Leaf patch clamp pressure probe measurements on olive leaves in a nearly turgorless state


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ABSTRACT

The non-invasive leaf patch clamp pressure (LPCP) probe measures the attenuated pressure of a leaf patch, \( P_p \), in response to an externally applied magnetic force. \( P_p \) is inversely coupled with leaf turgor pressure, \( P_c \), i.e. at high \( P_c \) values the \( P_p \) values are small and at low \( P_c \) values the \( P_p \) values are high. This relationship between \( P_c \) and \( P_p \) could also be verified for 2-m tall olive trees under laboratory conditions using the cell turgor pressure probe. When the laboratory plants were subjected to severe water stress (\( P_c \) dropped below ca. 50 kPa), \( P_p \) curves show reverse diurnal changes, i.e. during the light regime (high transpiration) a minimum \( P_p \) value, and during darkness a peak \( P_p \) value is recorded. This reversal of the \( P_p \) curves was completely reversible. Upon watering, the original diurnal \( P_p \) changes were re-established within 2–3 days. Olive trees in the field showed a similar turnover of the shape of the \( P_p \) curves upon drought, despite pronounced fluctuations in microclimate. The reversal of the \( P_p \) curves is most likely due to accumulation of air in the leaves. This assumption was supported with cross-sections through leaves subjected to prolonged drought. In contrast to well-watered leaves, microscopic inspection of leaves exhibiting inverse diurnal \( P_p \) curves revealed large air-filled areas in parenchyma tissue. Significantly larger amounts of air could also be extracted from water-stressed leaves than from well-watered leaves using the cell turgor pressure probe. Furthermore, theoretical analysis of the experimental \( P_p \) curves shows that the propagation of pressure through the nearly turgorless leaf must be exclusively dictated by air. Equations are derived that provide valuable information about the water status of olive leaves close to zero \( P_c \).

INTRODUCTION

Irrigation is the largest consumer of water in (semi-) arid countries. At present it is very often more cost-efficient for a farmer to over-irrigate than to risk the crop being stressed either early or at a later stage. However, it is well known (see e.g. Möller et al. 2007; Netzer et al. 2009) that proper management of irrigation can result in enhanced productivity and/or quality. Furthermore, as pressure on available freshwater resources will increase dramatically in the future, farmers have to find ways of improving water use efficiency. Installation of sensors in the field, which measure the water demands of the crop or of fruit trees in real time over the entire vegetation period, provides a sustainable and therefore smart solution for reduction of water consumption. By using sensitive indicators, the effects of irrigation can be gauged, and thus optimised. Plant-based sensors that measure sap flow, diurnal changes in trunk diameter or leaf thickness, stem and leaf water potential, stomatal conductance, time domain reflectometry and/or canopy temperature have been suggested by several authors as feasible indicators for smart irrigation (Scholander et al. 1965; Boyer 1967; McBurney 1988; Cardon et al. 1994; Smith & Allen 1996; Burgess et al. 2000; Zweifel et al. 2000, 2001; Fernández et al. 2001, 2006; Goldhammer & Fereres 2001; Green et al. 2003; Nadler et al. 2003, 2006; Naor et al. 2008). Routine implementation of these techniques in crop fields, orchards and forests, however, failed for several practical and technical reasons (see Blank et al. 1995; Jones 2004).

There is convincing evidence that the non-invasive, Internet-based leaf patch clamp pressure (LPCP) probe recently introduced by Zimmermann et al. (2008) could meet the demands for precisely monitoring leaf water status of plants in real time. When clamped correctly, the probe measures relative changes in leaf turgor pressure, \( P_c \). The measuring principle of the high-tech probe is quite simple. An external pressure, \( P_{clamp} \) (generated by springs or – more elegantly – by magnets) is applied to a small patch of an intact leaf. The

