIGHT IN CITRUS TREES

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Running title: Citrus trees water status determinations from canopy temperature

ABSTRACT

Alternative and more practical methods for plant water stress detection than stem water potential (ψ_s) and stomatal conductance (g_s) are needed when regulated deficit irrigation (RDI) strategies are applied. The aim of this experiment was to compare sap flow and canopy temperature (T_c) measurements with more classical methods like ψ_s or g_s to predict the effect of deficit irrigation on fresh fruit weight in citrus trees. The experiment was performed during the summer of 2011 in a “Clementina de Nules” orchard undergoing RDI. Sap flow was determined by means of the compensation heat pulse method in well-watered and RDI trees. T_c was measured continuously with infrared thermometers (IRTs) mounted over the canopies and also weekly with an infrared hand-operated thermographic camera taking frontal images of the sunlit side of tree crowns. Concurrently, ψ_s and g_s were also measured on all trees. Results showed that the evolution of the relative transpiration obtained with the sap flow sensors was in agreement with the plant water stress experienced. The values of T_c obtained with the fixed IRTs, normalized by air temperature ($T_c - T_a$) were in general poorly related with ψ_s and g_s. However, when T_c was obtained from thermal imaging, there was a good correlation with ψ_s in days of relatively high water stress (i.e. when ψ_s differences among treatments were > 1.0 MPa). The average fruit weight at harvest was significantly correlated with all the stress indicators, and the best correlation was that of thermographic T_c followed by ψ_s and g_s. Overall, results showed that in citrus trees T_c measurement obtained from thermal imaging is a good tool to predict the effect of water deficit on fresh fruit weight.

Key words: Infrared canopy temperature; Regulated deficit irrigation; Sap flow; Stem water potential; Stomatal conductance.

IINTRODUCTION

Citrus is one of the most important crops in Spain with more than 279.800 ha cultivated and an annual production of almost 5.3 million tons (MARM, 2010). Main citrus producing areas in Spain are located southeast, in semi-arid conditions, where water is a scarce resource which use should be optimized.

Regulated deficit irrigation (RDI) is an irrigation technique that has been successfully applied in many citrus species (Ruiz-Sánchez et al. 2010). The success of a RDI strategy, however, is dependent on the level of water stress reached by trees and, therefore, plant water stress monitoring becomes crucial. Stem water potential (Ψ_s) and stomatal conductance (g_s) measurements have been widely used in citrus orchards under deficit irrigation to determine the plant water status (Ballester et al. 2011, 2012). Nowadays, however, alternative methods less laborious and amenable for automation are being sought for use in commercial orchards (Jones 2007).

Sap flow measurement is one of the methods tested in citrus as a plant water stress indicator (Ballester et al., 2012; Gonzalez-Altozano et al., 1998; Ortuño et al., 2006). Sap flow sensors provide direct measurements of plant water flow, which is often reduced in deficit irrigated trees. In commercial orchards, the use of some plants irrigated at full water requirements as a reference allows the calculation of the relative transpiration, which can be used to indicate the level of water stress reached by trees (Ballester et al. 2013a ; Fernández et al. 2007).

Currently, the measurement of the canopy temperature (T_c) by infrared techniques as an indicator of both abiotic and biotic stresses has recently gained the attention of scientists (Jones et al., 2009). This method can be used remotely and allows the measurement of large areas when thermal imaging is employed. Plants under soil water deficit often close the stomata thereby reducing transpiration and therefore increasing leaf temperature. Recent studies have shown that canopy temperature measurements in citrus can be used to detect moderate to severe plant water stress (Ballester et al. 2013b; García-Tejero et al. 2011; Zarco Tejada et al., 2012). However, citrus trees are sensitive to the air vapor pressure deficit (VPD) and even well-watered trees often reduce transpiration in conditions of high VPD values what can make difficult the use of this method in such conditions.

A good water stress indicator must provide information of the plant water status but it would also be desirable that could provide a good prediction of the deficit irrigation effects on production. Many are the studies comparing different plant water stress indicators on the base of a signal to noise ratio (Ballester et al., 2013b; Goldhamer and Fereres, 2001; Ortuño et al., 2004; Remorini and Massai, 2003). However, fewer are the studies that assess the ability of water stress indicators to predict crop responses to deficit irrigation (Intrigliolo et al., 2006; Naor, 2000; 2004).

