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Usefulness of thermography for plant water stress detection in citrus and persimmon trees

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ABSTRACT

The feasibility of using canopy temperature (T_c) measured with a hand-operated infrared thermographic camera as a water stress indicator was evaluated in the field during two seasons on citrus and persimmon trees subjected to different levels of deficit irrigation. In both species, which differ in leaf anatomy and stomatal response to environmental conditions, T_c was compared with midday stem water potential (Ψ_s) measurements. In persimmon trees, leaf stomatal conductance (g_s) was also measured. In 2009, images were taken from the sunlit and shady sides of the canopies. Based on the results obtained, during the second experimental season images were taken from the sunlit side of the trees and also from above the canopy. In persimmon, trees under deficit irrigation had lower Ψ_s and g_s what resulted in a clear increase in T_c regardless of the position from where the pictures were taken. The maximum T_c difference between deficit-irrigated and control trees observed was of 4.4 ºC, which occurred when the stressed trees had Ψ_s values 1.1 MPa lower than the control ones. In persimmon trees, T_c was the most sensitive indicator of plant water status particularly due to the lower tree-to-tree variability as compared to Ψ_s and g_s. On the other hand, in citrus trees T_c was not always affected by plant water stress. Only in the second experimental season, when air vapour pressure deficit values were below 2.7 kPa and images were also taken from above the canopies, deficit-irrigated trees had higher T_c than the control ones, this difference being at most 1.7 ºC. Overall, the results show that hand-operated thermographic cameras can be used to detect plant water stress in both fruit tree species. Nevertheless, the use of T_c measurements to detect plant water stress appears to be more precise in persimmon than in orange citrus. This might be because persimmon trees have larger leaf size which determines higher canopy resistance allowing for higher increases in canopy temperature in response to water stress via stomatal closure.

Key words: Canopy temperature; CWSI, intra-crown temperature variability; regulated deficit irrigation; stem water potential; stomatal conductance.

1. Introduction

The perspectives for the foreseeable future point out that irrigation water demand will continue to increase leading to a shortage of water resources in many world regions (Fereres and González-Dugo, 2009). Thus, irrigation strategies that allow farmers to increase water use efficiency are becoming essential in irrigated agriculture. Among the irrigation strategies applied to fruit crops, regulated deficit irrigation (RDI) may allow substantial water savings without negatively affecting yield. The success of this strategy, however, is dependent on the timing and severity of the plant water stress. Surpassing a threshold value of plant water stress usually leads to a reduction in the final fruit size and in the economic return. Therefore, when RDI strategies are applied, it is important to frequently check the plant water status to avoid exceeding the threshold values.

Currently the plant indicators most commonly used to determine crop water status are the stem water potential (Ψ_s) and the stomatal conductance (g_s), but their measurements are labour-intensive and unsuitable for automation, characteristics that make the regular use of these methods difficult for farmers or even technicians in the field. Thus, methods for monitoring crop water status that could be automated are needed. In this sense, the possibility of using plant temperature as an indicator of soil water availability for plants is known since decades ago (Gates, 1964). Plants under soil water deficit often decrease stomatal conductance, thereby reducing transpiration and

increasing leaf temperature. The measurement of the infrared radiation emitted by the canopy can therefore be used as an indicator of plant water stress (Jackson, 1982; Jones, 1999; Merlot et al., 2002; Jones et al., 2002). However, it is important to keep in mind that stomatal aperture can be affected not only by soil water deficit, but also by other environmental and endogenous tree factors as well as biotic stresses such as pests and diseases (Jones et al., 2009). Besides, environmental conditions such as incoming radiative energy, air temperature and wind, plant morphology's aspects like canopy shape and leaf size, as well as plant-controlling transpiration mechanisms have a direct influence on canopy temperature (Scherrer et al., 2011).

Thermal sensing can be used remotely allowing a large crop area to be measured, especially when thermal imaging is employed (Jones, 2004). Images can be taken by thermographic cameras installed on airborne platforms (Berni et al., 2009) or by hand-operated cameras assisted with auxiliary devices as tripods, platforms or cranes (Möller et al., 2007). In the case of hand-operated cameras, these can take images of individual plants or even portions of them (shady or sunlit zones) with a higher spatial resolution than aerial images (Jiménez-Bello et al., 2011). With the involvement of a single operator a large number of images can be obtained. The subsequent analysis of the images to determine mean canopy temperature of each single tree can be automated and speeded with methodologies as the one developed by Jiménez-Bello et al. (2011), which allows the analysis of images taken on individual trees without the participation of an operator, saving almost 16 minutes per image with respect to the manual process. Besides mean canopy temperature, the measurement of the intra-crown standard deviation has also been suggested by some authors as an indicator of water stress (Fuchs 1990, González-Dugo et al., 2012). González-Dugo et al., 2012 observed in almond that the variability of T_c increased during the early stages of water stress while diminished

when the stress became more severe. However in other woody plants such as grapevines intra-canopy variations in T_c were not impacted by vine water status (Grant et al., 2007; Moller et al., 2007). Thus, studies in other perennial crops are needed to evaluate the feasibility of using intra-canopy T_c variability as an indicator of plant water status.

The general goal of this study was to explore the feasibility of canopy temperature measured with a hand-operated thermographic camera as a water stress indicator compared with common water status indicators as Ψ_s and g_s in persimmon and citrus tree crops. The specific aims were i) to assess the use of mean canopy temperature and temperature variability within the crowns as water stress indexes; ii) to test this water stress indexes in persimmon and citrus tree crops which were selected because of their differences in leaf anatomy (larger and thicker leaves in persimmon than in citrus) and differential stomatal response to air vapor pressure deficit (VPD) under favorable soil water conditions. It is well known that citrus trees tend to reduce stomatal conductance in response to high VPD (Oguntunde et al., 2007; Villalobos et al., 2009); while in Persimmon trees there is some evidence that stomatal conductance might be more insensitive to air VPD (Badal et al., 2010).

2. Material and methods

2.1. Plot characteristics and irrigation treatments

2.1.1. Persimmon experiment

The experiment was carried out in a 0.52-ha orchard located in Manises (Valencia, Spain) planted with eight-year-old Persimmon (*Diospyros Kaki*) trees, cv. "Rojo Brillante". Trees were planted at a spacing of 5.5 m x 4 m and grafted on *Diospyrus Lotus*. The soil was calcareous; of sandy loam to sandy clay loam texture with an

effective depth of 0.8 m. Trees were drip irrigated with two laterals per row and 8 emitters of 4 L h^{-1} per tree. At the beginning of the experiment, trees had a canopy ground cover of 39% of the soil surface area allotted per tree. Other orchard characteristics are described in Badal et al. (2010).

The experimental orchard was designed to test four irrigation regimes but only two of them were used for the purpose of this manuscript: i) control, irrigated at 100% of the estimated crop evapotranspiration (ET_c) during the whole season and, ii) water stressed (WS), irrigated at 50% ET_c from May 22nd, day of the year (DOY) 142, to August 18th (DOY 230) in 2009 and from May 21st (DOY 141) to August 27th (DOY 239) in 2010.

The experimental layout was a randomized complete block design with three replicates per treatment and 6-7 sampled trees per replicate. Perimeter trees were used as guard.

2.1.2. Citrus experiment

A field trial was performed in a 1.7-ha grove located in Chulilla (Valencia, Spain), planted at 6 m x 4 m with Navel Lane Late (*Citrus sinensis* (L) Osbeck) trees, grafted onto Carrizo citrange (*Citrus sinensis*, Osb. x *Poncirus Trifoliata*, Raf). The soil was of clay to clay loam texture, rich in calcium carbonate and with 11% by weight stones. Trees were drip irrigated with two laterals per row and 8 emitters of 4 L h^{-1} per tree. At the beginning of the experiment, trees had a canopy ground cover of 32% of the soil surface area allotted per tree. Grove characteristics are more detailed in Ballester et al. (2012).

