Usefulness of the ZIM-probe Technology for detecting water stress in Clementine and Persimmon trees

C. Ballester[1,a], M. Castiella[2,3], U. Zimmermann[2,4], S. Rüger[2,3], M.A. Martínez Gimeno[5] and D.S. Intrigliolo[1,5]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain; [2]ZIM Plant Technology GmbH, Hennigsdorf, Germany; [3]Present address: YARA ZIM Plant Technology GmbH, Hennigsdorf, Germany; [4]Department of Biotechnology, Universität Würzburg, Würzburg, Germany (from 31-12-2013); [5]Center for Applied Biology and Soil Sciences (CEBAS), Murcia, Spain.

Abstract

Further improvement on irrigation management requires continuous plant water status monitoring. The non-invasive ZIM-probe measures the pressure (P_p) transfer function through a patch of an intact leaf, which is inversely correlated with the turgor pressure. Data are sent wireless in real-time by telemetry to an Internet server via a mobile phone network where it is available to be analyzed. In this work, the detection of water stress by measuring relative changes in turgor pressure with the ZIM-probe was evaluated in clementine and persimmon trees. Ten trees of both species were equipped with two ZIM-probes each located at the east side of the canopy. The ZIM-probes were used over several months during which half of the trees were subjected to two drought cycles. Concomitant measurements of stem water potential (ψ_s) were taken at midday in both orchards during the drought periods. Additionally, determinations of ψ_s and stomatal conductance (g_s) were also performed during 1-2 days at hourly intervals in the clementine and persimmon orchards, respectively, to study the existing relationship of these classical indicators with the leaf turgor pressure. Results showed that diurnal P_p values increased in non-irrigated clementine trees when water restrictions were imposed. Persimmon drought-stressed trees, on the other hand, showed different P_p curve shapes (half and complete inverse curve) depending on the level of stress reached by the trees. There was a tight correlation between the hourly spot measurements of ψ_s and g_s with the probe data. Overall, results show that the ZIM-probe enables the detection of drought stress in clementine and persimmon trees. Nevertheless, different approaches for calculating the water stress level must be used in each of these species due to the higher tendency of persimmon leaves to the inversed P_p curve phenomena.

Keywords: citrus, deficit irrigation, drought stress, leaf turgor, pressure sensors, stem water potential

INTRODUCTION

Mediterranean ecosystems have high water requirements which are not compensated by the scarce rainfall typical from these semiarid areas. These climatic conditions make irrigation and optimization of the water resources essential for the proper management of the crops and, eventually, for the sustainability of the agricultural systems.

Several plant-based techniques have been studied during the last decades to continuously monitor the plant water status in crops of a high economical interest (Fernández, 2014). These techniques, such as the leaf/stem water potential, stem dendrometers or the sap flow measurements, among others, present however some practical issues that prevent their regular use in the field (Zimmermann et al. 2013a).

[a]E-mail: ballester_carlur@gva.es

Leaf turgor monitoring with a magnetic-based probe (ZIM-probe) has been pointed out as a promising alternative to the aforementioned techniques (Zimmermann et al. 2008). The non-invasive ZIM-probe measures the pressure (P_p) transfer function through a patch of an intact leaf, which is inversely correlated with the turgor pressure. The usefulness of the ZIM-probe to detect plant water stress has been studied on several horticultural and fruit crops (Westhoff et al. 2008; Zimmermman et al. 2009; Rüger et al. 2010a, b; Ehrenberger et al. 2012; Fernández et al., 2011; Rodríguez-Domínguez et al., 2012;). These studies showed that the ZIM-probe detected very sensitively changes in turgor pressure caused by variations in the microclimate and irrigation. Different approaches, however, may be used to determine the level of stress reached by the plants based on their physiological characteristics and their tolerance or sensitivity to drought stress.

This work aimed to assess the usefulness of the ZIM-probe to detect plant water stress in clementine and persimmon trees. Clementine is the most cultivated mandarin in Spain, which is the second world producer of this species (Faostat, 2012). Persimmon, on the other hand, is an emerging crop with a steadily increasing plantation in citrus producing areas (Perucho, 2015).