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pressure experienced by the cells is lower than $P_{\text{clamp}}$ because of losses due to the compressibility and deformability of structural elements (such as the cuticle, cell walls and intercellular air spaces). The attenuation factor $F_a$ for olive leaves is usually of the order of 0.2–0.3 and is assumed to be constant in the turgescent range of the leaf. Theory shows that the output pressure, $P_p$, sensed by the probe is dominated by the turgor pressure, $P_c$, of the cells. Both parameters are inversely coupled to each other. If the counter-acting turgor pressure is high (e.g. at pre-dawn), pressure transfer through the tissue is considerably attenuated; therefore, the output signal, $P_p$, is low. Vice versa, if the turgor pressure is low (e.g. at noon), a high $P_p$ value is recorded. The prediction of the theoretically postulated power function relationship between $P_p$ and $P_c$ has been verified for many plant species, such as olive, grapefruit, grapevine, lianas, eucalypts, banana plants and oak trees, by concomitant $P_p$ and leaf cell turgor pressure measurements using the minimal-invasive cell turgor pressure probe (Zimmermann et al. 2008, 2009; Westhoff et al. 2009; Rüger et al. 2010a,b). These measurements were performed in a range of $P_c$ values between ca. 50 and 550 kPa.

Case studies on several crop and fruit trees have shown that the profile of the diurnal curves of the output signals of the probe change in a characteristic manner upon ongoing drought, reflecting the increasing difficulty to compensate turgor pressure losses by water uptake. Several parameters, such as the rise time of the $P_p$ values in the morning ($=P_c$ loss), the peak $P_p$ values at noon (=maximum $P_c$ loss), the decrease rate of the $P_p$ values during the afternoon (=recovery phase of $P_c$) and/or the $P_p$ values reached during the night (=maximum $P_p$) are affected by water stress and can be used as sensitive indicators for irrigation.

When $P_c$ drops below ca. 50 kPa, the $P_p$ peak values measured at noon increase dramatically, quite often exceeding the recommended measuring range of the probe (up to 250 kPa). This increase is expected in the light of the current theory and can be used (together with the other $P_p$ parameters) as a clear-cut indication for severe water stress. Interestingly, olive trees (Ben-Gal et al. 2010; Fernández et al. 2011) under field conditions show a reversal of the diurnal $P_p$ curves upon approaching the plasmolytic point, i.e. at noon minimum $P_p$ values were recorded, whereas peak $P_p$ values occurred during the night. The reversal of the $P_p$ curves was completely reversible, even after a long period of drought. Upon watering, the diurnal $P_p$ changes measured usually on leaves are re-established within a very short time. The reversal of the $P_p$ curves cannot explicitly be explained by the current theory, suggesting that the $P_p$ signal is exclusively affected by $P_c$ changes (Zimmermann et al. 2008; Westhoff et al. 2009; Rüger et al. 2010a,b).

In this communication we show, theoretically, that the reversal of the $P_p$ curves observed in olive trees can easily be explained by assuming that the attenuation factor, $F_a$, is no longer constant at $P_p$ values close to zero due to an unavourable ratio of air to water. Thus, at very low $P_p$ values, $F_a$ and not $P_p$ is the dominant factor that affects the $P_p$ signals. Fundamentals for the theoretical framework were diurnal $P_p$ curves that were measured on small olive trees. These trees were subjected to several irrigation/non-irrigation regimes under constant laboratory conditions in order to exclude any effects of environmental factors on the $P_p$ values.

### MATERIAL AND METHODS

#### Plants

Probe measurements were performed under laboratory conditions on ca. 2-m tall olive trees (*Olea europaea*) planted in ca. 30-l pots filled with soil. The trees were subjected to a 9.5-h light/14.5-h dark regime. Ambient temperature and relative humidity were kept constant at 23°C and 55%, respectively. The light irradiation was ca. 196 μmol s⁻¹ m⁻² at the top and ca. 55 μmol s⁻¹ m⁻¹ at 1-m height.

Field experiments were made in 2010, in a hedgerow olive orchard with 4-year-old ‘Arbequina’ trees, close to Seville (37°15′N, 5°48′W). The tree rows were oriented north to south. Spacing between the rows was 4 m and between the trees 1.5 m (1667 trees ha⁻¹). The trees were, on average, 2.40-m tall and 2.12-m wide canopy. Some of the trees were subjected to a regulated deficit irrigation (RDI) treatment. The 60RDI treatments were scaled to a total irrigation amount of 60% of the crop evapotranspiration (ETc) demand.