The objective of this experiment was to compare sap flow and canopy temperature (T_c) measurements with more classical methods like ψ_s or g_s to predict the effect of deficit irrigation on fresh fruit weight at harvest in Clementina de Nules citrus trees

MATERIALS AND METHODS

Experimental plots and irrigation treatments

The study was conducted in a citrus commercial orchard located in Liria (40ºN, 0º W, elevation 300 m), Valencia (Spain) during the summer of 2011. The orchard was planted in 1999 with ‘Clementina de Nules’ (*Citrus clementina*, Hort ex Tan) grafted on

Carrizo citrange (*Citrus sinensis*, Osb. x *Poncirus Trifoliata*, Raf) at a spacing of 6 m x 4 m. Trees were drip irrigated with a double line leaving eight emitters of 4 L h^{-1} per tree. The soil was of clay to clay loam texture, rich in calcium carbonate and with 21% by weight stones. More details about the orchard's characteristics can be found in Ballester et al. (2011).

Irrigation treatments tested were: i) a control treatment, irrigated at full water requirements during the whole season; ii) an RDI treatment, in which trees were irrigated at 35% ET_c from mid July (DOY 200) to mid September (DOY 255) and at full dose during the rest of the season and; iii) a third treatment (NI) in which irrigation was withheld in three trees during five consecutive weeks and then irrigation resumed exactly as in the RDI treatment. The assessment of the different water stress indicators was carried out in a total of 11 trees: four control, four RDI and three NI trees.

Irrigation was scheduled weekly based on estimated crop evapotranspiration (ET_c = ET_o K_c). Reference evapotranspiration (ET_o) and other relevant weather information were obtained from a meteorological station located 4 km far from the orchard. The monthly crop coefficient (K_c) used was obtained based on the canopy ground cover (GC) according to Castel (2000).

Sap flow measurements

The four control and RDI trees were equipped with sap flow sensors to determine sap flow by the compensation heat pulse method (Swanson and Whitfield, 1981). Two trees of each treatment were instrumented with two sensors (TranzFlo NZ Ltd) per tree oriented north and south side, with thermocouples located at 5, 12, 21 and 32 mm below the cambium. The other two trees from each treatment were equipped with four sensors (IAS-CSIC, Córdoba) per tree located at north, south, east and west side of the trunk, with thermocouples at 5, 15, 25 and 35 mm depth from the cambium. More details about the sensors and methodology can be found in Testi and Villalobos (2009). Control trees had an average GC of 40.6% and a trunk diameter of 13.3 cm while in the RDI trees these values were of 39.7% and 13 cm, respectively.

A control box and a data-logger (model CR1000, Campbell Scientific Inc., Utah, USA) powered by a 12 V battery were used to drive the pulses and store the data every 30 minutes. The rest of details about the procedure used for sap flow calculations derived from heat velocity can be found in Ballester et al (2013a).

Canopy temperature measurements

Canopy temperature (T_c) was measured continuously in three trees from each of control, RDI and NI treatments with fixed infrared thermometer sensors (IRTs; Model PC21MT4 (Calex Electronics Limited, Leighton Buzzard, Bedfordshire, England)). Sensors were installed over the canopies to focus on the most exposed leaves to the solar radiation. In control and RDI trees, IRTs were installed approximately 0.9 m over the canopies allowing a field of view ($\alpha = 28.08^o$) of around 0.16 m^2. In NI trees, however, sensors were installed 0.6 m over the trees so the field of view was of 0.07 m^2. All the sensors were connected to a data-logger (model CR1000, Campbell Scientific Inc., Utah, USA) where data were stored every minute.

Apart from these measurements with IRTs, T_c was also measured periodically in all trees from each treatment with an infrared thermal camera TH9100 WR (NEC Avio Infrared Technologies Co., Ltd., Tokio, Japan). The camera had a precision of ± 2% of reading and was equipped with an angular field of view of 42.0º x 32.1º. It had a visible of 752 x 480 pixels and a 320 x 240 pixel microbolometer sensor, sensitive in the spectral range of 8 and 14 µm. The value used for the emissivity was 0.98, value indicated for healthy vegetation by Monteith and Unsworth (2008).

