PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON OLIVE IRRIGATION AND OIL QUALITY

Co-Conveners
S. Lavee
U. Yermiyahu

Nazareth, Israel
December 6-10, 2009

ISHS Section Nuts and Mediterranean Climate Fruits
ISHS Commission Irrigation and Plant Water Relations

Acta Horticulturae 888
February 2011
The Leaf Patch Clamp Pressure Probe: a New Tool for Irrigation Scheduling and Deeper Insight into Olive Drought Stress Physiology

S. Rüger, W. Ehrenberger and U. Zimmermann
Lehrstuhl für Biotechnologie, Biozentrum
Universität Würzburg
Am Hubland, D-97074 Würzburg
Germany

A. Ben-Gal and N. Agam
Environmental Physics and Irrigation
Gilat Research Center
Agricultural Research Organization
Israel

D. Kool
Irrigation and Water Engineering
Wageningen University
The Netherlands

Keywords: turgor pressure, water stress, monitoring, *Olea europaea* L.

Abstract

Leaf turgor pressure provides a very sensitive indicator of plant water status. Diurnal changes in turgor pressure of olives were measured over several months with a novel leaf patch clamp pressure (LPCP) probe. The LPCP probe is user-friendly, non-invasive, online-monitoring, robust and versatile, and is characterised by high precision, low-cost and automation suitability. Data are transferred wireless to an internet server via a mobile phone network for real-time evaluation and for remote regulation of irrigation or stress management. The probe measures the attenuated output pressure response \( P_p \) of a leaf patch upon application of an externally applied, constantly kept pressure, generated by two small magnets. \( P_p \) is sensed by a miniaturized pressure sensor embedded in silicon and integrated into one of the magnets. Concomitant measurements of balancing pressure values, \( P_b \) (i.e. stem water potential) using the pressure bomb technique revealed a very close correlation between \( P_b \) and \( P_p \). Both parameters depended inversely on cell turgor pressure as evidenced by direct measurements of leaf turgor pressure using the cell turgor pressure probe. Measurements on olive trees planted in weighing lysimeters that allowed continuous monitoring of water balance of individual trees subjected to varying conditions of water status demonstrated the potential of the LPCP probe for irrigation scheduling and for elucidation of drought stress physiology.

INTRODUCTION

Development of innovative sensors to provide sensitive and continuous plant water status will aid preventing losses by soil infiltration and evaporation. Water status monitoring of olives is of particular interest as stress conditions appear to be desirable for optimization of oil yield and quality (Ben-Gal et al., 2009; Dag et al., 2008). Information on plant water status can be obtained by leaf water potential (Ferreira et al., 1997), stem water potential (Naor et al., 1999), stomatal conductance (Tan and Layne, 1991), leaf temperature (Jones, 2004), variations in leaf thickness (Mc Burney, 1992) and plant organ diameter (Goldhamer et al., 1999), xylem sap flow (Massai et al., 2000) as well as cell turgor and xylem pressure probe measurements (Zimmermann et al., 2004; Tomos, 2000). Sensors monitoring soil water content and soil water potential in the root area have also been used to estimate crop water status (Smith and Mullins, 2000; Dane and Topp, 2002; Jones, 2004).

Even though the plant-based sensors currently employed have been useful tools for basic research in plant physiology, none of them have found wide-spread application in agriculture and horticulture. Many sensor systems are highly sophisticated and expensive, susceptible to wind and rain and/or too insensitive to monitor water status. Some instruments used for monitoring water stress require removal of leaves or other organs. Furthermore, most sensor systems do not allow online monitoring over an entire
vegetation period; automation and remote control of irrigation are very often difficult or even impossible.

A novel, non-invasive and inexpensive probe for online monitoring of cell turgor pressure changes with high precision has recently been described (Zimmermann et al., 2008, 2010; Westhoff et al., 2009). The so-called leaf patch clamp pressure (LPCP) probe (commercial name ZIM probe) consists of a miniaturised pressure sensor silicon-embedded and integrated into a magnetic clamp that is clipped to a patch of an intact plant leaf. The probe measures the pressure transfer function of the leaf patch, i.e. the attenuated output pressure, \( P_p \), in response to the clamp pressure, \( P_{\text{clamp}} \). The magnitude of the leaf pressure transfer function, and thus the attenuation of the constantly kept external pressure, is dictated by a plant-specific, turgor pressure-independent term (related with the compression of the silicone, the cuticle, cell walls and other structural elements) and a turgor pressure-dependent term. Thus, relative changes in turgor pressure are measured with high temporal resolution provided that the turgor pressure-independent term is kept constant. This was previously documented for leaves of grapevine and banana plants as well as for liana (Zimmermann et al., 2008, 2010; Westhoff et al., 2009). In this communication we demonstrate the potential for monitoring diurnal changes in leaf turgor pressure of olive trees using the LPCP probe.