#### Leaf patch clamp pressure (LPCP) probe

The measuring principle of the non-invasive, online-monitoring LPCP probe (commercial name: ZIM-probe) is described in details elsewhere (Zimmermann et al. 2008, 2009; Westhoff et al. 2009). Briefly, a relatively small patch of a leaf is used as a sensing element for turgor pressure changes in the entire leaf. To this end, the stomata in the patch must be closed; simultaneously, the patch must be in hydraulic contact with its surroundings. This is achieved by positioning of an intact leaf between two planar circular metal pads integrated into two magnets. The lower pad contains a receptacle for integration of the pressure sensor chip. Leaf turgescence is determined by measuring the pressure transfer function of the leaf patch, i.e. by measuring the output leaf patch pressure, $P_p$, upon application of a constantly maintained external clamp pressure, $P_{\text{clamp}}$ (up to 400 kPa). $P_{\text{clamp}}$ can be varied by changing the distance between the upper and lower magnet.

Probes, together with the components for telemetric and mobile network-based data transfer to the Internet, were purchased from the company ZIM Plant Technology GmbH (Henningsdorf, Germany). Real-time recording of the ZIM-probe data was provided by battery-powered telemetric transmitters, which were connected by cable to up to three probes. These transmitters sent wireless data together with the transmitter ID-code every 5 min *via* ISM (433 MHz) to a control station that logged and transferred the data with time stamps to a GPRS (*General Packet Radio Service*) modem linked to an Internet server, which provides the data in real time in chart and table form.

For proper function of the LPCP probe it is necessary that there is a homogeneous contact between the leaf patch and the pads of the two magnets. Only under these conditions can the pressure transfer function and thus turgor pressure be measured (see below). In the case of an inhomogeneous contact (e.g. point contacts), the probe is measuring changes in leaf thickness, which result in $P_p$ changes that are opposite to those induced by changes in turgor pressure. Changes in leaf thickness of plants subjected to water stress are much smaller than turgor pressure induced changes.
Cell turgor pressure probe

The principle of the cell turgor pressure probe is described in detail elsewhere (Teyrman et al. 2004; Zimmermann et al. 2004; Bramley et al. 2007). The probe was inserted from the adaxial or abaxial side of the leaves into the parenchyma cells close to the midrib. The probe was inserted most likely into the spongy tissue because of the penetration depth of the microcapillary. Adaxial and abaxial measurements yielded similar results; therefore, the data were pooled. The cell turgor pressure probe was also used for the extraction of air from the leaves. To this end, the microcapillary filled with oil up to the very tip (under very small overpressure) was introduced perpendicular to the leaf surface. After release of tiny amounts of oil into the tissue, air – if present – could enter the tip.

Microscopy

Small fragments of leaf (2 mm²) were fixed in 4% glutaraldehyde dissolved in 0.1 M cacodylate buffer, pH 7.2, for 3 h at 4°C and were post-fixed in 1% OsO₄ solution for 2 h at 4°C. Samples were dehydrated in a grade acetone series and embedded in Epon-812 (epoxy embedding medium; Sigma-Aldrich, St. Louis, MI, USA). Toluidine blue-stained semi-thin sections (0.5 μm) were viewed using a Leitz Aristoplan (Leica Mikroskopie and Systeme Gmbh, Wetzlar, Germany) light microscope.

Morphometric analysis

Forty cross-sections per group of treatments were investigated. The images were made with a digital camera (Leica DC-100, Leica Imaging Systems Ltd., Cambridge, England) and were analysed using a Leica Q-win program. Data were statistically evaluated with the Student-Newman-Keuls test. A statistically significant difference was considered when P ≤ 0.0001. Average data are given as mean ± standard error.