Images were taken weekly at solar midday during a total of 11 days. All images were taken at one meter distance from the sunny side of the trees, thus the thermal field of view was of 0.44 m^2. Images were analyzed automatically following the procedure of Jiménez-Bello et al. (2011) and T_c was obtained from the average of the total number of leaves pictured.

Plant water status measurements

Plant water status (e.g. Ψ_s and g_s) was periodically measured concurrently with the T_c camera measurements during the whole experiment.

Ψ_s was determined at midday in all the sampled trees with a pressure chamber (Model 600 Pressure Chamber, PMS Instrument Company, Albany, USA). Measurements were taken in two mature leaves per tree, bagged at least two hours previous to the measurements. Just previous to the Ψ_s measurements, g_s was also measured with a diffusion porometer (SC-1 porometer, Decagon, WA, USA) in a total of 10 leaves per tree fully exposed to the solar radiation.

Yield determination

Fruit from sampled trees were picked on 29th November. Yield from each individual tree was weighed and the number of fruit determined in order to obtain the average fruit weight.

Data analysis

Data were analyzed using analysis of variance procedure and means were separated by Dunnett's test and contrast between pair of treatments according to the mixed procedure of SAS (SAS Institute, 1994).

The relative transpiration (RT) derived from the continuous SF measurements in the RDI treatment was used for the water stress detection. RT was calculated by dividing average daily transpiration of the control trees by that of the RDI trees.

Linear relations between the average value of the different water stress indicators for the period of water restrictions and average fruit weight on a per tree basis were explored.

RESULTS AND DISCUSSION

Plant water status

Average daily ET_o and VPD values registered in the orchard during the experiment (DOY 200-255) were 4.1 mm and 1.5 kPa, respectively. VPD values for the days when Ψs and gs were also measured are shown in table 1. No rainfall events occurred during this period, thus, differences in plant water status between treatments were exclusively a consequence of the differential irrigation treatments applied.

During most of the experimental period, control trees had Ψ_s values around -1.08 MPa, values indicative of near optimum plant water status (Ballester et al. 2011). On the other hand, RDI trees reached minimum values of -1.84 MPa (Figure 1A), indicating that plant water stress experienced by RDI trees was moderately severe. Ψ_s values in NI trees fell down to -2.67 MPa and then increased to values similar to those registered in the RDI trees (DOY 242) when irrigation was resumed as in that treatment. The decrease in plant water status led to a reduction in stomatal conductance (Figure

1B). On average, control trees had g_s values of 119 mmol $m^{-2}s^{-1}$, while the corresponding values for RDI and NI trees were 96 and 77 mmol $m^{-2}s^{-1}$, respectively. Therefore, the plant water status recorded in the different treatments tested was in agreement with the watering regime applied.

Sap flow measurements

As mentioned in the previous section, sap flow measurements were performed only in control and RDI trees. The mean absolute sap flow value in control trees during the experimental period was of 1.0 mm day^{-1} while in the RDI treatment this value was 15% lower. The average daily absolute sap flow values obtained during the period of water restrictions were significantly correlated with Ψ_s (r^2 = 0.50**) and g_s (r^2 = 0.57***) measurements (Figure 2A). These correlations, however, were higher when relative values of sap flow were compared with Ψ_s (r^2 = 0.85***) and g_s (r^2 = 0.80**) measurements of the RDI trees (Figure 2B). Figure 1C depicts the RT decrease during the period of water restrictions, which closely followed the trends of Ψ_s and g_s observed in RDI trees. These results confirm previous findings in olive trees (Fernández et al., 2007; 2008). These authors also suggested that RT can be successfully used for water stress detection and even for automatic irrigation scheduling. The relationship observed between RT and Ψ_s in the present experiment (r^2 = 0.85), was similar although slightly tighter than that reported by Ortuño et al. (2006) in an experiment with lemon, where the water stress was more severe and trees reached Ψ_s values close to -3 MPa (r^2 = 0.95).

