PROCEEDINGS OF THE IX\textsuperscript{th} INTERNATIONAL WORKSHOP ON SAP FLOW

Convener

K. Steppe

Ghent, Belgium

June 4-7, 2013

ISHS Commission Irrigation and Plant Water Relations
ISHS Working Group Sap Flow

Acta Horticulturae 991
May 2013
Advanced Plant-Based, Internet-Sensor Technology Gives New Insights into Hydraulic Plant Functioning


ZIM Plant Technology GmbH, Germany

The UWA Institute of Agriculture, The University of Western Australia, Australia

Lehrstuhl für Biotechnologie, Universität Würzburg, Germany

Swiss Federal Institute for Forest, Snow and Landscape Research, Switzerland

Forest Protection, Forest Research Institute (SCION), New Zealand

Keywords: turgor pressure, water stress, plant hydraulics, ZIM-probe, patch pressure, water relations, drought

Abstract

Relative changes of turgor pressure of leafy higher plants and trees can be measured by the non-invasive leaf patch clamp pressure probe (so-called ZIM-probe) with high sensitivity. The ZIM-probe is characterised by operating convenience and robustness and, therefore, allows long-term measurements under field conditions. Probe data are sent by wireless telemetry and mobile network to an internet server from which the data can be downloaded, viewed and analysed in real time. Drought effects on the water supply of leaves can clearly be distinguished from those induced by climate factors (such as temperature or light irradiation). The temporal and spatial dynamics of plant water relations can be revealed on the scale of a single leaf but also at the whole plant (tree) level by simultaneous multiple probe readings at different heights and orientations. Furthermore, concomitant ZIM-probe, dendrometer and sap flow measurements have given intriguing insight into the complex non-linear sap flow-leaf turgor pressure pattern within tree canopies indicating possible roles of water storage compartments and complex adaptations to drought in maintaining water supply of leaves.

INTRODUCTION

Increasing worldwide shortages of fresh water, the continuous increase in water consumption by agriculture due to climate change, the increased demand for crop production to feed the world’s increasing population and the progressive salinization of arable land provoked by suboptimal irrigation are global concerns. These problems have highlighted the need for sensor technology for monitoring the temporal and spatial water needs of crops. There are currently numerous techniques available for measuring water status in plants such as the pressure chamber, psychrometers, sap flow techniques and stem dendrometers, as well as infrared thermometers for measuring canopy and/or leaf temperature (Wullschleger et al., 1998; Fernández et al., 2001; Zweifel et al., 2001, 2007; Steppe et al., 2006; Čermák et al., 2007). Even though these techniques are very useful for studies of water relations of plants, some practical difficulties of implementation have prevented routine field applications so far. As shown by many studies on various plant species under laboratory, greenhouse and field conditions (Zimmermann et al., 2008, 2010; Westhoff et al., 2009; Rüger et al., 2010a; Fernández et al., 2011; Lee et al., 2012; Barragán et al., 2012; Bramley et al., 2012; Raveh, 2012) the non-invasive magnetic leaf patch clamp pressure probe (ZIM-probe) is apparently an efficient sensor system for studying the dynamics of the water relations and drought stress physiology of plant species including Arabidopsis, cereals, fruit- and forest trees. Changes in leaf water status can be determined in real time and with high precision over long periods of time and is thus the proactive response to water shortage.

MATERIALS AND METHODS

The ZIM-probe technique measures the pressure transfer function through a patch...
of an intact leaf, i.e., the attenuation of an externally applied clamp pressure by the leaf tissue. The clamp pressure is generated by small magnets. High turgor pressure prevents pressure transfer through the leaf and, in turn, the patch pressure, $P_p$, measured by the probe is small. At very low turgor pressure, the applied pressure transferred to the pressure sensor is greatest and the patch pressure assumes a maximum value. Turgor pressure and patch pressure are hence, inversely correlated with each other (for details, see Zimmermann et al., 2008).