Three irrigation treatments were studied in this case: i) control, irrigated at 100% ET_c during the whole season; ii) mild water stressed (MWS), irrigated at 50% ET_c from last July to mid September and at full dose during the rest of the season; and iii) severe water stressed (SWS), irrigated at 35% ET_c during the same period as MWS.

The experimental layout was a randomized complete block design with four replicates per treatment and at least 10 sampled trees per replicate. Perimeter trees were used as guard.

2.2. Plant water status measurements

During the period of water restrictions plant water status was periodically measured in both orchards by means of stem water potential, and canopy temperature. In addition, in persimmon trees stomatal conductance was also measured.

Stem water potential (Ψ_s) was measured at solar midday with a pressure chamber (Model 600 Pressure Chamber, PMS Instrument Company, Albany, USA) following the recommendations of Turner (1981). Leaves were enclosed in plastic bags covered with silver foil at least two hours prior to the measurements. Measurements were performed in two mature leaves per tree, in three trees per replicate in the persimmon experiment and two trees per replicate in the citrus one. Thus, Ψ_s was measured in a total of 24 and 18 trees in the citrus and persimmon orchards, respectively.

Stomatal conductance (g_s) was measured at noon only in the persimmon orchard with a leaf porometer (SC-1 Porometer, Decagon, WA, USA). Measurements were carried out in five fully exposed leaves per tree and three trees per replicate.

2.3. Image acquisition and processing

Canopy temperature (T_c) was measured at noon 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 emissivity was set at 0.98, value indicated for healthy vegetation by Monteith and Unsworth (2008).

In 2009, T_c was measured in both sunlit ($T_c$sunlit) and shaded ($T_c$shady) sides of the crowns by taking frontal thermal images from a distance of 3 m in persimmon trees and 1 to 2 m in the citrus ones. Pictures were taken in four representative days for persimmon (DOY 170, 205, 226 and 240) and in seven days for citrus (DOY 204, 218, 225, 232, 239, 246 and 253).

Based on the results obtained in 2009, images were only taken from the sunlit side of the trees in 2010. During this season pictures were taken in nine days for the persimmon orchard (DOY 138, 155, 169, 176, 190, 204, 211, 218 and 232) and five days for the citrus one (DOY 216, 224, 238, 246 and 258). Additionally, in the citrus experiment, the camera was assisted with a tripod and mounted 1 m above the canopy pointing vertically downward to take pictures of the leaves most directly exposed to the solar radiation on DOY 224, 238 and 246. Due to the orchard characteristics, these pictures were only taken in the control and the most stressed trees (SWS treatment). Furthermore, during DOY 239 for persimmon and 253 for the citrus orchard, pictures of the sampled trees were taken by an operator mounted on a truck-crane pointing downward from a height of 12 m above the ground. Given the camera optical and resolution characteristics and that the average canopy height was 2.5 m, a picture taken from 12 m height represented a pixel size of 5.1 cm^2 at canopy level.

Images were processed with the ArcGIS 9.3 software (ESRI, Readlands, USA) according to the method described by Jiménez-Bello et al. (2011). For the general data analysis, T_c of each single tree was obtained from the average temperature of all the leaves integrated in the image.

In addition, temperature variability within the crowns (standard deviation) was analyzed in days with Ψ_s differences between treatments above 0.7 MPa. Standard deviation (σ) within trees was calculated as:

$$\sigma = \sqrt{\frac{\sum(x_i - \bar{x})^2}{(n-1)}} \quad (1)$$

where n is the number of pixels, x_i is the temperature value for a given pixel and $\bar{x}$ is the mean temperature of all the pixels from the canopy.

The crop water stress index (CWSI) was also determined only in the second experimental season of the citrus experiment. CWSI was calculated according to Idso et al. (1981):

$$CWSI = \frac{(T_c - T_a) - (T_c - T_a)_{LL}}{(T_c - T_a)_{UL} - (T_c - T_a)_{LL}} \quad (2)$$

where $(T_c - T_a)$ is canopy – air temperature differential, $(T_c - T_a)_{LL}$ the expected lower limit of $(T_c - T_a)$ in the case of a tree transpiring at the potential rate, and $(T_c - T_a)_{UL}$ the expected differential in the case of a non-transpiring canopy. The upper and lower limits of $(T_c - T_a)$ were obtained by using wet and dry reference surfaces as suggested by Jones et al., (2002). As a reference for $(T_c - T_a)_{LL}$, wetted leaves sprayed with water previous to the measurements were employed. $(T_c - T_a)_{UL}$, was obtained from leaves impregnate with petroleum-jelly.

2.4. Data analysis

The 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 different water stress indicators (Ψ_s, gs, T_c and CWSI) were assessed by means of a sensitivity analysis (sensitivity defined as signal to noise ratio) based on that proposed by Goldhamer and Fereres (2001). Thus, when there were significant differences between treatments, the value "signal" for Ψ_s, T_c and CWSI was calculated as the ratio between the average value for the water stress and control treatment while for g_s it was obtained from the ratio between the average value for the control and the water stress treatment. In all cases the "noise" was obtained as the average coefficient of variation among trees from the same treatments as the signal value.

3. Results

3.1. Meteorological conditions

During the first year of the persimmon experiment, the air temperature (T_a) during the hours in which measurements were taken ranged between 30.1 (DOY 170) and 34.6 ºC (DOY 205). Wind speed was similar among the different days (on average 1.6 ± 0.3 m s^{-1}). In 2010, average T_a was 30.2 ± 4.8 ºC being DOY 239 the warmest day and DOY 138 the coolest (Table 1).

For the first year in the citrus experiment, the average T_a for the days in which thermal images were taken was 32.9 ± 1.7 ºC and DOY 204 was the warmest day (34.8 ºC). This day was also the windiest with a wind speed of 5.9 m s^{-1}. In 2010, T_a values were lower than in the first experimental season, 30.8 ± 3.1 ºC on average, being DOY 238 the warmest day (37.1 ºC). Wind speed was similar among the different days (on average 2.4 ± 0.5 m s^{-1}, Table 2).

3.2. Persimmon experiment

During the experimental period of 2009, persimmon control trees had an average Ψ_s value of -0.73 ± 0.17 MPa (Figure 1A). The average g_s measured in these trees was 151 ± 29 mmol m^{-2} s^{-1} (Figure 1B). WS trees had significantly lower Ψ_s and g_s values than the control ones, with average values for the whole period of -1.42 ± 0.59 MPa and 111 ± 29 mmol m^{-2} s^{-1}, respectively (Figure 1A,B).

Thermal images from both sides of the canopy (sunlit and shaded side) detected the existing differences in water status between control and WS trees (Figure 1C,D). On average for the period of water restriction, control trees had $T_c$sunlit values of 31.2 ± 3.7 ºC while WS trees had values significantly hotter (33.3 ± 4.8 ºC). $T_c$shady values were slightly lower than those obtained from the sunlit side of the canopies. In this case, control trees had an average $T_c$shady value of 30.6 ± 3.4 ºC while WS trees were 2.0 ºC hotter. During this first experimental season, T_c in control trees was always between 1.0 ºC above and 2.5 ºC below T_a. Nevertheless in WS trees, T_c was always warmer than ambient temperature (Figure 1C,D). Maximum T_c differences between treatments (ΔT_c) were observed on DOY 205 when WS trees had Ψ_s values of -1.92 MPa and were almost 6 ºC warmer than T_a. These maximum ΔT_c values varied slightly depending on the canopy side from where images were taken, and were of 4.4 and 4.1 ºC respectively for the sunlit and shady sides. When water restrictions ended and irrigation was resumed to normal dose (DOY 240), WS trees returned to Ψ_s, g_s and T_c values similar to those of the control trees (Figure 1).