Canopy temperature measurements

T_c values obtained from thermal images were also in agreement with the evolution of Ψ_s. RDI and NI trees had higher canopy temperature minus air temperature, (T_c - T_a), values than control trees during the period of water restrictions (Figure 1D). The maximum differences in T_c - T_a (+2.6 ºC) were detected between control and NI trees on DOY 236, which was the day with the highest differences in plant water status between treatments as indicated by the Ψ_s and g_s measurements (Figure 1A). On this day, RDI trees were less stressed than the NI ones and consequently the difference in temperature compared to the control trees was lower, +1.7 ºC, a value similar to that reported for Navel Lane Late RDI trees by Ballester et al. (2013) although lower than the differences reported by García-Tejero et al. (2011). T_c - T_a differences observed between water-stressed and well-watered trees on DOY 236, were lower than those predicted by a leaf energy balance model, as the one developed by Prof. Kevin Tu (http://landflux.org/Tools.php), using the corresponding leaf (shape, dimensions, absorptance, angle from the horizontal, emissivity and stomatal resistance) and environmental parameters (short wave radiation, relative humidity, wind speed and air temperature). In our experiment we did not measured the leaf angle from the horizontal. However, initially we used a value of 35º, characteristic for well-watered citrus trees (Cohen and Fuchs, 1987). We can then speculate that the differences between measured and model predicted values could be explained by a change in the leaf angle of water-stressed trees that allow them to intercept lower solar radiation. In fact, the T_c - T_a differences of 2.6 and 1.7ºC observed between NI and RDI trees compared to the control would be equal to the model predictions if leaf angles of 55º and 49º, respectively are introduced in the model. Our visual observations indicate that citrus leaves under severe water stress (as in NI trees) tend to roll and also become more erectophylic, corroborating this hypothesis.

There was a noticeable decrease in T_c - T_a from DOY 242 in all the treatments and more so in water stressed trees, compared to the previous five days. These lower T_c - T_a values in all the trees during late August were probably due to the lower values of midday solar radiation and relative humidity registered from that moment onwards.

A significant relationship was found between T_c - T_a and the absolute values of sap flow in days with values of VPD lower than 3 kPa (Figure 2C). T_c - T_a was also well related with Ψ_s and g_s measurements in those individual days where Ψ_s differences between treatments were higher than 1 MPa (Table 1) and when data for each individual tree was averaged for the period of water restrictions (Figure 2D). However, the relationship observed was poorer, although still significant, for the data of the entire experimental period (Figure 3A), pointing out the difficulty of using T_c measurements in absolute terms as a water stress indicator in citrus. A relatively poor relationship between these parameters has also been reported in Navel Lane Late trees under RDI (r^2 = 0.42; Ballester et al., 2013), and in Powell Navel oranges and Clemenvilla mandarins (r^2 = 0.34; Zarco-Tejada et al., 2012) in which T_c was obtained with a thermal camera from an unmanned aerial vehicle in Seville (Spain). In contrast, other authors (García-Tejero et al., 2011) have reported higher correlations in Navelina orange deficit irrigated trees (r^2 = 0.75).

The good relationships observed for individual days when T_c was obtained from thermal imaging were in contrast with the results obtained from the IRTs. T_c values registered at solar midday by the IRTs, when Ψ_s and g_s measurements took place, were poorly related to them on any single day of determinations (Table 1). Only when data were grouped by treatments, a significant relationship between Ψ_s and (T_c – T_a) was observed in the RDI treatment (r^2 = 0.68**). However, no significant relationship was observed for the control neither for NI trees despite the fact that this latter treatment reached the lowest values of Ψ_s (Figure 3B). The higher variability and lower target area focused by the IRTs, particularly in the NI treatment, compared with the thermal camera clearly hampered the detection of changes in T_c in water stressed trees with the IRTs. The sample of leaves included in the measurement with the thermal camera was larger than with the IRTs and consequently should be better related with Ψ_s and g_s measurements. Moreover, thermal images were taken from the sunlit side of the canopies while IRTs were pointing from above. This fact could impair T_c measurements with the IRTs since they were focused on the most exposed leaves to the solar radiation, but perhaps also on some shaded areas from inside the canopy. The manual and also the automatic processing of the thermal images allows the operator to select the leaves (sunlit or shaded) or even portions of the canopy to be analyzed, avoiding then those areas that could introduce significant noise in the results. These facts, highlight the advantage of thermal images as compared to IRTs indicating that methods that integrate a larger number of leaves for the temperature measurements, as in the thermal imaging, are more appropriate than methods that rely only in a few leaves from a specific location of the canopy as occurred with the fixed infrared thermometer sensors.