Data are sent wireless by transmitters to a GPRS modem which is linked to an internet server via mobile phone network. The plant signals are stored on a data bank and can be downloaded by smartphones, tablets or laptops. If desired, probes for measuring ambient temperature, relative humidity and light irradiation close to the plant, as well as soil temperature and moisture, can also be connected to the transmitters thus giving a complete view about the microclimate and the soil properties.

RESULTS AND DISCUSSION

Environmental Effects on Leaf Turgor Pressure

Concomitant measurements on many plant species using the pressure chamber and the ZIM-probes showed that both techniques yield in principle the same results despite of the frequent high value variability of the pressure chamber data under field conditions (see e.g., Rüger et al., 2010b; Bramley et al., 2012). Several reports have shown (Westhoff et al., 2009; Zimmermann et al., 2010) that calibration of both technologies against the cell turgor pressure probe yielded a clear correlation of the ZIM-probe and pressure chamber data with cell turgor pressure. This can be taken as evidence that both techniques measure relative changes of turgor pressure (or leaf water potential if the osmotic pressure remains constant).

ZIM-probes are very responsive to changes of turgor pressure induced by changes in the environment. Figure 1A shows the $P_p$ response of a wheat leaf (under growth chamber conditions) upon an increase in temperature, $T$, in the dark followed by illumination. $P_p$ increased with $T$ (see also Feller, 2006), but more rapidly upon illumination. Pressure chamber values showed a similar increase with $T$ as the patch pressure values (Fig. 1B) indicating that the effects recorded by the ZIM-probe resulted from an increase in transpiration and dehydration of the leaf, but not from temperature effects on the ZIM-probe. The ZIM-probe is almost temperature-independent as shown by control experiments (1-3 kPa over a T-range of 30°C and see Bramley et al., 2012).

Effects of Drought on Leaf Turgor Pressure

ZIM-probes are also highly sensitive to relative changes in leaf turgor pressure in response to soil moisture levels. Effects of watering and non-watering can clearly be distinguished from microclimate effects. Most plant species (cereals, fruit trees, etc.) show a very similar response in $P_p$, and thus, turgor pressure when watering is stopped. Examples of typical $P_p$ readings are given in Figure 2 for a 5-m tall almond tree. It is obvious that with progressing drought the amplitude of the $P_p$ peaks at noon continuously increases. Concomitantly, but sometimes a few days delayed, the $P_p$ night values also increase, indicating insufficient rehydration and recovery of the original turgor pressure during the night. Decrease of ambient temperature during drought can slow down the increase of the noon and night $P_p$ values. If water is still available in the soil and the water pathways in the plant are intact, temporary decreases of temperature can also lead to partial recovery of the original $P_p$ peak and night values. However, the overall trend of increasing $P_p$ peak amplitudes at noon and $P_p$ night values will continue with drought. Frequently, a dramatic increase of the noon and night $P_p$ values is observed when the turgor pressure drops below ca. 100 kPa (as revealed by calibration of the ZIM-probe against the microcapillary cell turgor pressure probe, see Ehrenberger et al., 2012a). A plateau value is reached soon and the plant dies if not watered.

Some species, e.g., almond, eucalyptus, olive, avocado and orange trees, exhibit a
change in the shape of the \( P_p \) curves (Fig. 2). This is the case when turgor pressure values approach ca. 50 kPa (Ehrenberger et al., 2012a); \( P_p \) assumes minimum values during the day and maximum values during the night. The reason for this is most likely a very unfavourable water to air ratio within the leaves (for details, see Ehrenberger et al., 2012a). At turgor pressure values close to zero the compression of the air-filled spaces mostly determines the response of the probe (Ehrenberger et al., 2012a). The transition from the turgescent \( P_p \) profiles (so-called state I) into inverse \( P_p \) profiles (state III) can occur over several days and is characterised by half-inversed \( P_p \) curves (Fig. 2, state II). Olives and some eucalyptus are very drought-tolerant and can remain in state III for more than two months. For olives, maintenance in state III occurs at the expense of yield and fruit size (Fernández et al., 2011; unpublished data). Irrigation or rain leads rapidly to the re-establishment of the turgescent state I. By contrast, almonds and the other plant species mentioned above have to be watered immediately upon occurrence of half-inverse \( P_p \) curves because the plant is irreversibly damaged if it reaches state III. Thus, the ultimate threshold for irrigation is the occurrence of state II. When this procedure is followed, water can be saved by up to 40%.