On days when control and WS treatments had Ψ_s differences above 0.4 MPa (DOY 170, 205 and 226), $T_c - T_a$ measured on either side of the canopy was well correlated with Ψ_s and g_s (Table 3).

In 2010, the water stress experienced by WS trees was lower than in 2009. The average Ψ_s value for the control trees was -0.49 ± 0.13 MPa while in the WS treatment it was -0.88 ± 0.43 MPa (Figure 2A). Similarly, average g_s values were of 134 ± 26 mmol m^{-2} s^{-1} and 118 ± 20 mmol m^{-2} s^{-1} for the control and WS trees, respectively (Figure 2B).

As mentioned before, based on the results obtained during 2009 for the effect of canopy side on T_c, in 2010 only $T_c$sunlit was measured. During this year, T_c in control trees remained always below T_a. WS trees, however, surpassed ambient temperature on DOY 204 by 1.0 ºC (Figure 2C), day in which trees from this treatment reached the lowest Ψ_s values (-1.66 MPa) and the maximum ΔT_c (1.5 ºC).

The best correlations between $T_c - T_a$ and Ψ_s or g_s were found in days with differences of Ψ_s between treatments higher than 0.3 MPa (Table 3).

Pooling data from each entire experimental season $T_c - T_a$ was significantly related with Ψ_s ($P<0.001$, Figure 3) although no clear relationship with g_s was observed (results not shown).

The day in which T_c was measured from a truck-crane at 12 m above the canopies (DOY 239), control and WS trees had Ψ_s values of -0.99 MPa and -1.91 MPa, respectively. On average T_c was of 38.8 ºC in the control treatment and 41.3 ºC in the WS one. There were statistically significant ($P<0.05$) correlations between T_c - T_a, measured from the crane, and g_s or Ψ_s measurements (Figure 4).

3.3. Citrus experiment

In 2009, Ψs values registered in the control trees were quite similar during the seven days in which images were taken with an average value of -1.00 ± 0.10 Mpa (Figure 5A). Trees from both water stressed treatments showed Ψ_s values significantly more

negative than the control ones reaching minimum values of -1.47 MPa in the MWS treatment and of -1.67 MPa in the SWS one (Figure 5A).

In spite of these important differences in Ψ_s, neither images taken from the sunlit side of the canopy nor those taken from the shady side detected T_c differences between control and water stressed trees. Only on DOY 218 there was a significant, but weak correlation between T_c - T_a and Ψ_s (Table 4).

In general, trees from all treatments, regardless their water status, maintained T_c values between 1 ºC above and 2 ºC below ambient temperature during this season with the exception of the last measurement day (DOY 253) in which T_c - T_a decreased to values of -5 ºC (Figure 5).

During 2010 frontal images were only taken from the sunlit side of the canopies. Furthermore, thermal images from 1 m above the canopies were also taken in three different days (DOY's 224, 238 and 246) and in one additional day from 12 m above trees with a truck-crane (DOY 253).

During this experimental season there were also significant differences in Ψ_s between treatments (Figure 6A). Control trees had an average value of -0.94 ± 0.09 MPa while in water stressed trees it was of -1.11 ± 0.17 and -1.34 ± 0.29 MPa in the MWS and SWS treatments, respectively. In days with differences in Ψ_s, with the exception of DOY 238, T_c - T_a was significantly higher in water stressed trees than in control ones. The first day of measurements (DOY 216) T_c of all the trees was 3.0 ºC warmer than T_a. Henceforth, the canopy to air temperature difference in control trees ranged between 0.5 and -2.2 ºC while in both deficit irrigated treatments ranged between 2.0 and -2.2 ºC (Figure 6B).

When images were taken from 1 m above the canopies, differences in Ψ_s of 0.35 MPa between control and stressed treatments represented an increase of T_c in SWS trees

of 1.36 ºC. On DOY 253, when images were taken from the trunk-crane, Ψ_s in SWS trees was 0.97 MPa lower than the control ones. In this case, the ΔT_c between both treatments was higher than in the other cases, 1.73 ºC.

The best correlations between $T_c - T_a$ and Ψ_s (on average $r^2 = 0.51^{**}$) were found on DOY's 238 and 246 when images were taken from 1 m above the canopies and control and water stressed trees showed differences in Ψ_s higher than 1 MPa (Table 4). For this experimental season and pooling data from days with similar VPD values, $T_c - T_a$ had a significant relationship with Ψ_s, with $r^2 = 0.42^{*}$ when each single measurement was taken into account, and $r^2 = 0.76^{**}$ when data were grouped by treatments (Figure 7).

The CWSI ranged from 0.33, value registered in the control treatment, to 0.51, registered in the most stressed one. MWS trees differed significantly from the control ones on DOY 246 and 258 as well as SWS trees which also differed on DOY 224 (Table 5). Pooling data from all days of measurements CWSI was significant but poorly related with Ψ_s ($r^2 = 0.15^{***}$, results not shown).

3.4. Sensitivity of the indicators

In both experiments, T_c was the water stress indicator that showed less variability among trees from the same treatment and it was also the most sensitive (Table 6). Differences in sensitivity between T_c and the rest of the indicators were more marked during the second experimental season in which Ψ_s, g_s and CWSI had similar values.

3.5. Assessment of intra-crown temperature variability for water stress detection

In those days where the intra-crown temperature variability was determined there were clear differences in plant water status among treatments (Table 7). Despite this, the intra-crown temperature variability did not differ significantly between treatments

(Table 7). This is because the coefficient of variation observed within treatments in both orchards for the intra-crown temperature variability ranged from 22 to 133%.

4. Discussion

4.1 Canopy temperature sensitivity to water stress in persimmon and citrus trees.

The canopy temperature of persimmon trees showed great responsiveness to variations in plant water status. To the best of our knowledge, the results reported here are the first evidence of using canopy temperature as a water status indicator for this woody perennial crop. The maximum ΔT_c observed in this species was of 4.4 ºC, that occurred when the WS trees had Ψ_s values 1.1 MPa higher than the control ones. In pistachio trees, another woody crop with large leaves like persimmon, Testi et al. (2008) reported T_c differences of as much as 6.0 ºC between well-irrigated and stressed trees when nadir-view radiometric temperature was measured with infrared thermometers.

Among the water stress indicators evaluated in persimmon trees, T_c was clearly the most sensitive mainly as a consequence of the much lower tree-to-tree variability compared to Ψ_s and g_s (Table 6). The use of a thermographic camera along with an automated program to process the images allows for a large number of leaves per tree to be measured. Ψ_s and g_s, however, are usually determined by measuring a small number of leaves per tree (in this study two for Ψ_s and five for g_s), which can increase the variability due to the important heterogeneity found in the intra-crown variation of leaf water status and particularly of stomatal conductance as a consequence of differences in hydraulic resistance among different parts of the tree (González-Dugo et al., 2012).

In the experiment with citrus, T_c response to water stress was different for each experimental season. In 2009 Tc did not allow detecting the existing differences in plant water status, but in 2010 water stressed trees had significantly higher T_c than the control ones with differences of up to 1.7 ºC. As a consequence, in 2010, T_c was the best water

stress indicator studied while Ψ_s and CWSI showed similar sensitivity between them. In any case the differences in temperature between well-watered and stressed trees reported here are low compared to another study in sweet orange trees (García-Tejero et al., 2011), which reported differences of up to 4.8 ºC. However, in García-Tejero et al. (2011) plant water stress reached by the deficit irrigated trees was more severe (Ψ_s of -2.0 and –2.4 MPa) than in the present study in which the Ψ_s values reached by the stressed trees were more moderate and in the range of what it is suggested for application of regulated deficit irrigation in commercial orchards (Ballester et al., 2012). Our results allowed then to test the feasibility of using canopy temperature for plant water status detection under moderate stress levels that can be more frequently applied in commercial orchards. In olive trees, another plant like citrus with small leaves, Sepulcre-Cantó et al. (2009) found similar differences in T_c that reached 2.0 ºC between irrigated and non-irrigated trees.