MATERIALS AND METHODS

Experimental plots and treatments

The experiment was performed during 2014 and 2015 in two commercial orchards planted with *Citrus clementina*, Hort ex Tan 'Arrufatina', and *Diospyros kaki* 'Rojo Brillante'. The clementine orchard was located in Alberique (39º 7' 31.33" N, 0º 33' 17.06" W), Valencia (Spain). Trees were grafted onto Citrange Carrizo (*Citrus sinensis*, Osb. x *Poncirus trifoliata*, Raf.) at a spacing of 5 m x 4 m and had an average canopy ground cover at the beginning of the experiment of 33% of the area allotted per tree. Irrigation was applied with two drip lines leaving 8 emitters (2.2 L h^{-1}) per tree. The persimmon orchard was located in Liria (40° N, elevation 300 m), Valencia (Spain) where trees were grafted onto *Dyospiros Lotus* at a spacing of 5 m x 2.5 m. At the beginning of the experiment persimmon trees had an average trunk perimeter of 23.6 cm. Drip irrigation was applied with two drip lines leaving 10 emitters (4.5 L h^{-1}) per tree. Two irrigation treatments were applied in both orchards: (i) control treatment, irrigated at 100% of crop evapotranspiration (ET_c) during the whole season and; (ii) non-irrigated treatment (DS), in which trees were subjected to drought cycles (from July 23rd to July 31st and from August 28th to September 6th in the clementine orchard, and from August 18th to August 23rd and August 29th to September 3rd in the persimmon plot). Each treatment was made up of five trees in which stem water potential (ψ_s), stomatal conductance (g_s) and leaf turgor measurements were performed.

Leaf turgor and plant water status determinations

Two ZIM-probes were installed in mature leaves at the east side of all the selected trees (10 ZIM-probes/treatment) in order to monitor the leaf turgor. All the pressure sensors were previously tested under laboratory conditions to ensure that ambient temperature (T_a) did not have any influence on their readings. Relative humidity (RH) and T_a sensors were also installed in both orchards (two RH and four T_a sensors in the clementine grove and two RH and T_a sensors in the persimmon orchard) to distinguish relative changes in leaf turgor pressure due to microclimate variations from those caused by drought stress (Zimmermann et al. 2013b). The ZIM-probes, T_a and RH sensors were wired to transmitters (one per tree) which sent the data via wireless to a GPRS modem which in turn was linked to an internet server via mobile phone network.

Concomitant measurements of midday ψ_s were carried out in both orchards during the drought cycles to determine the plant water status. Measurements were performed at noon with a Scholander pressure chamber in 2-4 mature leaves from all the trees equipped with the ZIM-probes as described in Turner (1981). Additionally, hourly cycles of ψ_s (May 4th and 5th, 2015) and g_s (August 19th, 2014) measurements were performed in the clementine and persimmon orchards, respectively. The g_s measurements were performed in a total of five sunny leaves per tree with a leaf porometer (SC-1 porometer, Decagon, WA, USA).

Statistical analysis

Data were analyzed using Origin (OriginLab, Northampton, MA). For statistical comparison of the treatments two-tailed Student's *t*-test ANOVA was used. A value of $P < 0.05$ was considered to be statistically significant. Average data of the daily maximum and minimum patch pressure ($P_p$max and $P_p$min) slopes and ψ_s are presented as mean ± standard error. The relationship between P_p, ψ_s and g_s during the daily cycles of measurements was explored by correlation analyses.

RESULTS AND DISCUSION

Citrus experiment

At the beginning of the experiment, control and DS trees had similar values of ψ_s (around -1.09 MPa). When water restrictions began, however, differences in plant water status arose between treatments. DS trees reached minimum values of -1.88 and -1.67 MPa during the first (from July 23rd to July 31st) and second (from August 28th to September 6th) drought cycles, respectively, while control trees did not surpass in any case the -1.27 MPa (Fig.1).