Prediction of fresh fruit weight

On the base of a signal to noise ratio, T_c has been shown as a high sensitive water stress indicator for citrus trees due to the much lower tree-to-tree variability compared to other methods like Ψ_s or g_s (Ballester et al., 2013b). Apart from the sensitivity to the water stress, from the agronomical point of view, it would be a desirable aspect for a water stress indicator to be a good predictor of the water deficit effects on yield. The Photochemical Reflectance Index (PRI) monitored by high spatial resolution multispectral airborne imagery has been proven as a water stress indicator significantly correlated with some orange fruit quality parameters such as total soluble solids (TSS), titratable acidity (TA) and the ratio TSS/TA (Suárez et.al 2010; Stagakis et al. 2012). These authors suggest using this indicator to remotely measure plant water stress and to estimate the internal fruit quality parameters in commercial orchards. The PRI index could be used to schedule harvest based on the estimation of these quality parameters in order to maximize gross revenues in those places that value fruit quality over fruit size. However, in the Mediterranean area, almost the whole citrus production is commercialized as fresh fruit and, in these markets, fruit size is more valued than internal fruit quality, being therefore fresh fruit the major yield value determinant. Studies with plum (Intrigliolo et al., 2006; Naor, 2004), peach (Naor, 2000) and almond (Shackel et al., 1997) have reported tight relationships between fruit weight and Ψ_s or g_s. In our study, the relationships between fruit weight and Ψ_s or g_s were significant although with lower fit than those reported for the crops mentioned above (Table 2). T_c or (T_c – T_a) obtained from the IRTs were not significantly related with fruit weight. These relationships, however, were highly significant (r^2 = 0.72**) when canopy temperature was obtained from thermal imaging. In fact, T_c from thermal images was the water stress indicator that better predicted the effect of the water restrictions applied on fruit weight at harvest. A good correlation between canopy temperature and fruit size in orange trees was also observed by other authors (Suárez et al. 2010) for the cv. Navelina in which T_c was measured with thermal imagery. However, in Suárez et al., (2010), the correlation observed was lower (r^2 = 0.47*) than that obtained in this experiment probably due to the low level of stress reached by their trees. For our experiment, an increase in (T_c – T_a) of 1 ºC on average for the period of water restrictions resulted in a reduction of 5.3 g in fruit weight.

The good performance of T_c measured by a thermal camera for estimating fruit weight reductions as consequence of plant water stress is probably due to the fact that thermal images allowed the integration of large portions of the tree canopy, obtaining then a reliable determination of the actual whole tree canopy temperature. This is one of the main advantages of thermal imaging that, if combined with tools for automatic imaging analysis, can be used to remotely determine temperature of large areas. Indeed, the present study is among the first to corroborate that T_c measurements allow estimating with sufficient precision the effects of plant water stress on average fruit weight at harvest, which as mentioned before is a critical determinant of the fruit commercial value.

In the past, much effort was done to explore the feasibility of using trunk diameter variations as a potential continuous water stress indicator (Ortuño et al. 2010, Férnandez and Cuevas 2010). It seems that the effort is now more concentrated on using T_c to that end. The present results justify this new trend since T_c provide for a more direct assessment of plant water status trough stomatal regulation of transpiration and therefore leaf evaporative cooling. The possibility of using this type of tools will indeed facilitate the more widespread adoptions of RDI techniques, which at least in ‘Clementina de Nules’ under Mediterranean costal environment have been proven to be useful in commercial orchards for increasing tree water use efficiency (Ballester et al. 2011). Using canopy temperature measurements related with air ambient temperature will allow growers to better control the actual water stress reached during summer irrigation restrictions thereby avoiding that it could become too severe and negatively affect fruit weight.