Although g_s was not measured in the citrus orchard, it is well known that even well-watered trees respond to air dryness with partial stomatal closure and therefore with a reduction in transpiration (Oguntunde et al., 2007; Villalobos et al., 2009). In this same plot, measurements with sap flow methods (Ballester et al., un-published results) showed that transpiration of well-watered trees was weather dependent and had a negative relationship with VPD, i.e., days with high VPD corresponded with low transpiration values. In our experiment of citrus during 2009, in which there were no significant differences in T_c between treatments, the days of thermographic measurements had higher VPD values than those of 2010. Furthermore, in 2010 T_c was significantly different between treatments for all the days except on DOY 238 that had a VPD value (4.5 kPa) similar to those registered in the first experimental season (Table 2). The possible reduction in transpiration in the control trees during the days with high

evaporative demand along with the low increase in T_c observed in the water stressed trees respect to the control ones for the two experimental seasons, could explain the lack of consistence in the response of canopy temperature to water stress observed between years.

The different effect that plant water stress had in canopy temperature between citrus and persimmon trees can be explained considering two physiological differences between crops. On one hand, persimmon trees have larger leaf size than citrus. Leaf size and wind speed are the main factors affecting the air boundary layer next to a leaf, which influence heat exchange and hence the temperature of the leaf. Under conditions of low wind speeds (<10 m s^{-1}), larger leaf size leads to thicker air boundary layers, less convective heat loss, and consequently greater differences from air temperature than smaller leaves (Nobel, 2009). Thus, under a certain stomatal closure level, crops with larger leaves like persimmon will tend to raise its temperature more than plants with smaller leaves like citrus. On the other hand, we should consider the effect that other factors, apart from soil water deficit, might have on stomatal closure. Contrarily to the already mentioned stomatal closure in response to VPD in citrus trees (Oguntunde et al., 2007; Villalobos et al., 2009), in the experiment performed in the persimmon orchard where g_s was measured, a positive relationship between g_s and VPD (0.51**) was observed. This feature of persimmon allowed that even in days with high evaporative demand (like DOY 239 with VPD of 6.9 kPa) T_c was well correlated with Ψ_s. The relationship found between T_c and Ψ_s for this day, when images were taken from a crane, was best-fitted by a polynomial curve (Figure 4), indicating that lower Ψ_s values corresponded with higher T_c values up to -2.2 MPa, point in which the canopy temperature stops increasing.

4.2 Comparisons of different canopy temperature indexes

In persimmon trees, both frontal (sunlit or shaded) and zenithal images clearly detected the higher T_c - T_a of WS trees with regard to the control ones. In this crop, either side of the canopy was suitable for measuring the temperature. In citrus trees, however, the results obtained suggest that images from the leaves most directly exposed to the solar radiation are more appropriate than frontal images to detect plant water stress. In fact, in a day with high evaporative demand during the second experimental season (DOY 238) when frontal images did not detect any differences in T_c between the SWS and the control trees, zenithal thermal images detected significant differences between them (Figure 6B,C). In addition, the highest correlations between T_c - T_a and Ψ_s were obtained when pictures were taken from 1 m above the canopies (Table 4).

In this two-year study, images were taken on each experimental season at least in 5 different days. For both orchards, T_c - T_a and Ψ_s or g_s were well correlated in some particular days. The highest correlations were always those between T_c - T_a and Ψ_s which had a coefficient of correlation of up to 0.90 and 0.56 for persimmon and citrus trees, respectively (Tables 3 and 4). The correlations obtained in particular days for citrus between T_c - T_a and Ψ_s when images were taken from 1 m above the canopies (Table 4), are similar to those reported by Sepulcre-Cantó et al. (2006) in olive trees, in which canopy temperature was measured with fixed infrared sensors installed 1 m above the tree crowns. However, when data from several days were pooled together, the relationships between T_c-T_a and Ψ_s or g_s were not tight suggesting that other environmental and endogenous factors also affected the relationships between canopy temperature and plant water status. Similarly, in citrus trees day-to-day differences in CWSI were not tightly related with Ψ_s measurements. The CWSI normalizes T_c measurements taking into account the day-to-day differences in the environmental

conditions, but it does not consider possible on-tree factors affecting stomatal conductance such as seasonal changes in the sink:source relationships and in leaf age.

The relationships between $T_c - T_a$ and Ψ_s observed in persimmon for each experimental season (Figure 2) and in citrus for the second one (Figure 7), are similar to those reported for olive trees by Sepulcre-Cantó et al. (2006) who obtained relationships with r^2 ranging between 0.25 and 0.62. Nevertheless our relationships show a generally lower fit than those reported by other authors in peach (Wang and Gartung, 2010), with r^2 of 0.70, or sweet orange (García-Tejero et al., 2011), with r^2 of 0.75. These authors also found lower regression coefficients between T_c - T_a and g_s suggesting that this fact could be due to the difficulty of relating the average temperature of multiple differently-oriented leaves with stomatal conductance of individual ones. In our experiment, however, there were no significant relationships between these parameters.

Finally, an effort was made to explore if the intra-crown temperature variability could be also used for water stress detection. Recently, González-Dugo et al. (2012) in almond trees found that this indicator was mainly related with differences in soil water content, rooting depth and irrigation distribution; while the environmental conditions did not affect much the seasonal variation of this indicator. However, in the present experiment in citrus and persimmon trees the intra-crown temperature variability was not different among irrigation treatments. Thus this indicator does not seem useful to detect plant water stress in persimmon and citrus trees. Our results are more in agreement with those reported in grapevines by Möller et al. (2006) or Grant et al. (2007), who also found that temperature variability within a canopy was not different between well watered and water stressed grapevines. It seems then than the usefulness of the intra-crown temperature variability index for plant water stress detection might be different according to the plant species. An analysis of the absolute values of standard

deviation values shows that species with apparent low sensitivity of intra-crown temperature variability have higher absolute values (1.6 to 3.8 ºC for grapevines, 2.1 to 2.4 ºC for persimmon and 1.1 to 1.9 ºC for citrus) than almond trees where the standard deviation values of canopy temperature varied from 0.6 to 1.8 ºC. It is difficult to find an explanation for this different behaviour among species since many physiological responses such as stomatal patchiness, leaf angle distribution, cavitation and branch or shoot autonomy behaviour, among others, can determine intra-crown temperature variability when soil water limitations are imposed.

5. Conclusions

This study shows that canopy temperature variations in response to water stress in persimmon and citrus trees can be detected with a hand-operated thermographic camera. Nevertheless, the use of T_c measurements to detect plant water stress is more suitable for crops like persimmon that are not highly sensitive to vapour pressure deficit and in which leaf characteristics such as leaf size that determine the aerodynamic resistance, allow higher increases of canopy temperature. In crops like citrus, the reduction in transpiration in well-watered trees as consequence of high VPD values could negatively affect the sensitivity of T_c as a water stress indicator. While in persimmon trees thermal images taken from either side of the canopy allowed detecting differences in temperature between treatments, in orange trees thermal images of the most exposed leaves to the solar radiation seemed to be more appropriate to detect plant water stress than frontal images. In any case, since $T_c - T_a$ and the CWSI did not predict well Ψ_s for a whole season the use of canopy temperature as a water stress indicator in commercial persimmon and citrus orchards should be used in relative terms using control plants irrigated at potential evapotranspiration as a reference.