RESULTS

Experimental

Figure 1 shows part of a typical, long-term probe measurement on an olive tree subjected to cycles of irrigation/non-irrigation. About 4 days after stoppage of watering, the peak $P_p$ values, which were reached at the end of the light phase (05:00 h), increased continuously over the following days, indicating continuous turgor pressure, $P_o$, loss. After some days, the increase in the peak $P_p$ values was also accompanied by an increase of the $P_p$ values during the dark phase. The original $P_p$ values were obviously not restored during the dark phase, because of a water shortage. This was also reflected in an increase in the time needed for $P_p$ recovery in the afternoon and at night (see enlargement in Fig. 2A and B). The turgor pressure recovery process starting in the afternoon could be approximated by an exponential function. The time constant, $\tau$, of the exponential decreases of the $P_p$ values increased from 54 min (just after watering) to 256 min, when the peak $P_p$ value reached a maximum of ca. 95 kPa at the end of the light phase after 7 days (see the filled squares in Fig. 3). The increase of $\tau$ with ongoing non-irrigation could be fitted by an exponential function characterised by a time constant of 2.6 days.

Cell turgor pressure probe measurements showed the well-known inverse relationship between $P_p$ and $P_o$, taking into account the difficulties of $P_p$ measurements on olive leaves. Inspection of the calibration curve in Fig. 4 reveals that a value of $P_p = 95$ kPa corresponded to a $P_o$ value of ca. 50 kPa, providing evidence that the leaf cells were still turgid.

Cell turgor pressure probe measurements also reflected in an increase in the time needed for $P_p$ recovery after non-irrigation. This state was characterised by a continuous increase of the $P_p$ values during the dark phase (non-transpiration) and a continuous decrease of the $P_p$ values during the light phase (transpiration).

On day 7 after stoppage of watering, the $P_p$ profile changed dramatically (Figs 1 and 2C). After switching on the light, the peak $P_p$ value of 95 kPa was reached very rapidly compared to state I (3 h versus 11 h; Figs 1 and 2) and then decreased until at the end of the light phase a minimum value was reached. In the following dark hours the $P_p$ values increased again. A peak value was reached at midnight, then again a decrease of the $P_p$ values was observed until the light was switched on at 07:30 h. This diurnal curve shape of the $P_p$ values was also recorded qualitatively on the following day. Measurements on the next day showed (Fig. 2D) that this state was intermediate (termed state II), because the diurnal $P_p$ values overturned into a stable state III measured also in the following days of non-irrigation. This state was characterised by a continuous increase of the $P_p$ values during the dark phase (non-transpiration) and a continuous decrease of the $P_p$ values during the light phase (transpiration). Both curves could be approximated by exponential functions. The time constant, $\tau$, in dependency of the time after reaching state III, are given in Fig. 3 for the decrease of the $P_p$ values during the light phase and for the increase of the $P_p$ values during the dark phase (open circles and filled triangles, respectively). It is obvious from the figure that the $\tau$ values of the dark phase in state III reached values of up to

![Fig. 1. Leaf patch clamp pressure probe measurements on a 2-m tall olive tree subjected to irrigation/non-irrigation cycles under well-defined laboratory conditions. The figure shows part of a 2-month recording of the output patch pressure, $P_p$. Irrigation was stopped several times (marked by white areas above the panel). Note that ongoing non-irrigation resulted in a dramatic increase of the $P_p$ value during the dark phase and of the peak $P_p$ value during the light phase after 4 days. Note further reversal in the diurnal $P_p$ profiles after 7 days of non-irrigation. At this time, $P_p$ takes maximum values during the dark phase and minimum values during the light phase. Upon irrigation, the original $P_p$ diurnal profiles are restored and measured again.](https://example.com/figure1.png)
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Fig. 2. Pressure transfer through the leaf patch under irrigation and non-irrigation conditions as predicted from equation (1). State I (turgescence state; upper graphs): $P_p$ is inversely coupled to $P_c$ ($P_p = f(1/P_c)$); the attenuation factor $F_{a,\text{corr}}$ related to turgor pressure-independent structural elements (such as cuticle, cell walls and air-filled spaces) is practically constant. Thus, the magnitude of $P_p$ peaking at noon and the night $P_p$ values depends exclusively on $P_c$ (A: well-watered leaf; B: leaf subjected to 4-day drought). State II (very low turgor pressure values; C): $F_a$ is no longer constant because of an unfavourable ratio of air to water in the leaf. Thus, $P_p$ becomes a linear function of $F_{a,\text{corr}}$, see equation (6), but still depends on $P_c$, to some extent ($P_p = f(F_{a,\text{corr}}, 1/P_c)$). State III (turgor pressure values close to zero): $P_p$ depends exclusively on $F_a$ ($P_p = f(F_{a,\text{corr}})$) which assumes a minimum value during light phase (=large air spaces; maximum damping of pressure transfer) and a maximum value during dark phase (decrease of the air spaces by some water uptake and/or by a decrease in temperature: improvement of pressure transfer; D). Subsequent irrigation (arrow in E) resulted in an instant increase of the $P_p$ values, followed by $P_p$ decreasing during the dark phase and then by $P_p$ peaking during the light phase (state I) on the following day (E). The amplitude of the $P_p$ peaks decreased in the following 2 days, reaching the value measured under well-watered conditions (compare F with A).