Conclusions

This experiment shows the link between canopy temperature measured by thermal imaging and the fruit weight at harvest in summer deficit irrigated citrus trees. Among the water stress indicators studied, T_c was the one which better correlate with fruit weight followed by Ψ_s and g_s. These results suggest that canopy monitoring by thermal imaging can be a useful tool to avoid exceeding plant water stress levels in citrus trees under deficit irrigation that could reduce fruit weight and therefore revenues obtained by growers. Methods like thermal imaging, which allow the measurement of a high number of trees and integrate a large number of leaves in the measurement, seem to be more appropriate than the use of fixed IRTs that only focused a specific zone of the tree. Furthermore, this work confirms the results of previous experiments (Ballester et al. 2013a; 2013b) indicating that sap flow and canopy temperature can be used in relative terms as water stress indicators in citrus orchards, particularly in days with moderate to severe plant water stress (differences between treatments above 1 MPa).

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Table 1 Relationships between canopy temperature measured with a hand-operated thermographic camera, minus air temperature (T_c – T_a), stem water potential (Ψ_s) and stomatal conductance (g_s). For each day, the range of Ψ_s, air vapor pressure deficit (VPD), radiation (Rd), relative humidity (RH) and wind speed (WS) during the hours of the measurements are also shown.

DOY	**207**	**216**	**223**	**229**	**236**	**242**	**249**	**256**	**259**	**264**	**270**
Ψ_s vs. T_c - T_a (Thermal imagery)	0.09	0.08	0.32	0.64	0.70	0.52	0.52	0.41	0.07	0.19	0.04
g_s vs. T_c - T_a (Thermal imagery)	0.02	0.37	0.14	0.78	0.76	0.18	0.38	0.34	0.22	0.05	-
Ψ_s vs. T_c - T_a (IRTs)	0.10	0.28	0.41	0.14	0.24	0.01	0.03	0.03	0.00	0.24	0.20
Ψ_s vs. g_s	0.51	0.23	0.11	0.81	0.70	0.56	0.56	0.78	0.59	0.13	-
Ψ_s range	0.30	0.41	0.53	1.19	1.44	1.01	1.15	1.10	0.33	0.49	0.30
VPD	3.14	2.23	2.15	2.94	2.67	3.15	2.22	3.05	2.60	2.32	2.28
Rd (W m^{-2})	900	857	861	836	830	807	804	770	795	801	761
RH (%)	32.53	48.16	46.29	41.25	43.81	35.32	44.77	32.83	38.69	37.13	40.20
WS (m s^{-1})	2.19	1.47	1.80	1.91	1.76	2.18	1.67	1.60	1.14	1.38	1.50

Table 2 Relationships between fruit weight (FW) at harvest and the average value of the different water stress indicators: stem water potential (Ψ_s), canopy temperature normalized by air temperature (T_c – T_a) either measured by thermal imagery or by fixed infrared sensors (IRTs), sap flow measurements and stomatal conductance (g_s). Data are individual tree values.

	R^2	**n**
FW vs. Ψ_s	0.57**	11
FW vs. (T_c-T_a) (Thermal imagery)	0.70**	11
FW vs. g_s	0.45*	11
FW vs. (T_c-T_a) (IRTs)	0.26	9
FW vs. Sap flow	0.34	4