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Table 1. Average values of air temperature (T_a), solar radiation (Rad), wind velocity (V) and vapor pressure deficit (VPD) from 13:00 to 15:00h for each day of measurements in the persimmon orchard.

	DOY	T_a (ºC)	Rad (Wm^{-2})	V (ms^{-1})	VPD (kPa)
2009					
	170	30.1	788.6	1.9	2.3
	205	34.6	793.2	1.5	2.8
	226	34.5	766.2	1.2	3.8
	240	30.7	696.0	1.8	1.8
2010					
	138	24.3	836.6	2.6	1.6
	155	29.7	851.8	2.4	1.9
	169	26.2	663.2	2.0	1.5
	176	28.5	751.0	2.5	2.3
	190	31.9	823.4	1.9	2.7
	204	28.9	530.6	1.7	1.5
	211	30.2	683.2	1.8	1.8
	218	29.1	723.2	1.5	1.6
	232	30.5	781.2	1.1	1.9
	239	42.4	740.2	2.0	6.9

Table 2. Average values of air temperature (T_a), solar radiation (Rad), wind velocity (V) and vapor pressure deficit (VPD) from 13:00 to 15:00h for each day of measurements in the citrus orchard.

	DOY	T_a (ºC)	Rad (Wm^{-2})	V (ms^{-1})	VPD (kPa)
2009					
	204	34.8	854.0	5.9	4.5
	218	33.2	793.2	2.8	3.0
	225	31.7	794.6	2.9	3.3
	232	34.0	788.8	3.0	4.2
	239	31.6	749.6	3.3	2.7
	246	34.7	748.0	2.1	4.7
	253	30.5	734.8	2.4	3.5
2010					
	216	29.9	634.2	3.3	2.0
	224	30.3	777.4	2.4	2.3
	238	37.1	760.5	2.1	4.5
	246	29.2	739.3	2.0	2.5
	253	28.1	715.1	2.0	2.6
	258	30.2	662.1	2.4	2.4

Table 3. Relationships between the different water status indicators in the persimmon experiment.

	R^2						
DOY	**$T_c$sunlit - $T_a$vs. Ψ_s**	**$T_c$shady - T_a vs. Ψ_s**	**$T_c$sunlit - T_a vs. g_s**	**$T_c$shady - T_a vs. g_s**	**Ψ_s vs. g_s**	**n**	**Ψ_s range**
Persimmon 2009							
170	0.76***	0.72***	0.79***	0.76***	0.69**	12	1.1
205	0.47**	0.65***	0.52*	0.80***	0.53**	16	1.3
226	0.90***	0.81***	0.70***	0.66***	0.90***	18	1.5
240	0.00	0.24	0.02	0.12	0.05	18	0.4
Persimmon 2010							
138	0.09	-	0.08	-	0.10	17	0.2
155	0.03	-	0.01	-	0.23	16	0.2
169	0.00	-	0.35*	-	0.00	18	0.2
176	0.00	-	0.02	-	0.25	18	0.1
190	0.66***	-	0.42*	-	0.43*	17	0.8
204	0.57***	-	0.19	-	0.30*	22	1.4
211	0.40**	-	0.11	-	0.16	17	0.7
218	0.59***	-	0.60***	-	0.20*	16	0.9
232	0.00	-	0.17	-	0.01	18	0.3
239	0.70***	-	0.46**	-	0.71***	18	1.8

*, **, *** and ns denote significant differences at P<0.05, P<0.01, P<0.001 and non significant differences, respectively, by Dunnett's test.

Table 4. Relationship between T_c – Ta and Ψ_s in the citrus experiment for each season.

	R^2			R^2		
DOY	**$T_c$sunlit – Ta vs. Ψ_s**	**$T_c$shady – Ta vs. Ψ_s**	**N**	**$T_c$zenith – Ta vs. Ψ_s**	**n**	**Ψ_s range**
Citrus 2009						
204	0.01	0.00	24	-	-	0.5
218	0.20*	0.21*	24	-	-	1.1
225	0.01	0.06	24	-	-	0.7
232	0.02	0.13	24	-	-	0.9
239	0.08	0.09	24	-	-	1.4
246	0.04	0.01	24	-	-	1.1
253	0.01	0.04	24	-	-	1.3
Citrus 2010						
216	0.03	-	24	-	-	0.5
224	0.27**	-	33	0.23*	20	0.7
238	0.14*	-	29	0.43	8	1.0
246	0.32***	-	35	0.56***	20	1.1
253	-	-	-	0.29**	25	1.6
258	0.20*	-	24	-	-	1.2

*, **, *** and ns denote significant differences at P<0.05, P<0.01, P<0.001 and non significant differences, respectively, by Dunnett's test.

Table 5. Stem water potential (ψ_s, MPa), canopy temperature (T_c, ºC) and the crop water stress index (CWSI) of each treatment during the second experimental season (2010) in the citrus orchard.

	ψ_s	T_c	CWSI
DOY 224			
Control	-0.93a	30.44a	0.43a
MWS	-0.98a	30.66a	0.41a
SWS	-1.15b	31.42b	0.48b
DOY 238			
Control	-0.90a	34.89	0.49
MWS	-1.20b	34.93	0.49
SWS	-1.53c	35.19	0.51
DOY 246			
Control	-0.94a	29.72a	0.33a
MWS	-1.08a	30.67b	0.40b
SWS	-1.36b	30.73b	0.39b
DOY 258			
Control	-0.92a	30.64a	0.40a
MWS	-1.32b	32.06b	0.48b
SWS	-1.59c	32.11b	0.48b

Values followed by different letters are significantly different at $P<0.05$ from ANOVA.

Table 6. Sensitivity of the different water stress indicators for each species and experimental season.

	Persimmon			Orange		
	T_c	g_s	Ψ_s	T_c	Ψ_s	CWSI
2009						
Signal	1.08	1.36	1.94	-	1.40	-
Noise	0.15	0.26	0.41	-	0.20	-
Sensitivity (signal/noise)	7.20	5.23	4.73	-	7.00	-
2010						
Signal	1.04	1.38	1.86	1.05	1.48	1.11
Noise	0.05	0.33	0.37	0.06	0.20	0.16
Sensitivity (signal/noise)	20.80	4.18	5.03	17.5	7.40	7.07

Table 7. Average values for intra-crown temperature variability (σ), coefficient of variation (C.V.), stem water potential (Ψ_s) and canopy temperature (T_c) in citrus and persimmon trees for each treatment and season.