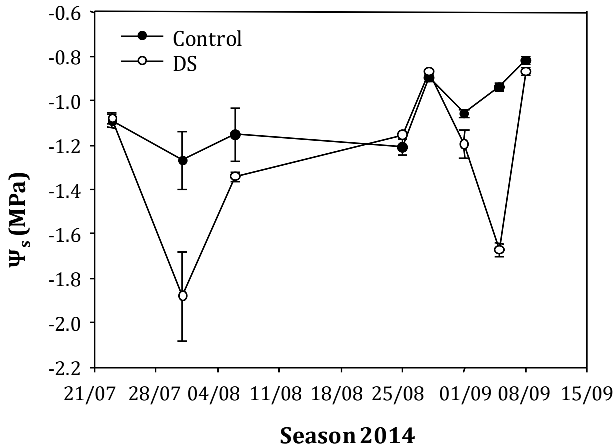

Figure 1. Stem water potential (ψ_s) evolution in citrus control and non-irrigated trees (DS) during the drought cycle periods. Each point is the average of 10-20 leaves (5 trees/treatment). Vertical bars represent ± standard error.

Differences in the P_p profile between control and DS trees were also detected with the ZIM-probes during the drought cycles. Irrigation withholding increased $P_p$max values in DS trees as ψ_s decreased (Fig. 2). Similar P_p profiles have been reported for a large number of crops such as grapevine, olive, banana, and almond trees when subjected to drought stress (Zimmermann et al., 2013b). In almond, orange (Zimmermann et al. 2013b), and olive (Ehrenberger et al., 2012; Fernández et al., 2011) trees, continuous water stress led to different leaf turgor status, which were identified by a half or complete inversion of the P_p curve, i.e. higher P_p values during night and lower values during the day (Ehrenberger et al., 2012). In this experiment, an inversion of the P_p curve was not commonly observed and just two out of ten ZIM-probes monitored a half inversion of the P_p curve. Prolonged drought stress has also been related with an increase in the $P_p$min (recorded at night), which has been suggested as other possible water stress indicator when using the ZIM-probes (Zimmermann et al., 2013b). Here in clementine trees, $P_p$min did not significantly increase in the DS treatment along the drought cycles, which suggests that rehydration of leaves during the night, was not affected (Fig. 2; table 1). These results point out the $P_p$max increase as the most useful water stress indicator for clementine trees. In fact, notable differences were observed between treatments in the $P_p$max slopes (Table 1). Nevertheless, these differences were statistically significant only for the second drought cycle due to the high variability observed among ZIM-probes within the same treatment (Table 1).

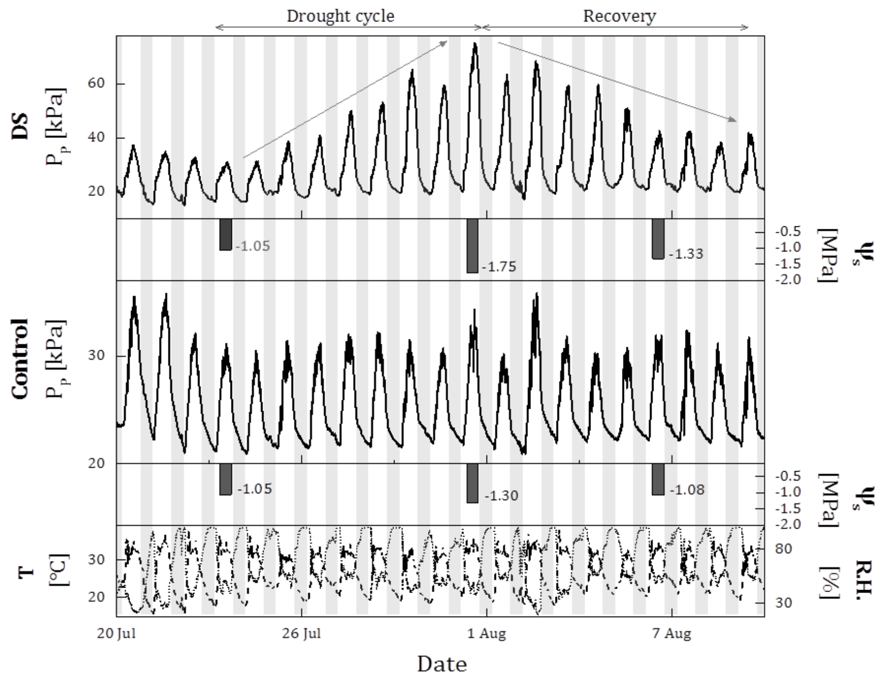

Figure 2. Air temperature (T, *dashed line*), relative humidity (R.H., *dotted line*) and patch pressure (Pp, *solid line*) evolution in a drought-stressed (DS) and control tree during the 1st drought cycle and the recovery period. Average stem water potential (ψ_s) measurements are shown as vertical columns ± standard error, n=2. The shaded background columns indicate the nocturnal hours.