MATERIALS AND METHODS

Plant Material

Olives, *Olea europaea* ‘Barnea’, were planted in 15 2.5-m³ volume lysimeters filled with a loamy sand soil at the Gilat Research Center in Israel. LPCP probes were attached to leaves of 3 year old non-bearing trees. The trees were normally irrigated daily with 120% of their measured evapotranspirative consumption. During the summer of 2009, the irrigation to 5 replicate trees was ceased for a period of one week (July 23 to July 28, inclusive) as available soil water was allowed to reach negligible levels while the other 10 trees continued with full irrigation.

Leaf Patch Clamp Pressure Probe: Principles and Theoretical Considerations

The basic idea underlying the LPCP probe (Fig. 1 A) is that a small leaf patch is used as a sensing element of turgor pressure changes in the surrounding uncovered tissue. A pre-condition is that the stomata of the sensing elements must be closed in order to avoid water loss (see the analogy to stem water potential measurements). This is achieved by clamping a leaf between two planar circular pads made of (non-transparent) aluminium as shown schematically in Figure 1 B. The lower pad contains an integrated pressure sensor chip. The clamp pressure is generated magnetically; the pad containing the sensor chip is fixed on a small toric magnet (see inset of Fig. 1B), and the counter pad on a magnet that can be moved along a threaded rod. The distance between the two magnets dictates the clamp pressure. Variation of the distance allows adjustment of the magnetic force to the rigidity and thickness of the leaves.

Theoretical considerations have shown that the output patch pressure \( P_p \) sensed by the sensor chip upon application of an external clamp pressure, \( P_{\text{clamp}} \), is a power function of the turgor pressure, \( P_c \) (Zimmermann et al., 2008; Westhoff et al., 2009):

\[
P_p = \left( \frac{b}{aP_c + b} \right)^{1/a} \cdot F_a \cdot P_{\text{clamp}} \tag{Eq. 1}
\]

where \( F_a \) is the attenuation factor and takes into account turgor pressure-independent losses due to the compressibility of the silicone of the sensor chip and leaf-specific structural elements such as cuticle, cell walls and intercellular air spaces. \( a \) and \( b \) are constants and equal or larger than unity. Eq. 1 does not hold for turgorless leaf tissue and was derived by assuming that the volumetric elastic moduli of the cell walls depend linearly on turgor pressure and are not temperature-dependent. The inverse relationship
between \( P_p \) and \( P_e \) becomes particularly obvious if the constant \( a \) is equal to unity and \( b \) is much smaller than \( P_e \). In this case, Eq. 1 becomes \( P_p = k/P_e \) where \( k = b \cdot F_a \cdot P_{clamp} \), i.e. \( P_p \) and \( P_e \) show opposite changes. \( F_a \) is usually in the order of 0.2 to 0.3 and can be assumed constant over a large range of temperature and turgor pressures. However, upon approaching the plasmolytic point, \( F_a \) can vary considerably during day and night because of the dramatic water loss and the associated large midday temporary accumulation of air in the leaves. Under these conditions \( F_a \) assumes very small values, i.e. the leaves are more compressible than during the night, and Eq. 1 loses validity.

Data Acquisition

The LPCP probe values are continuously remotely recorded. The pressure sensor chip is connected via a cable to a battery-powered wireless telemetric transmitter (teleBITcom, Teltow, Germany). This transmitter sends the pressure signals together with the transmitter ID-code via an ISM band to a radio control unit located up to 500 m away (NTTB, Zeuthen, Germany). If needed, the distance between the transmitter and the radio control unit can be further increased using repeaters to amplify the transmitted signals. The radio control unit transfers the data with a time stamp to a GPRS modem linked to an internet server where the data are stored and subsequently analysed in a control centre. If the evaluation shows that the plants are subjected to drought, growers can be informed (e.g. by SMS or e-mail) or irrigation pumps/valves can be activate.