	σ	**C.V.**	**Ψ_s**	**T_c**
Citrus 2009				
Control	1.28a	0.28	-0.97c	33.2a
MWS	1.08a	0.36	-1.31b	32.8a
SWS	1.17a	0.39	-1.57a	33.1a
Citrus 2010				
Control	1.78a	1.33	-0.92c	32.0a
MWS	1.87a	1.09	-1.09b	32.5a
SWS	1.92a	1.03	-1.33a	32.7a
Persimmon 2009				
Control	2.08a	0.22	-0.86b	34.1b
WS	2.36a	0.26	-1.96a	37.5a
Persimmon 2010				
Control	2.31a	0.32	-0.54b	28.8b
WS	2.39a	0.29	-1.13a	29.6a

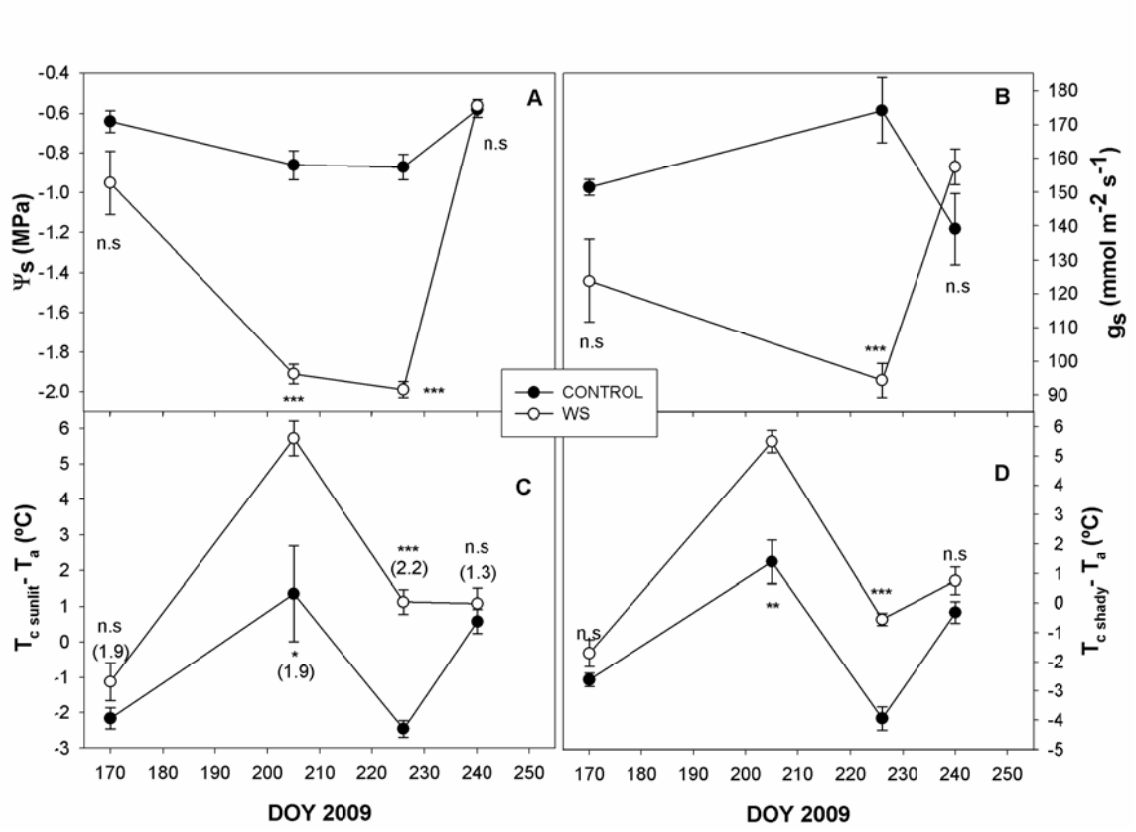

Figure 1. Evolution of stem water potential (Ψ_s; A), stomatal conductance (g_s; B) and canopy temperature (T_c), measured on the sunlit (C) and shady (D) side of the trees, for the different treatments in the persimmon orchard in 2009. *, **, *** and n.s denote significant differences at $P<0.05$, $P<0.01$, $P<0.001$ and non significant differences, respectively, by Dunnett's test. In graph C, average daily air vapour pressure deficit values of each day are shown between brackets.

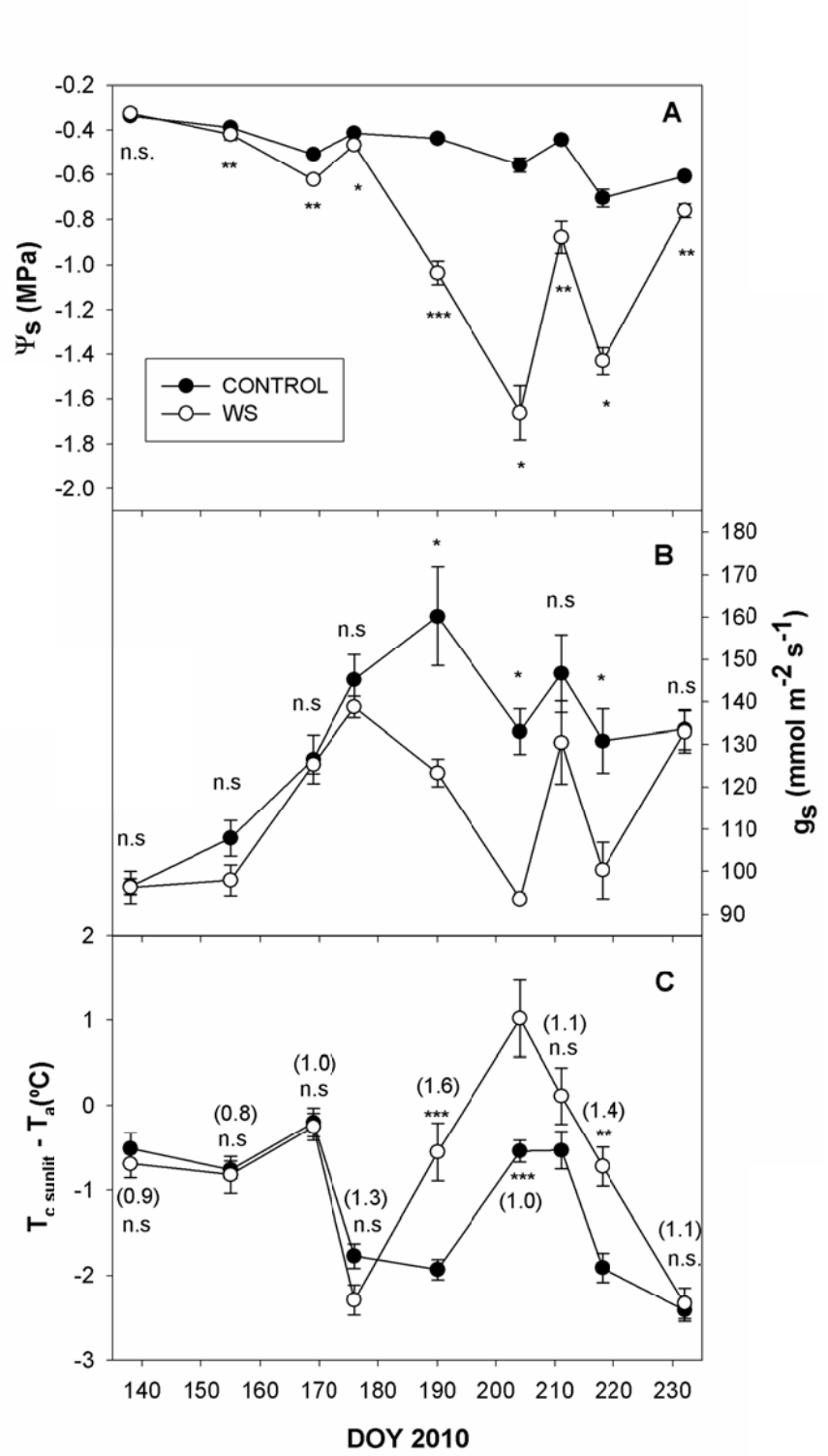

Figure 2. Evolution of stem water potential (Ψ_s; A), stomatal conductance (gs; B) and canopy temperature measured on the sunlit side of the trees ($T_c$sunlit; C) for the different treatments in the persimmon orchard during 2010. *, **, *** and n.s denote significant differences at P<0.05, P<0.01, P<0.001 and non significant differences, respectively, by Dunnett's test. In graph C, average daily air vapour pressure deficit values of each day are shown between brackets.