When many plant species experience mild water stress they respond by periodically opening and closing their stomata (e.g., olives, orange, wheat, etc.). This is reflected in corresponding \( P_p \) and, thus, in turgor pressure changes. An example is given in Figure 3 for orange trees treated with low salinity water. Avoidance of stomatal oscillations by appropriate irrigation scheduling can increase fruit yield by up to 30%, as shown for grapevine (unpublished data).

**Dynamics of Tree Water Relations**

Multiple probe readings on trees can give deep insights into the temporal and spatial water supply of the trees. Typical measurements on a 5-m tall, well-watered avocado tree, in Australia, are shown in Figure 4. Characteristic of the southern hemisphere, north-facing leaves experience longer and more intense sun exposure and should hence encounter drought stress more rapidly than south-facing leaves. After sunrise, a rapid decline in turgor pressure (corresponding to an increase in \( P_p \)) is observed on the tree’s east side. A high \( P_p \) plateau value, corresponding to low turgor pressure, is reached within about 2 hour. On the north side, but also on south side (much less exposed to sunshine) the \( P_p \) values increase much more slowly, but almost in parallel in order to reach peak values at around 13:50. Water loss on the tree’s western side occurs very slowly and increases only significantly in the afternoon, when the leaves become exposed to the sun. However, subsequent turgor pressure recovery in the western side occurs much faster than in the other sides of the tree.

Clamping of multiple probes over the entire height yields information about cavitation or embolism because water supply will be reduced and is reflected immediately in probe readings (unpublished results). Interruption of water pathways due to drought and, in turn, insufficient supply of the leaves can also be detected by concomitant ZIM-probe and dendrometer measurements. A typical measurement performed on 1.5-m tall, oak trees is given in Figure 5, showing the daily course of leaf turgor changes and water-dependent stem shrinkage (see also Ehrenberger et al., 2012b). In well-watered oak trees, the time between \( P_p \) peaking (minimum turgor pressure) and minimum trunk diameter is about 2 h, but increases up to 9 h when the trees were subject to drought for 20 days.

Concomitant trunk sap flow and ZIM-probe measurements can give further intriguing insights into the complex non-linear water-flow pattern within trees. For example, measurements on 2.4-m high olive trees showed (Rodriguez-Dominguez et al., 2012) that sap flow was usually positively correlated with \( P_p \) values. However, depending on watering and weather conditions, the onset of sap flow after sunrise could be significantly delayed compared to the immediate transpiration-induced changes in \( P_p \). Furthermore, peak sap flow did not always coincide with peak \( P_p \). In drought stressed trees, the transition of \( P_p \) curves from the turgescent state I into the inverse state III was not reflected in sap flow, which decreases continuously with proceeding drought. The
results strongly suggested that short-distances forces and local water storage reservoirs play an important role for leaf water supply (see Meinzer et al., 2001; Zimmermann et al., 2004). Concomitant ZIM-probe and sap flow measurements on a 5.5-m *Banksia menziesii* tree performed during the hot and dry West Australian summer, support this view (Bader et al., manuscript submitted). As observed for other trees (Fig. 2), the drought stress symptoms were reflected in increasing daily \( P_p \) amplitudes and incomplete turgor recovery at night (upward baseline shift). *Banksia menziesii* trees did not show an inversion of the diurnal \( P_p \) curves under severe drought stress. Multiple ZIM-probe recordings across a *Banksia menziesii* tree crown revealed an opposing signal pattern between opposite cardinal directions (Fig. 6). Abating drought stress in the north was paralleled by a deterioration in the south suggesting that during hot rainless periods water is reallocated from the least drought-affected southern crown section to relief drought stress symptoms in the most severely drought-affected northern part of the crown (see also Fig. 4). However, peak sap flow measured in the trunk about 1.3 m above the ground remained largely unaffected during these days, but returned much more slowly to zero nighttime values after sunset. This hysteresis is obviously indicative of incipient drought stress.