Table 1. Statistical analysis (significance differences at *p* value <0.05) for the maximum patch pressure ($P_p$max), minimum patch pressure ($P_p$min) and stem water potential (ψ_s) measurements obtained in the citrus experiment.

	P_p max		P_p min		ψ_s	
	slope (kPa day^{-1})	T-test	slope (kPa day^{-1})	T-test	slope (kPa day^{-1})	T-test
1st drouht cycle						
Control	0.96 ± 0.83	0.07	0.23 ± 0.97	0.08	0.20 ± 0.10	**<0.001**
DS	2.43 ± 2.29		0.83 ± 0.34		0.95 ± 0.30	
2nd drouht cycle						
Control	1.19 ± 0.99	**<0.001**	0.77 ± 1.20	0.05	0.05 ± 0.04	**<0.001**
DS	3.20 ± 1.87		1.74 ± 1.79		1.00 ± 0.14	

Once water restrictions ended and irrigation was resumed in the DS treatment, ψ_s and $P_p$max recovered in a few days to similar values as those of control trees (Fig. 1 and 2).

Daily P_p values yielded in general a good correlation with ψ_s (coefficient of determination, r^2, ranging from 0.40 to 0.74) when data from both days of measurements were pulled together. Within each day, the weakest correlations were obtained in May 4th when T_a and consequently P_p sharply decreased from 15:00 until 21:00h (Fig. 3). In May 5th, the P_p drop during the afternoon was much more moderate than the previous day yielding its relationship with ψ_s an r^2 that ranged from 0.65 to 0.88; these values are more similar to those reported in other studies with eucalyptus, birch or wheat (Westhoff et al. 2008, Zimmermann et al. 2010, Bramley et al. 2012).

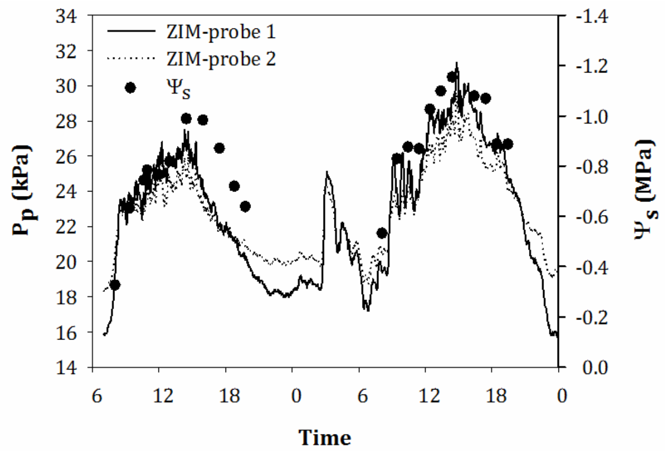

Figure 3. Hourly cycle of stem water potential (ψ_s) and patch pressure (P_p) evolution monitored in two ZIM-probes installed in the same tree during two consecutive days (May 4th and 5th 2015). Each point is the average of three ψ_s measurements.

Persimmon experiment

Control and DS trees had a mean ψ_s at the beginning of the experiment of -0.66 MPa. Once irrigation treatments began, ψ_s in control trees remained around values of -0.65 MPa during the whole experiment while it sharply dropped in DS trees from -0.66 to -1.73 MPa and from -0.99 to -1.97 MPa during the first and second drought cycles, respectively (Fig. 4).