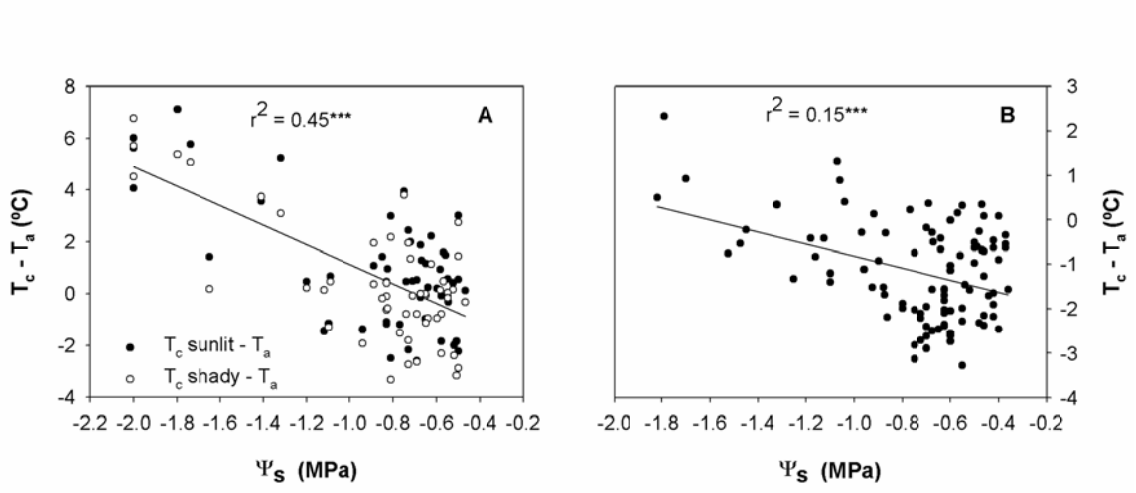

Figure 3. Relationship between $T_c - T_a$ and Ψ_s in persimmon trees for 2009 (A) and 2010 (B). Each value is a single tree measurement (n = 105 in figure A and 98 in B).

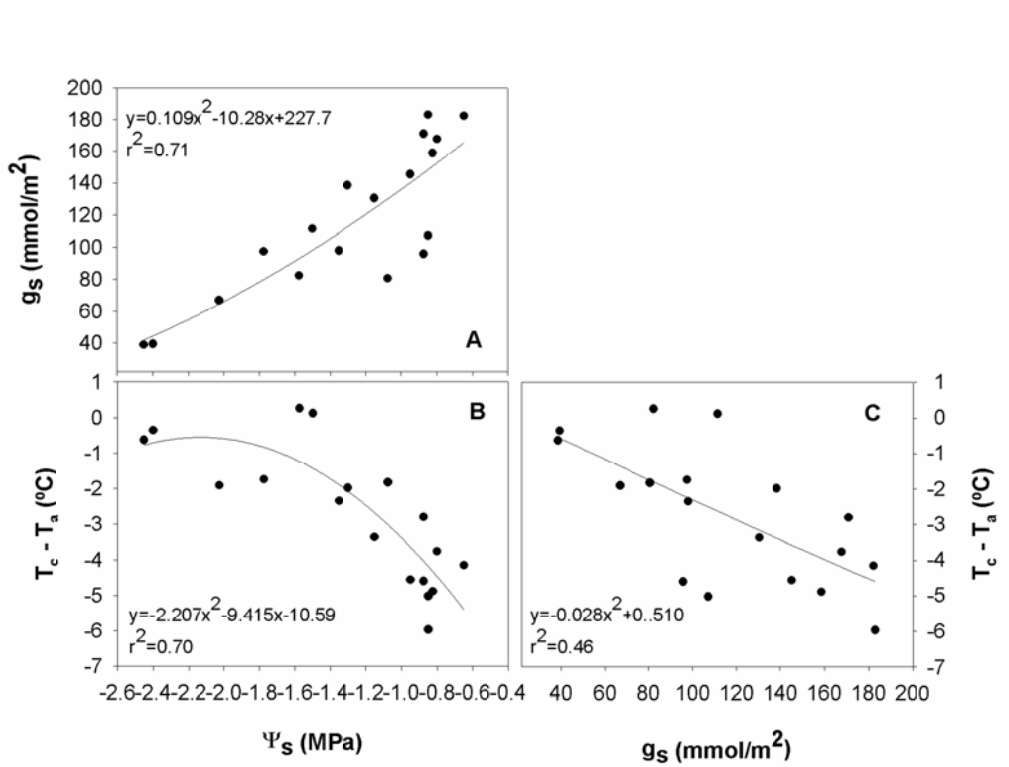

Figure 4. Relationships between g_s and Ψ_s (A), T_c and Ψ_s (B) and T_c and g_s (C) for DOY 239 when thermographic images were taken from a crane in the persimmon orchard. Each value is a single tree measurement.

