A non-invasive probe for online-monitoring of turgor pressure changes under field conditions

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INTRODUCTION

Increasing worldwide shortages of fresh water, the continuous increase in water consumption by agriculture and the progressive salinisation of arable land provoked by irrigation are global problems (Läuchli & Lütge 2002; Olesen & Bindi 2002; Jones et al. 2005; Fuchs 2007; Iglesias et al. 2007). Drip irrigation methods have saved water consumption in agricultural crops and have slowed down soil salinisation, but have highlighted the need for sensors to monitor sub-optimal water status of the crop. Water status can be determined by plant-based (e.g. xylem pressure/sap flow, turgor pressure, stomatal conductance, photosynthesis, transpiration, leaf temperature/thickness) and/or by soil-based (e.g. water content and water potential) indicators (Jones 2004). Plant-based sensing has many potential advantages over soil-based sensing, but a large number of practical difficulties have limited routine field application.

The water status of plants is usually determined by determination of the leaf water status using the pressure chamber (Scholander et al. 1965). The method is simple and thus very popular, but massively invasive, time-consuming and unsuitable for automation. A further drawback is that the number of leaves that can be measured is rather limited and, therefore, data can be misrepresented of the overall in situ conditions (due to variability in height, sun exposure, microclimate conditions, canopy circumference etc.). Most importantly, and frequently ignored, the readings cannot always straightforwardly be

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ABSTRACT

An advanced non-invasive, field-suitable and inexpensive leaf patch clamp pressure probe for online-monitoring of the water relations of intact leaves is described. The probe measures the attenuated output patch clamp pressure, P_p, of a clamped leaf in response to an externally applied input pressure, P_{clamp}. P_{clamp} is generated magnetically. P_p is sensed by a pressure sensor integrated into the magnetic clamp. The magnitude of P_p depends on the transfer function, T_f, of the leaf cells. T_f consists of a turgor pressure-independent (related to the compression of the cuticle, cell walls and other structural elements) and a turgor pressure-dependent term. T_f is dimensionless and assumes values between 0 and 1. Theory shows that T_f is a power function of cell turgor pressure P_c. Concomitant P_p and P_c measurements on grapevines confirmed the relationship between T_f and P_c. P_p peaked if P_c approached zero and assumed low values if P_c reached maximum values. The novel probe was successfully tested on leaves of irrigated and non-irrigated grapevines under field conditions. Data show that slight changes in the microclimate and/or water supply (by irrigation or rain) are reflected very sensitively in P_p.
interpreted in terms of xylem pressure and/or turgor pressure (Zimmermann et al. 2004; Zimmermann et al. 2007). Both parameters are linked hydraulically to each other in turgent cells and can quite accurately be measured by the probe techniques pioneered by Zimmermann and co-workers (Zimmermann et al. 1969; Balling & Zimmermann 1990). Even though these techniques are non-destructive and allow very accurate measurements on the single xylem vessel or cell level of intact plants, they are not suitable for long-term outdoor applications because of their susceptibility to gusting winds and heavy rain. There is a similar problem with ball tonometry, a non-destructive method by which cell turgor pressure is recorded by application of an external pressure (Lintilhac et al. 2000; Geitmann 2006). Online measurements of leaf thickness have been suggested as an indirect indicator for turgescence of leaves (e.g. Burquez 1987; McBurney 1992; Malone 1993). A number of instruments are commercially available for the routine monitoring of leaf thickness. However, leaf thickness sensors as well as other plant-based sensors have not found widespread applications in irrigation scheduling. The main reasons for this are the frequent insensitivity of leaf thickness to changes in the water status of the leaves and the anisotropy of leaf shrinkage (Jones 2004). Thus, development of a simple, inexpensive and field-suitable sensor for precise online monitoring of leaf turgor pressure or, generally speaking, of leaf water status of intact plants continues to be an important and indispensable aspect of any optimisation of irrigation management.

In this communication, an advanced online operating, non-invasive, field-suitable and inexpensive leaf patch clamp pressure probe is described. This probe measures the attenuated pressure of a leaf, \( P_p \), in response to an external clamped pressure, \( P_{clamp} \). The attenuation of \( P_{clamp} \) depends on the leaf transfer function. The magnitude of the leaf transfer function, and thus attenuation of external pressure signals, is determined by a plant-specific, turgor pressure-independent term (related to the compression of the cuticle, cell walls and other structural elements) and a turgor pressure-dependent term, \( T_e \). The potential of the leaf patch clamp pressure probe for long-term measurements of leaf cell turgor pressure is demonstrated by measurements on various grapevine cultures under different climate and irrigation conditions. Concomitant pressure chamber and cell turgor pressure measurements (using the cell turgor pressure probe) demonstrated that this novel probe exceeds these techniques and other plant-based sensor technologies in all relevant performance criteria (see also Jones 2004).