Differences in plant water status between treatments were also detected by the ZIM-probes. The typical P_p curve characterized by a steady increase during morning, peak around midday and a gradual decrease during the afternoon was observed in control trees during the whole experiment (Fig. 5). DS trees showed the same profile as control trees at the beginning of the water restrictions and after the recovery period. Nevertheless, similar to the aforementioned for almond and olive trees and contrary to the results obtained in the clementine experiment, different states of turgor pressure (state I, II and III) were observed in DS trees as ψ_s decreased (Fig. 5). Values of ψ_s above -0.90 MPa were associated with the typical P_p curve observed in control trees, which corresponded to the state I of leaf turgor (Fig. 5). As plant water status impaired, the P_p curve shape changed most likely due to the presence of air bubbles within the leaves (see Ehrenberger et al., 2012 for more detail). The intermediate state of leaf turgor, identified by a half inversion of the P_p curve, was associated with ψ_s values ranging from -0.90 to -1.20 MPa (Fig.5). Below -1.20 MPa, a complete inversion of the P_p curve (state III) was recorded in seven and six out of ten ZIM-probes during the first and second drought cycles, respectively. These results are different from those reported for olive trees (Fernández et al., 2011) in which state III of leaf turgor was associated with ψ_s values below -1.70 MPa, pointing out the different sensitivity to drought stress exhibited by these two crops.

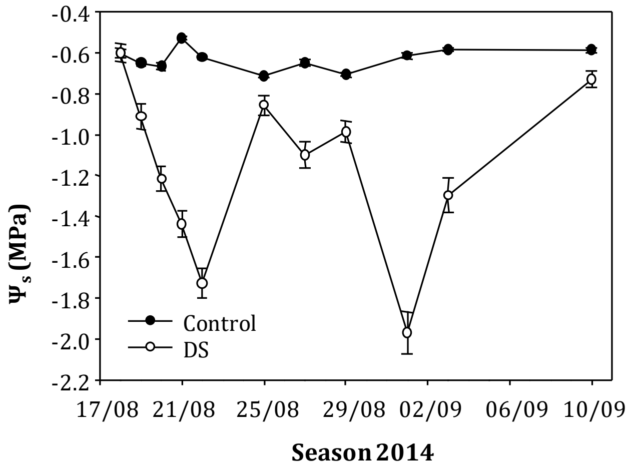

Figure 4. Stem water potential (ψ_s) evolution in the control and non-irrigated trees (DS) during the drought cycle periods applied to the citrus trees. Each point is the average of 10-20 leaves (5 trees/treatment). Vertical bars represent ± standard error.