600 min, whereas the $\tau$ values during the light phase were in the range measured for the $\tau$ values recorded during the turgor pressure recovery phase in state I. Subsequent irrigation (see arrow in Fig. 2E) resulted in an instant increase of the $P_p$ values, followed by $P_p$ decreasing during the dark phase and then peaking during the light phase on the following day. Peaking was nearly as high as observed the day before the overturning phenomena had started (compare Fig. 2E with 2B). After a further 2 days, the amplitude of the peak $P_p$ values during the light phase was comparable to that measured on the well-irrigated plants (Fig. 2F versus 2A). Similarly, the $\tau$ values decreased accordingly after re-watering and reached the original values of well-watered plants after 3 days (Fig. 3).

The phenomena described above were completely reversible, as shown by several irrigation/non-irrigation cycles, and were also found for different olive trees under field and laboratory conditions. It is also worthwhile to note that the reversal of the diurnal $P_p$ curves upon severe drought could also be observed under field conditions; an example is given in Fig. 5. Whereas the leaves of the control trees remained in state I (Fig. 5A) over the summer period, trees that received only 60% of the total irrigation amount most of the time exhibited diurnal changes of $P_p$ related to state II and state III, respectively (Fig. 5B).

Inspection of cross-sections of leaves subjected to severe drought, i.e. of leaves showing inverse diurnal $P_p$ changes, had much larger areas of air spaces in the spongy mesophyll compared to well-watered leaves (0.5496 ± 0.012 mm$^2$ versus 0.399 ± 0.008 mm$^2$; Fig. 6). This finding was supported by increased extraction of air from the leaves using the cell turgor pressure probe (a few microliters versus <1 µl in well-watered leaves).

Changes in leaf thickness as a possible reason for the reversal of the $P_p$ curves in response to ongoing drought could be excluded. Leaf thickness measurements on well-watered leaves showed $P_p$ curves that were opposite to those depicted in Figs 1 and 2. With ongoing drought, the $P_p$ peaks decreased continuously towards their disappearance (data not shown).

Theoretics

From a thermodynamic standpoint, the leaf patch can be considered as a black box consisting of turgescence cells and turgor-independent compressible structural elements, such as the cuticle, cell walls and air spaces. The output patch pressure $P_p$ sensed by the sensor chip upon application of an external clamp pressure, $P_{\text{clamps}}$, is only determined by the leaf transfer function, $T_r(V)$, where $V$ is the leaf patch volume (Zimmermann et al. 2008; Westhoff et al. 2009):
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\[ P_p = T_f(V) \cdot F_{a,\text{const}} \cdot P_{\text{clamp}} \]  \hspace{1cm} (1)

where \( F_{a,\text{const}} \) is the leaf-specific attenuation factor that takes into account that only a fraction of \( P_{\text{clamp}} \) may arrive at the cell level due to \( P_c \)-independent pressure losses arising from the compressibility of the silicone used for the embedding of the sensor chip into the magnets and the leaf-specific structural elements.