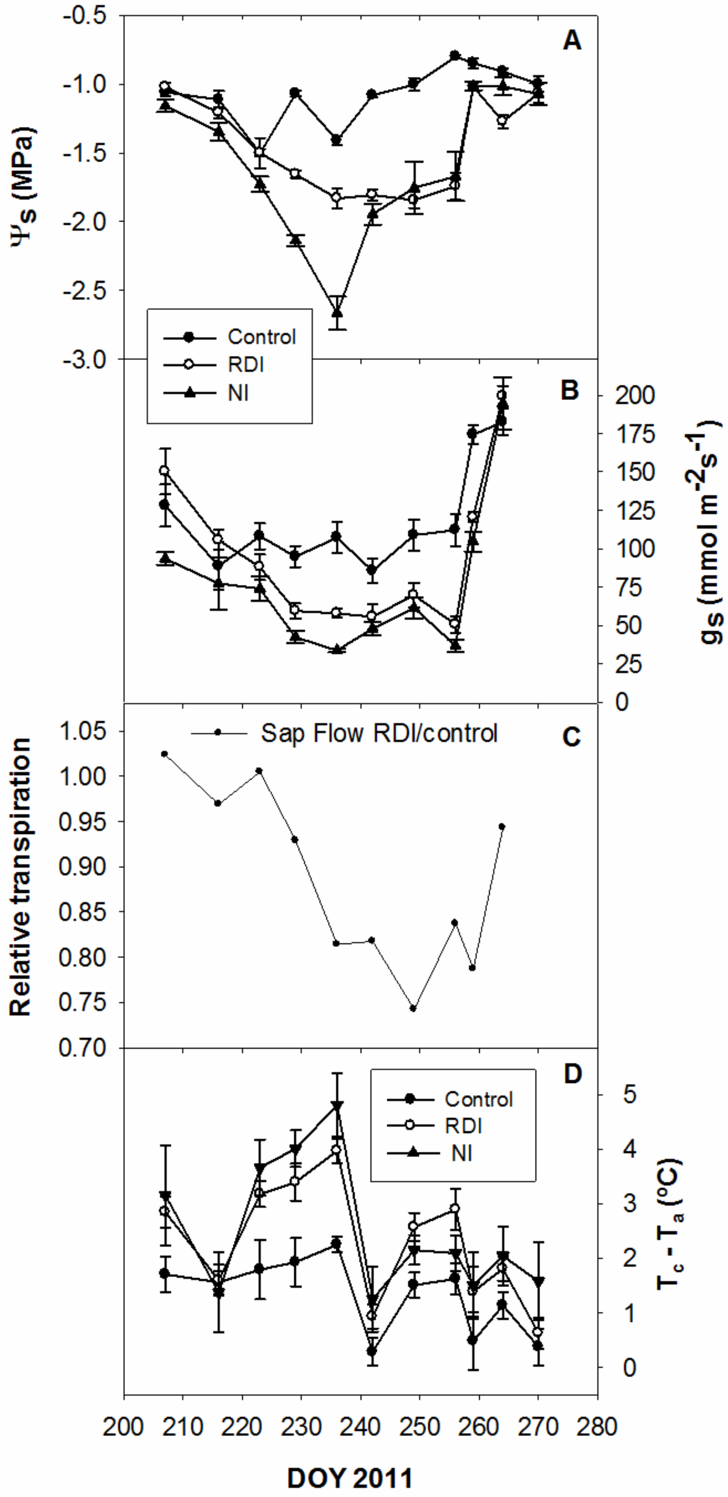

Fig.1 Evolution of the stem water potential (Ψ_s; A), stomatal conductance (g_s; B), relative transpiration (C) and canopy temperature normalized by the air temperature ($T_c - T_a$; D) during the period of water restrictions. Vertical bars represent ± the standard error.