**CONCLUSIONS**

In conclusion, ZIM-probes can be employed for many different applications, such as when to irrigate crops, to investigating mechanisms of drought adaptation and survival in forest trees. Concomitant multiple ZIM-probe readings combined with other technology such as dendrometer and sap flow reveal dynamic and localized responses within canopies of individual trees, demonstrating the complexities of the hydraulic network in maintaining the water balance of tall trees.

**ACKNOWLEDGEMENTS**

The work presented here was supported by the Group of Eight Australia - DAAD German Research Cooperation Scheme to KS and ZIM Plant Technology (Project-ID: 54387088) and by a grant of the Europäischer Fonds für regionale Entwicklung (EFRE 80145650; State Brandenburg) to ZIM Plant Technology GmbH. Funding for HB and research costs were provided by the UWA Institute of Agriculture and research facilities by CSIRO Plant Industry as part of the UWA/CSIRO PI partnership. The ZIM-probe measurements on almonds were performed in the orchard of Andres Hasbún, Agricola Prodalmen Limitada, Chile. The authors are very grateful to him for his interest and cooperation.

**Literature Cited**


Ehrenberger, W., Rüger, S., Fitzke, R., Vollenweider, P., Günhardt-Goerg, M., Kuster, T., Zimmermann, U. and Arend, M. 2012b. Concomitant dendrometer and leaf patch pressure probe measurements reveal the effect of microclimate and soil moisture on...


Zweifel, R., Item, H. and Häsl er, R. 2001. Link between diurnal stem radius changes and
tree water relations. Tree Physiol. 21:869-877.
Zweifel, R., Steppe, K. and Sterck, F.J. 2007. Stomatal regulation by microclimate and
tree water relations: interpreting ecophysiological field data with a hydraulic plant

Figures

Fig. 1. (A) Typical example of the effects of illumination and ambient temperature (T; 
dotted line) on patch pressure (Pp; black line). (B) Relationship between balancing
pressure (Pb; closed symbols) and Pp (open symbols) on ambient temperature (T; 
growth cabinet). The examples shown are for a wheat leaf.

Fig. 2. Typical patch pressure (Pp) readings of a leaf on an insufficiently watered almond
tree together (lower panel) with the corresponding profiles of temperature (solid 
line) and relative humidity (dotted line). Nocturnal hours are marked by grey bars.
Fig. 3. Typical $P_p$ oscillations (period: 21.4±5.4 min; $n=23$) measured on a leaf of an orange tree irrigated with low salinity (21.55 mM) water. Nocturnal hours are marked by grey bars.

Fig. 4. Multiple probe readings on upper leaves of a 5-m tall well-watered avocado tree in Australia performed on a sunny day. Arrows mark sunrise and sunset. Probes were clamped in the cardinal directions. For details, see text and Rüger et al., 2010b.

Fig. 5. Concomitant patch pressure ($P_p$) and changes in water-dependent stem diameter variations, $\Delta W$, measurements on a well-watered 1.5-m tall oak tree (A) and a tree subjected to drought for 20 days (B). The dendrometer was connected to the tree trunk at about 10 cm above ground. Nocturnal hours are marked by grey bars.
Fig. 6. Diurnal variation in patch pressure (Pp) of north- and south-facing leaves in the upper crown of a 5.5-m tall *Banksia menziesii* tree growing in a Banksia woodland in Western Australia (A, B). Normalized sap flow (C), air temperature (solid line) and relative humidity (dotted line) in the upper crown (D). Data are part of a 4-month recording and show a hot and rainless summer period from 6-10 February 2012. Grey bars indicate nighttime hours.