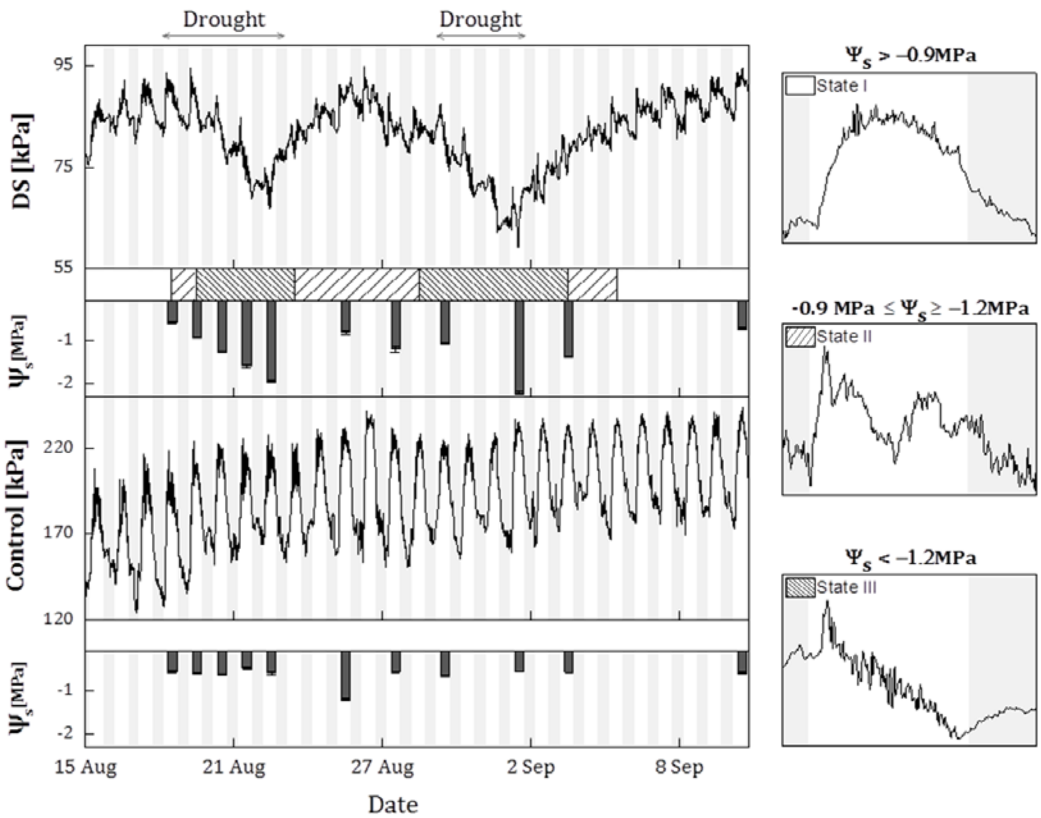

Figure 5. Average stem water potential (ψ_s, *columns*) and patch pressure (P_p, *solid line*) evolution in a drought-stressed (DS) and control tree during the two drought cycles applied in the persimmon orchard (1st drought cycle: August 18th to August 23rd; 2nd cycle: August 29th to September 3rd). Different P_p curve shapes associated to different plant water status are also identified between the P_p and ψ_s measurements for each treatment (state I ▭; state II ▨ and; state III ▩). ψ_s values are means and the error bars indicate the standard errors, n=4. The shaded background columns indicate the nocturnal hours.

The gs measurements showed a similar daily pattern as that of the P_p curve in most of the cases. An example of the daily g_s and P_p pattern measured in one of the control persimmon trees is depicted in figure 6. Both, g_s and P_p showed the lowest values early in the morning, increased thereafter to reach a peak at noon and steadily decreased during the afternoon. There was in general a good correlation between g_s and P_p yielding r^2 values above 0.66 in nine out of eleven probes (one of the ZIM-probes was out of range).

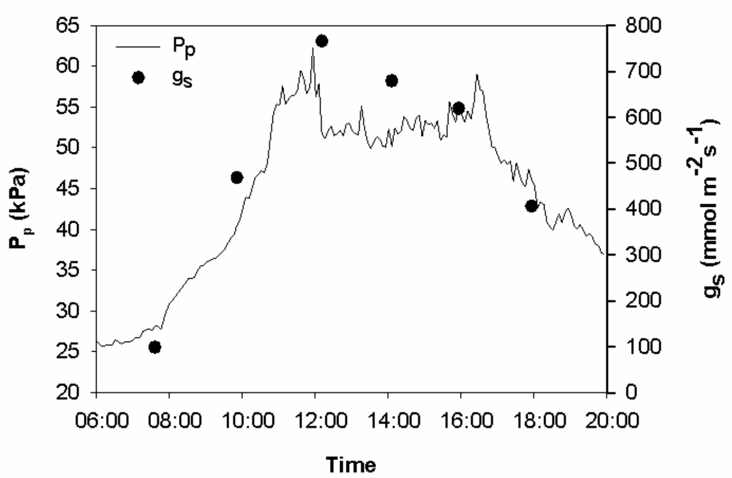

Figure 6. Stomatal conductance (g_s) and patch pressure (P_p) evolution monitored in a same tree during a sunny day (August 19th, 2014). Each point is the average of five g_s measurements.

CONCLUSIONS

Overall, the results from this experiment show that the non-invasive ZIM-probe technology enabled the detection of water stress in clementine and persimmon trees. Nevertheless, they also show that different approaches must be used for each of these species. On one hand, the assessment of the $P_p$max seemed to be a good water stress indicator for clementine trees although more research on this species would be needed in order to determine the level of stress reached by the plants. Persimmon trees, on the other hand, were more prone than clementine trees to the P_p curve inversion phenomena, which allowed the identification of P_p curve shapes to different levels of plant water status. This technology could be used then to properly manage plant water status in this crop and subject trees to moderate water stress during certain periods in order to reduce summer fruit drop and avoid impairing crop production.

The high leaf-to-leaf variability observed within trees points out the need of studying how leaf age or elasticity may influence P_p. More research on the influence of other parameters apart from water status such as rootstock, seasonal variability in tissue water relations or crop load would be also of great interest.

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