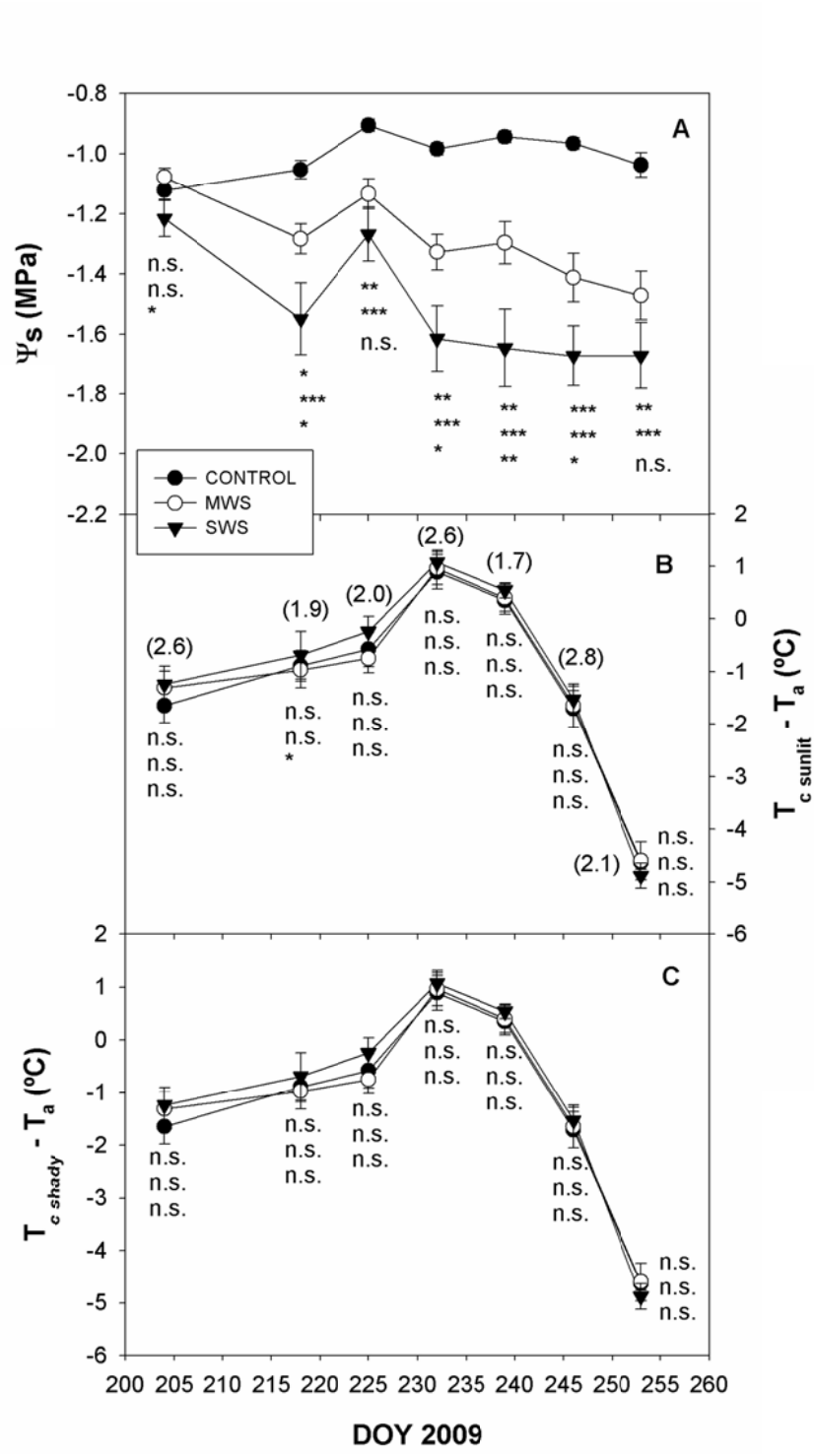

Figure 5. Evolution of stem water potential (Ψ_s; A) and canopy temperature (T_c), measured on the sunlit (B) and shady (C) side of the trees, for the different treatments in the citrus orchard during 2009. *, **, *** and n.s. denote significant differences at P<0.05, P<0.01, P<0.001 and non significant differences, respectively, by Dunnett's test. For each day, the top asterisks or n.s. indicate differences between control and MWS, the middle ones between control and SWS and the bottom ones between MWS and SWS. In graph B, air vapour pressure deficit (kPa) values of each day are shown between brackets.

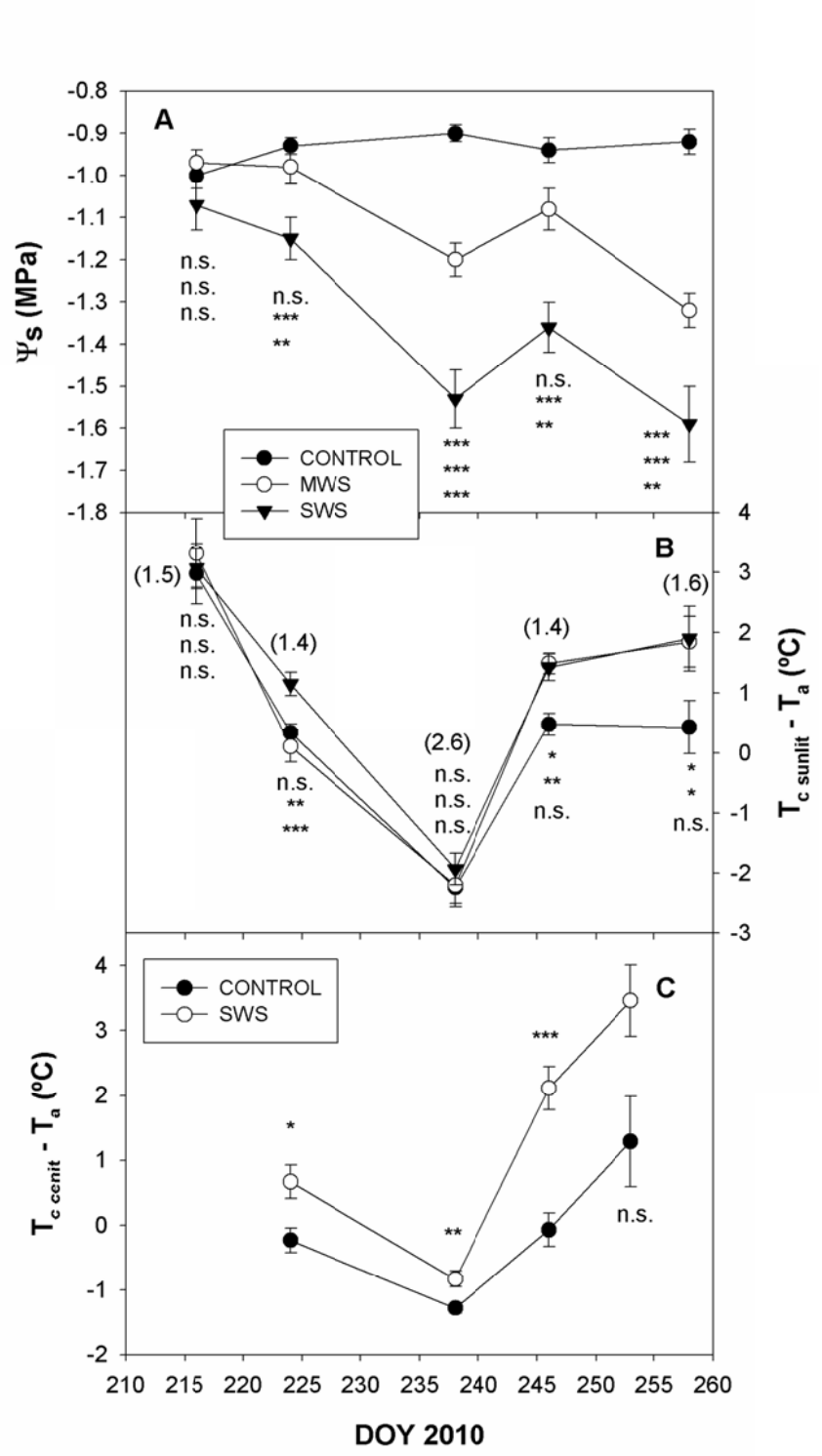

Figure 6. Evolution of stem water potential (Ψ_s; A) and canopy temperature (T_c) measured on the sunlit side of the trees (B) and from 12 m above the canopy (C) for the different treatments in the citrus orchard during 2010. *, **, *** and n.s. denote significant differences at $P<0.05$, $P<0.01$, $P<0.001$ and non significant differences, respectively, by Dunnett's test. For each day, the top asterisks or n.s. indicate differences between control and MWS, the middle ones between control and SWS and the bottom ones between MWS and SWS. In graph B, air vapour pressure deficit values of each day are shown between brackets.

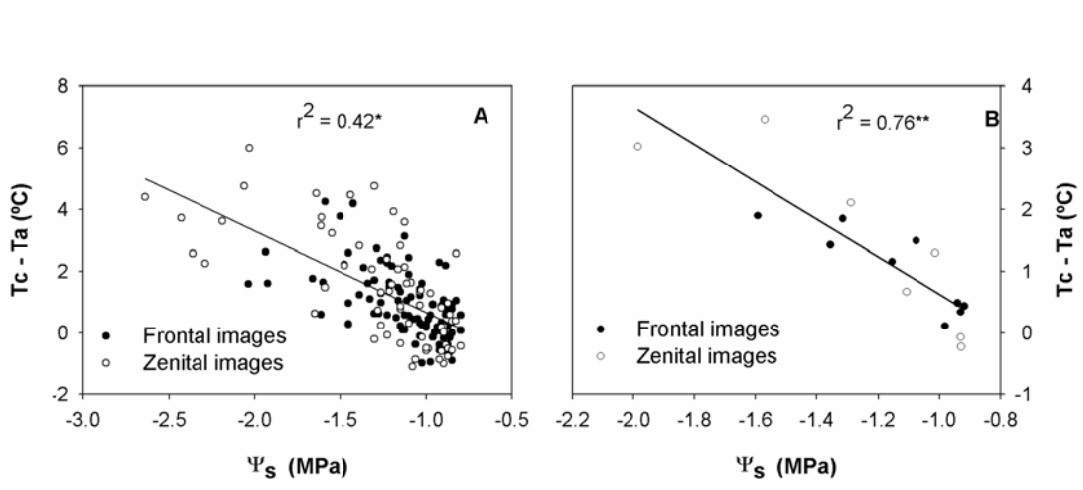

Figure 7. Relationship between $T_c - T_a$ and Ψ_s in the citrus orchard for the 2010 season. In figure A each point is a single tree measurement (n = 94). In figure B data are grouped by treatments (n = 16).