LPCP Calibration

Calibration of the LPCP probe was performed using a cell turgor pressure probe. The principle of this probe is described in detail elsewhere (Zimmermann et al., 2004). Briefly, a microcapillary, sealed to a small Plexiglas chamber containing a pressure transducer, is inserted into a leaf cell. Microcapillary and chamber are filled with incompressible oil that transfers the turgor pressure to the pressure transducer. Because of their rigidity and due to logistic reasons, calibration was not done on olive leaves but was performed on leaves of a range of different perennial trees including grapevine, banana, liana and eucalyptus.

Pressure Chamber

The Scholander pressure bomb (MRC, Israel) method was applied as described by Shackel et al. (1997). For determination of balancing pressure (stem water potential) values, leaflets were enclosed in darkened bags for at least two hours and allowed to equilibrate with the xylem water potential before measurements.

RESULTS

As observed for leaves of other species (Zimmermann et al., 2008, 2010; Westhoff et al., 2009) homogenous contact between leaf and probe was a prerequisite for turgor pressure monitoring on olive leaves. Probe clamping partly on the thick midrib of leaves led to erroneous results, because under these conditions, changes in the thickness of the midrib were measured. As expected, the initial \( P_{clamp} \) and, in turn, the \( P_p \) values and ranges had no effect on the diurnal \( P_p \) profiles of olive leaves. This was also proved by multiple probe readings on adjacent leaves exposed to the same microclimate (data not shown). Removal of the probes after 3 to 4 months showed no impressions or lesions. The adaxial and abaxial areas beneath the pads maintained the same colour as the surrounding tissue (Fig. 2). After longer clamping times, the patch area can become somewhat brighter indicating some slight decrease in chlorophyll content. This had no apparent effect on the hydraulic connection of the cells in the patch with the surrounding uncovered cells.

Probe versus Cell Turgor Pressure and Stem Water Potential Measurements

According to Eq. 1 the LPCP probe measures relative changes in leaf turgor pressure. This was proved by concomitant LPCP and cell turgor pressure probe measurements on leaves of various plant species (Fig. 3). It is evident that the relationship
between \( P_p \) and \( P_c \) could be described quite well by Eq. 1 for all plant species independent of the \( P_p \) range determined by the selected clamp pressure \( P_{clamp} \).

Figure 4A shows a typical diurnal profile of \( P_p \) measured on an olive leaf of a well-watered tree together with balancing pressure values \( P_b \) (corresponding to negative values of stem water potential) on a sunny day (air temperature and relative humidity at noon: \( T_a = 35^\circ C, \text{RH} = 26\% \)). It is evident that the \( P_b \) values measured on several cut groups of leaflets show a similar diurnal trend as the \( P_p \) values measured on a single leaf. The small delay in response of \( P_b \) after sunrise and subsequent onset of transpiration resulted most likely from the fact that the leaves for the \( P_b \) measurements were taken from the north side of the tree. Leaves at this side became sun-exposed much later than the leaves located on the east side.

Despite the good agreement between the changes in the \( P_b \) and \( P_p \) values, incidents of variable weather and times when irrigation was ceased showed that the resolution of the LPCP probe was finer than that of the stem water potential measurements. An example is given in Figure 4B for another well watered tree. Under hot and dry conditions and/or water stress, oscillations of stomatal aperture occurred when turgor pressure dropped. These oscillations were reflected in the \( P_p \) values. Olives, similar to other plants (Farquhar and Cowan, 1974; Zimmermann et al., 2010), apparently keep transpiration water loss to a minimum under water shortage at the expense of photosynthetic productivity.

**Drought Effects on LPCP Probe Readings**

Probe readings from periods in which olive trees were exposed to short-term extreme drought conditions caused by cessation of irrigation are depicted in Figure 5. Irrigation was stopped on 23 July and reinstated on 29 July. Full tree transpiration decreased incrementally daily from the first day without irrigation and was less than 20% of that of fully irrigated trees on 28 July. From the first day of suboptimal conditions of soil water (24 July), an increase in the peak \( P_p \) value was recorded at noon, indicating lower turgor pressures compared to trees with optimal soil moisture (full irrigation). The time of turgor pressure recovery during the afternoon increased as the period of drought continued and the stress conditions increased. After 2 days (July 25) tree-scale transpiration was 50% of well watered trees and the \( P_p \) value established during the night due to water uptake via the roots also increased significantly, indicating that night-time high turgor pressure was not restored. Interestingly, when severe stress was reached after 4 days of drought (30% of transpiration relative to well watered trees), a reversal in the diurnal \( P_p \) profiles was observed, i.e. \( P_p \) reached minimum values at noon and maximum values at night (see inset of Fig. 5). All the phenomena related to changes in turgor pressure due to drought stress were reversible and, following a single day of re-irrigation, diurnal \( P_p \) profiles of the previously stressed trees were identical to those measured before the drought period.