**MATERIALS AND METHODS**

**Measuring sites**

Data collection on grapevine (used for the production of table grapes) was conducted during June and September 2007 at the Lachish Research and Development station near Quiryat-Gat, Israel (31°36’15.6” N; 34°47’25.9” E; elevation 146 m). The vineyard was irrigated by drip irrigation that could be switched off and on according to the experimental needs. The measuring site was characterised by hot and dry weather during the day (up to 38 °C) and by still relatively high temperatures during the night (about 18 °C). Diurnal changes in temperature (T) and relative humidity (RH) were practically identical over the entire week of experimentation. Drizzle occurred sometimes before predawn because of a relative humidity of 100%. During the day, RH dropped to about 40%. Field experiments on grapevines (used for the production of white wine grapes) were also conducted at Gedera, Israel (31°46’25.2” N; 34°44’46.8” E; elevation 50 m) in June/July 2008. Grapevines were irrigated according to an irrigation regime used by the owner of the vineyard in previous years. Maximum and minimum T and RH values were comparable to those recorded at Quiryat-Gat during the day and the night, but subject to more significant variations (see below). Measurements were also performed during August 2007 at the vineyards of the Schmachtenberger Farm close to Würzburg, Germany (49°46’6.7” N; 9°58’12.0” E; elevation 260 m). These vineyards were not irrigated. The rainfall was often extremely high during the experimental period (see below).

**Plants and planting conditions**

**Vineyard Quiryat-Gat**

Leaf patch clamp pressure, pressure bomb and cell turgor pressure measurements were performed on *Vitis vinifera* L. cv. *Superior Seedless*. The grapevines were about 2-m tall. Spacing between the rows was 3.5 m and between the plants, 2 m. The plant density was 1428 grapevines per ha. The first, outer row was equipped with drainage lysimeters. The plants in this row were irrigated daily between 06:00 h and 11:00 h (EET, Eastern European Time). For irrigation of the grapevine rows, fresh water was used, except for the second row, which was irrigated with effluent. Before and during the experimental period in June the irrigation amount per grapevine and day was 70 l. After harvest of the grapes in August, irrigation water was reduced to 50 l per plant and day. The leaf area index of the lysimeter-treated plants and of plants irrigated with effluent varied between 3.4 and 5.7.

Irrigation of the fifth row located in the centre of the vineyard and east of the ‘lysimeter row’ was stopped on June 18th and started again on June 24th at 11:25 h. The irrigation amount per grapevine and day was 19 l of fresh water and was reduced to about 10 l per day after harvest. The leaf area index of these plants varied between 3.9 (within the row) and 4.7 (at the end of the row).

An automatic weather station was installed at the vineyard and used to record solar radiation (CM-11, Kipp & Zonen, Delft, the Netherlands), wind speed and direction (type 05103; R. M. Young, Traverse, MI, USA), air temperature and RH (type HMP 45C; Campbell Scientific, Inc., Logan, UT, USA). During the experimental periods...
in June and September, ambient T and RH were recorded close to the sites of the leaf patch clamp pressure probes using thermistors (Tinytag; RS Components GmbH, Mörfelden-Walldorf, Germany). Data were collected every 5 min.

**Vineyard Gedera**

Leaf patch clamp pressure and cell turgor pressure measurements were performed on *V. vinifera* L. cv. French Colombard. The grapevines were about 1.7-m tall. Spacing between the rows was 3.0 m and between the plants, 1.5 m. Each row contained 92 vines (2200 grapevines per ha). Plants were irrigated once a week from 10:00 h to 15:00 h. The irrigation amount per grapevine was about 50 l. The leaf area index of the plants was about 1.1. T and RH were recorded close to the sites of the leaf patch clamp pressure probes using Tinytag thermistors. Data were collected every 5 min.

**Vineyard Würzburg**

Leaf patch clamp pressure probe and pressure bomb measurements were performed on *V. vinifera* L. cv. Bacchus. The grapevines were about 1.8-m tall. Spacing between the rows was 1.6 m and between the plants, 1.35 m. The plant density was 4600 grapevines per ha. The leaf area index was about 1.4. T and RH were recorded close to the sites of the leaf patch clamp pressure probes using Tinytag thermistors. Data were collected every 5 min.

**Leaf patch clamp pressure probe**

For patch clamp pressure measurements, a leaf was positioned in the space between two planar circular pads, where one of the pads contained a receptacle (area: $4 \times 2.5$ mm$^2$, height: 0.8–1.2 mm) for integration of the pressure sensor chip. The principle of operation and a probe clamped to a leaf are shown schematically in Fig. 1a. The clamp pressure was generated magnetically. The pad containing the sensor chip was made of nickel and was fixed on a toric magnet (NdFeB, axial magnetised). The counter pad was made of aluminium (diameter: 8 mm). A further toric magnet with an inside thread (type M 4) could be moved along a threaded rod fixed on the back of the counter pad. The variation in the distance between the pad and the movable magnet allows adjustment of the magnetic force according to requirements (see Fig. 1b). The maximum force measured by a tensiometer was in the order of 400–600 g.