\[ T_f \] depends on the cellular volume of the leaf patch \( V \), which, in turn, depends on \( P_c \). The magnitude of volume changes upon changes in \( P_c \) is dictated by the average volumetric elastic modulus of the cells of the tissues, \( \varepsilon_p \) (Philip 1958):

\[ \frac{\partial P_c}{\partial V} = \varepsilon_p \]  \hspace{1cm} (2)

\( \varepsilon_p \) is a function of \( P_c \) and is given by equation (3) at constant temperature, \( T \) (Murphy & Ortega 1995):

\[ \varepsilon_p = \varepsilon_0 - (\varepsilon_0 - \varepsilon_\infty) \cdot e^{-kP_c} \]  \hspace{1cm} (3)

where \( k \) is a constant, \( \varepsilon_0 \) and \( \varepsilon_\infty \) are the volumetric elastic moduli at \( P_c \approx 0 \) and \( P_c \approx \infty \), respectively. According to equation (3), \( \varepsilon_p \) reaches a plateau value for large \( P_c \) values. For smaller \( P_c \) values, \( i.e. P_c < 1/k \), \( \varepsilon_p \) can be approximated by a linear dependency on \( P_c \):

\[ \varepsilon_p = aP_c + b \]  \hspace{1cm} (4)

where \( a \approx k (\varepsilon_\infty - \varepsilon_0) \) and \( b = \varepsilon_\infty \). Both constants are equal or larger than unity.

Combination of equation (1) with equation (2) and equation (4) yields:

\[ P_p = \left( \frac{b}{aP_c + b} \right)^{1/2} F_{a,\text{const}} \cdot P_{\text{clamp}} \]  \hspace{1cm} (5)

Equation 5 demonstrates that \( P_p \) is a power function of \( P_c \). This means that \( P_p \) increases more or less linearly with decreasing \( P_c \) over a large range of turgor pressures. However, at very low \( P_c \) (ca. \( <100 \text kPa} \) values, \( P_p \) increases over-proportional with a further decrease in \( P_p \) provided that the attenuation factor can still be assumed to be constant. Equation (5) describes state I quite well, as shown by fitting of the data in Fig. 4 using appropriate values for the elastic constants \( a \) and \( b \). For \( P_c \approx 0 \), equation (5) becomes equation (6):

\[ P_p = F_{a,P_c=0} \cdot P_{\text{clamp}} \]  \hspace{1cm} (6)

In order to explain the experimental results, we have to assume that the attenuation factor in equation (6), \( F_{a,P_c=0} \), is no longer constant and becomes a function of time, \( t \), around \( P_c \approx 0 \). The most likely reason for this is the diurnal variable accumulation of air in the leaf, as found experimentally (see Fig. 6). Thus, in the light of equation (6), we are driven to the conclusion that \( P_p \) becomes a linear function of this parameter because there is no physical reason to assume that \( P_{\text{clamp}} \) is changing upon approaching \( P_c = 0 \).

State II (Fig. 2C) obviously reflects the transient pressure range below \( ca. 50 \text kPa} \), where changes in \( F_{a,P_c=0} \) start to contribute to \( P_p \), thus partly compensating for the inverse effect of \( P_c \) on \( P_p \).

Inspection of the \( P_p \) curves of state III (Fig. 2D) shows that the increase of \( P_p \) during the dark regime and the decrease of \( P_p \) during the light regime can be approximated very well by assuming an exponential change of \( F_{a,P_c=0} \) with time. The mathematical analysis yields, for the increase of \( P_p \) during the dark regime:

\[ F_{a,P_c=0} = (F_{a,\text{max}} - F_{a,\text{min}}) \cdot (1 - e^{at}) + F_{a,\text{min}} \]  \hspace{1cm} (7)

and for the decrease of \( P_p \) during the light regime:

\[ F_{a,P_c=0} = (F_{a,\text{max}} - F_{a,\text{min}}) \cdot (e^{at}) + F_{a,\text{min}} \]  \hspace{1cm} (8)

where \( F_{a,\text{max}} \) and \( F_{a,\text{min}} \) correspond to the maximum and minimum \( P_p \) values, respectively, and \( a_t \) and \( a_d \) are the time constants of the corresponding exponential functions. In light of the experimental results, there are some good reasons to assume that \( F_{a,\text{max}} \) is equal or very similar to \( F_{a,\text{const}} \).