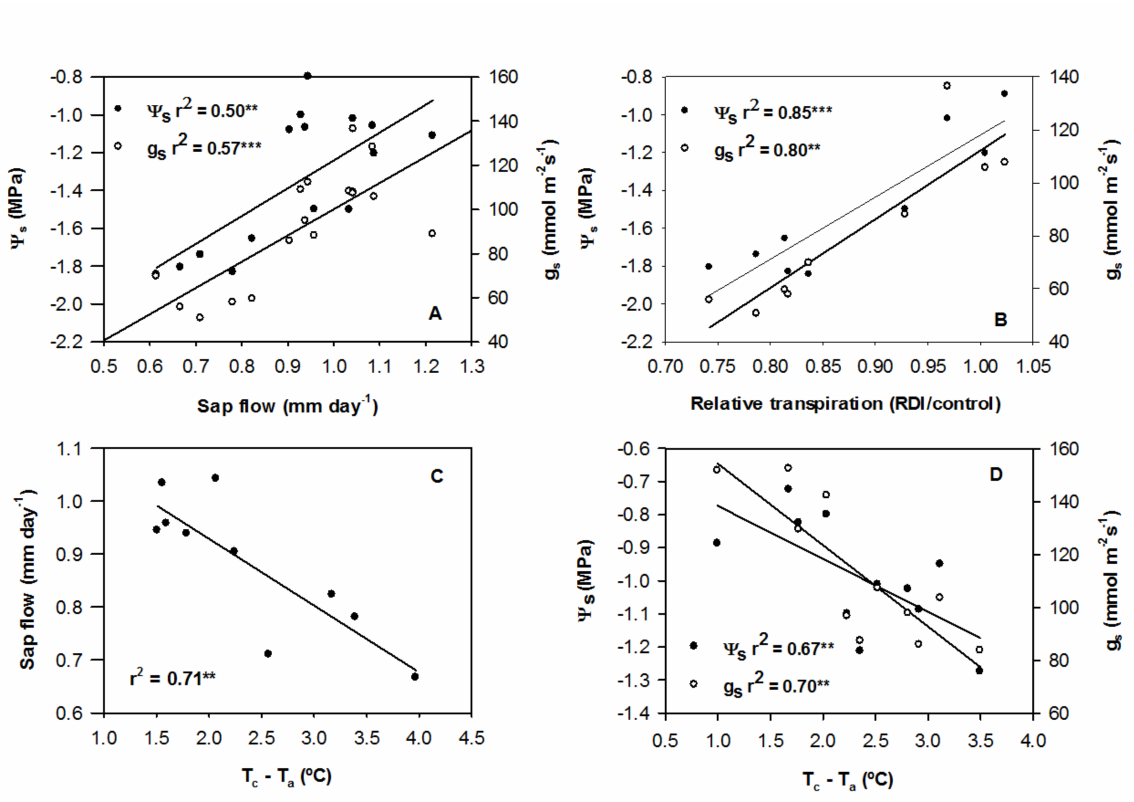

Fig.2 Relationships between stem water potential (Ψ_s) and stomatal conductance (g_s) with the absolute sap flow values (A) and relative transpiration (B) and; relationship between T_c-T_a and sap flow (C), and Ψ_s and g_s (D).

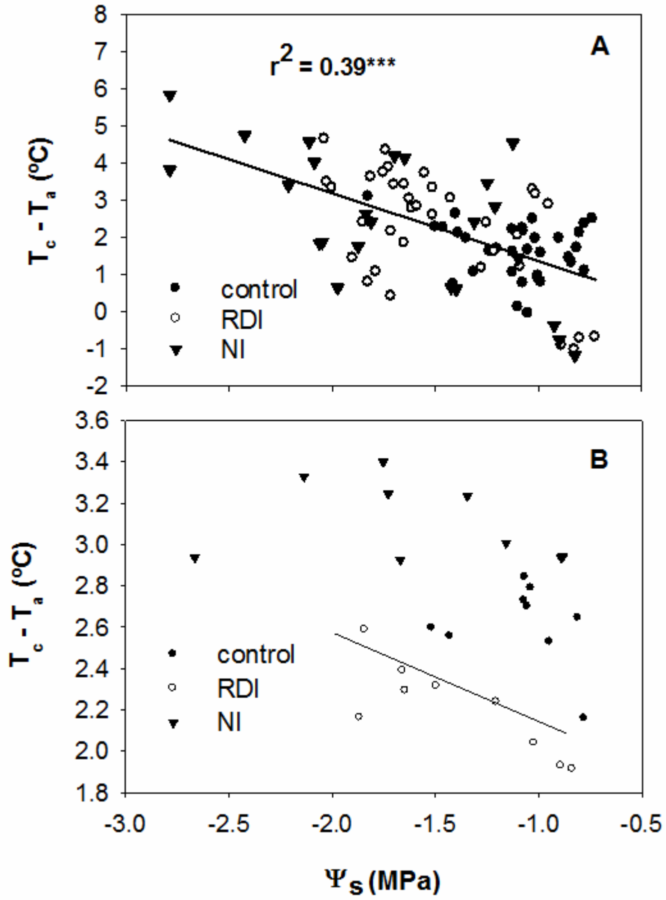

Fig.3 Relationship obtained between stem water potential (Ψ_s) and canopy temperature minus air temperature ($T_c - T_a$) for each treatment when T_c was obtained by thermal imaging (A; data from all the measurements pooled together, n=93) and by fixed infrared thermometer sensors (B, r^2 for the RDI treatment was 0.68**).