**DISCUSSION**

The data presented here demonstrate that the patch clamp pressure probe can provide sensitive online monitoring of effects of transpiration and/or water stress on olive tree leaf water status. Experimental evidence is given that the LPCP output pressure \( P_p \) is inversely correlated with turgor pressure and is correlated linearly with balancing pressure values \( P_b \) (stem water potential). This suggests that both the pressure bomb and the LPCP probe measure relative changes in turgor pressure. We further suggest that the probe allows the definition of reliable threshold values for triggering irrigation or for maintaining desired conditions of water stress. Possible indicators of water deficits in olive trees (and other plants) are the increase of the peak \( P_p \) values at noon and of the base \( P_p \) values at night as well as the increase of the time of turgor pressure recovery during the afternoon. The occurrence of \( P_p \) oscillations at severe turgor pressure losses is another important criterion for water deficits and particularly for fruit yield, because periodic closure of the stomata and, in turn, of \( \text{CO}_2 \) assimilation, will certainly affect yield.
Reversal of the diurnal changes of \( P_p \) as observed here for severely stressed olive leaves can be taken as evidence that the leaf cells were nearly turgorless and contained a large amount of air. Under such conditions the LPCP probe no longer sensed turgor pressure but rather changes in thickness were recorded (i.e. the attenuation factor \( F_a \) in Eq. 1 was extremely low during noon and higher at night). Interestingly, this dramatic change in the ratio of air to water in the leaf was not reflected in the \( P_p \) values (data not shown).

The determination of sub-optimum leaf water supply in real time opens up possibilities for water management. For irrigation monitoring calibration of the LPCP probes against the cell turgor pressure probe is not stringent. On the basis of the data depicted in Figure 5 it is sufficient to know the peak \( P_p \) values at noon and the minimum \( P_p \) values at night under appropriate irrigation conditions.

**ACKNOWLEDGEMENTS**

This work was supported by a grant from the AIF (no. KF 0054703WM8) to U. Z. We would like to thank P. Geßner, G. Zimmermann, A. Yafe and E. Presnov for their support of the field studies and E. Stepień-Böttsch for her great help in evaluation of the LPCP datasets.

**Literature Cited**


Figures

![Fig. 1. The magnetic leaf patch clamp pressure (LPCP) probe. (A) Photograph of a probe clamped on an olive leaf in the field; (B) schematic diagram of the measuring principle of the probe ($P_{clamp} =$ applied clamp pressure; $P_c =$ turgor pressure) and inset a schematic view of the lower part of the probe with the embedded sensor chip and silicone membrane.](image-url)
Fig. 2. Appearance of the adaxial (A) and abaxial (B) patches (marked by circles) of olive leaves upon removal of the probes after 3 months.

Fig. 3. Calibration of the output pressure, $P_p$, values measured by the LPCP probe against the cell turgor pressure, $P_t$, using the cell turgor pressure probe. Measurements were performed on banana plants (*Musa acuminate*: open squares), oak (*Quercus robur*: filled circles) eucalyptus trees (*Eucalyptus gomphocephala*: open triangles) and on grapevine (*Vitis vinifera*: filled triangles). Lines were fitted to data points by Eq. 1.
Fig. 4. (A) Concomitant measurements of output patch pressures, $P_p$ (solid line), and balancing pressure, $P_b$, values (using a Scholander pressure bomb) on a well watered olive (28 July, 2009). The $P_b$ values (circles) are averages, error bars are standard deviations, $n=5$. Note that $P_b$ values reflect negative values of stem water potential. (B) Typical $P_p$ oscillations of an olive leaf of a well watered tree measured by the LPCP probe (8 November, 2009).

Fig. 5. Part of a 4-month $P_p$ recording on an olive tree under field conditions at the Gilat Research Center, Israel. The tree shown was subjected to drought by ceasing irrigation on 23 July and was re-irrigated on 29 July, 2009. Note the reversal in the diurnal $P_p$ profiles after 3-days of non-irrigation: at this time $P_p$ assumes maximum values during the night and minimum values at noon (see the enlargement in the inset).