Figure 7A represents the theoretically expected change of \( F_{a,P_c=0} \) with time using equations (7) and (8), respectively, and Fig. 7B is the correlation between the \( P_p \) values and the corresponding \( F_{a,P_c=0} \) values of Fig. 7A. Inspection of Fig. 7B shows that a linear correlation exists between these two parameters, as expected in light of equation (6).

**DISCUSSION**

Direct turgor pressure measurements on olive leaves using the cell turgor pressure probe have verified (Fig. 4) that the patch pressure \( P_p \) measured with the LPCC probe is inversely coupled to \( P_c \) over a large \( P_c \) range, as predicted by equation (5). This was also found for other plant species (see literature quoted above). In the \( P_c \) range where equation (5) holds (termed state I; Figs 1 and 2), \( P_p \) peaking occurs during the

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**Fig. 4.** Calibration of the leaf patch clamp pressure, \( P_p \), measured in state I through short-term measurements of cell turgor pressure, \( P_c \). Each \( P_c \) data point is an average turgor pressure value (±SD) taken from a 2- to 5-min measurement; the corresponding \( P_p \) values represent the mean (±SD) of at least three measuring points. Data were fitted using equation (5), with \( F_{a,\text{const}} = 0.29 \), \( P_{\text{clamp}} = 398 \text kPa} \), \( a = 1.0 \), \( b = 244 \text kPa} \). \( R^2 = 0.87 \). The dependency of \( P_p \) on \( P_c \) was found for more than 30 cells measured on different days.
light phase (transpiration) and the minimum $P_p$ values are recorded during the dark phase (non-transpiration).

We have demonstrated here for olive leaves that a reversal of the $P_p$ curves occurred towards low turgor pressure values. The direct turgor pressure measurements have shown that the reversal of the diurnal $P_p$ curves of olive leaves started below a turgor pressure, $P_c$, of about 50 kPa (state III). The transition from $P_p$ peaking during the light phase (state I) to $P_p$ peaking during the dark phase (state III) took place within 2–3 days (state II) under laboratory conditions. The reversal of the $P_p$ curves was completely reversible after re-watering and was also found for olive trees under field conditions (Fig. 5). Measurements of the pressure transfer function, and thus of $P_c$, require a uniform contact between the leaf patch and the pads of the magnetic probe. In the case of a non-uniform contact, mainly transpiration-induced changes in leaf thickness are recorded (Westhoff et al. 2009). Leaf thickness

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**Fig. 5.** Part of a long-term measurement of diurnal changes of $P_p$ measured on east-oriented leaves of a control tree (A) and a 60RDI tree (B; RDI = regulated deficient irrigation) under field conditions. Irrigation amounts (IA) are denoted in grey bars; nocturnal hours are marked as grey columns. C: Ambient temperature ($T$; solid line) and relative humidity (RH; dotted line). D: 30-min averages of solar global radiation ($L_o$). Note that below the curves the state of turgescence of the leaves is given: state I = turgor pressures $>\text{ca. 50 kPa}$ ($P_p$ peaking at noon, minimum $P_p$ values during the night), state II = turgor pressures $<\text{ca. 50 kPa}$ (half inverse state: second peaking in late afternoon) and state III = very low turgor pressures (inverse state: minimum $P_p$ values at noon and maximum values during the night). For further details, see text.

**Fig. 6.** Typical images of cross-sections of olive leaves under well-watered conditions (A; state I) and severe water stress (B; state III); bar = 100 μm. (1) Lower epidermis, (2) spongy mesophyll, (3) air space, (4) palisade mesophyll, (5) upper epidermis, (6) cuticle.
assumes minimum values at high transpiration and vice versa maximum values at non-transpiration. Therefore, one possible explanation for the low-turgor pressure reversal of the $P_p$ curves is that the probe is measuring changes in leaf thickness upon approaching $P_c \approx 0$ rather than the pressure transfer function of the leaf (equation 1). However, this explanation is very unlikely. Changes in leaf thickness are only expected to increase then significantly with ongoing non-irrigation.

The increase of $\tau$ can be described by an exponential function with a time constant of 2.6 days. Extrapolation of the exponential curve of state I to state III shows (see Fig. 3) that the $\tau$ values of the $P_p$ increase recorded during the dark phase (but not the $\tau$ values of the $P_p$ decrease recorded during the light phase) can be fitted by the same function. This suggests that the $P_p$ increase phase reflects the phase of some turgor pressure regeneration close to $P_c \approx 0$. The build up of turgor pressure is obviously superimposed by a second, dominating process in the opposite direction.

This process is most likely initiated by the air in the leaves. Due to its high compressibility, air attenuates the pressure transfer through the leaf. Transpiring leaves will generally contain larger air spaces than non-transpiring ones (due to water uptake, and under field conditions due to lower temperatures during the night). Thus, attenuation of the external magnetic pressure will, in principle, be larger in transpiring plants than in non-transpiring ones. When the water supply of the leaves is sufficient and, in turn, the turgor pressure is quite high, the diurnal changes in the air amount in the leaves will be negligible. Thus, it is justified (and was verified experimentally) to assume that the attenuation factor, $F_a$, which takes – among other things – mainly pressure losses by compression of air spaces into account, is constant for a first and good approximation. However, the total amount of air within the leaf tissue is apparently increased dramatically with decreasing $P_c$ below ca. 50 kPa. Support for this assumption was obtained from analysis of cross-sections through well-watered leaves and leaves subjected to severe drought. As shown in Fig. 6, the volume occupied by air increased considerably in leaves exhibiting inverse $P_p$ curves (state III) compared to turgescent leaves (state I). Increased amounts of air were also found through extraction of air by using the cell turgor pressure probe.
As already mentioned above in the theoretical section, these findings and observations lead to the conclusion that the attenuation factor, \( F_a \), can no longer be assumed to be constant at low \( P_c \) values. Since the contribution of \( P_t \) to the \( P_p \) signals is practically negligible [see the denominator of equation (5) and set \( P_t = 0 \)], \( P_p \) becomes exclusively a linear function of \( F_a P_{a,0} \). When \( state \ III \) is reached [see equation (6) and Fig. 7B], Theory and the experiments show consistently (equations (7), (8) and Fig. 7A) that \( F_a P_{a,0} \) changes exponentially with time. \( F_a P_{a,0} \) reaches a maximum value (minimum air-related losses of the external magnetic pressure) during the dark phase and a minimum value (minimum air-related losses of the external magnetic pressure) during the light phase. The maximum \( F_a P_{a,0} \) value of 0.26 reached at the end of the dark phase corresponds quite well with the \( F_a \) value determined for \( P_t > ca. \) 50 kPa, supporting the view that during the dark phase some turgor pressure is built up by water uptake through the roots or by water movement within the plants.

Taken together, the above considerations demonstrate that the turgor pressure information that can be deduced from LPCP probe measurements is not restricted to the normal turgor pressure range. Rather, the theory shows that valuable information about the water supply to the leaves can also be extracted from measurements at extremely low turgor pressures. The surprising finding that the \( P_r \) reversal phenomenon was completely reversible after re-wetting suggests that the air spaces play an important role in the water supply of olive leaves under severe water stress. It is well-known (see the review article of Zimmermann et al. 2004) that air spaces can create interfacial water flow (termed Marangoni streaming) through which water can still be shifted effectively to the leaf cells, even if the xylem is interrupted by gas bubbles due to cavitation. Future experiments must elucidate the proposed role of air spaces in more detail. Nevertheless, for agricultural water management, it is sufficient to point out that the \( P_r \) reversal phenomenon can be used as a powerful indicator for determination of the water stress state of olive trees.

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REFERENCES


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