Two types of pressure sensors were used for recording the transfer function of the leaf. One was purchased from Raumedic AG (Helmbrchts, Germany) and the other from Keller AG (Druckmesstechnik, Winterthur, Switzerland). Both sensors are based on an electronic chip strain gauge coated with a thin silicone membrane. The sensors were calibrated by pressurisation in a pressure chamber equipped with an integrated manometer (LEO 1, Keller AG, Winterthur, Switzerland).

**Data acquisition**

The sensor signals were acquired by the telemetric system SENBIT (teleBITcom gmbh, Teltow, Germany). The transmitters read and amplified the analogue signals of the leaf patch clamp pressure probe. The digitised data were sent together with the transmitter ID code every 90 s or 5 min via the ISM band of 433 MHz to a RF receiver unit over a distance of up to 400 m. The receiver was connected via an RS-232 interface to a personal computer or to a GPRS modem linked to an Internet server (NTBB Systemtechnik GmbH, Zeuthen, Germany) via mobile phone network. The telemetric system allowed, in principle, simultaneous data processing of 32 sensors. Up to 17 sensor/transmitter units were installed in the vineyards for leaf patch clamp pressure recordings.

**Pressure chamber**

Diurnal changes in the balancing pressure ($P_b$) values of irrigated and non-irrigated grapevines were measured in parallel to online leaf patch clamp pressure measurements on 2 days in the vineyard at Quiryat-Gat and on 3 days in the vineyard close to Würzburg. The pressure bomb method is described in detail elsewhere (Scholander et al.).
1965). For the measurements performed at Quiryat-Gat, a pressure bomb purchased from ARI (Kfar-haruv, Israel) was used. Measurements on the grapevines located at Würzburg were performed with a home-built device equipped with a high-resolution digitised manometer [LEO 1, Keller AG; for details, see Zimmermann et al. (2007)].

Balancing pressure values were determined on sun-exposed and shaded leaves. Leaves were sampled nearest to the site of the leaf patch clamp pressure probe.

**Turgor pressure probe**

Diurnal changes in cell turgor pressure were recorded concomitantly with online leaf patch clamp pressure measurements on 2 days in the vineyard at Quiryat-Gat and at Gedera using the cell turgor pressure probe (Zimmermann et al. 1969). The pressure probe technique is described in detail elsewhere (Zimmermann et al. 2004). The probe was inserted from the abaxial side of the leaves into the parenchymal cells close to the main vein. Leaf patch clamp pressure probes were clipped to nearby leaves and to leaves further away, and partly also to leaves of other branches. Turgor pressure measurements in the vineyard close to Würzburg failed because of tip clogging of the microcapillary of the pressure probe by abundant mucopolysaccharides.

**Leaf area index**

Leaf area of the vines was estimated using a non-destructive Sunscan canopy analysis system (model SS1-R3-BF3; Delta-T Devices, Cambridge, UK). The method (‘gap fraction inversion’) is based on PAR (photosynthetically active radiation) measurements under the canopy and parallel reference measurements above canopy (Cohen et al. 1997). Under each grapevine, 18 radiation measurements were taken (spaced every 20 cm) covering the soil surface completely under a given grapevine. Detailed information and instrument calibration is available in Netzer et al. (2005).

**THEORETICAL CONSIDERATIONS**

The input pressure seen by the cells of the leaf patch, $P_{in}$, is only equal to the external clamped pressure, $P_{clamp}$, if the pressure signal is transferred without loss to the cells. However, losses usually occur due to the compressibility and deformability of the silicone surrounding the sensor chip as well as the compressibility of the cuticle and other structural elements of the leaf. Therefore, theory shows that only a fraction of $P_{clamp}$ is seen by the cells, i.e. the attenuation factor, $F_a = P_{in}/P_{clamp}$, is smaller than unity. $F_a$ depends on the individual leaf properties. In the case of the rigid leaves of vines, $F_a$ is c. 0.3 as evidenced by control experiments (data not shown). $F_a$ can be assumed to be constant if the structural elements are completely pre-compressed by application of an appropriate $P_{clamp}$ and if $P_{clamp}$ is kept constant during the following experimental period. Thus the output patch clamp pressure, $P_p$, depends only on the cell transfer function, $T_f (V)$, where $V$ is the patch leaf volume. $T_f (V)$ determines the fraction of $P_{in}$ that is sensed by the probe (i.e. $P_p$). $T_f$ is dimensionless and assumes values between zero and unity:

$$P_p = T_f(V) \cdot P_{in} \quad (1)$$

The function of $T_f$ on leaf volume, $V$, is given at constant ambient temperature, $T$, by Equation (2):

$$T_f = -\left(\frac{\delta T_f}{\delta V}\right) \cdot \frac{V}{T} \quad (2)$$

The relative volume change $\delta V/V$ of the leaf patch is correlated to the turgor pressure change, $\delta P_c$, by the average volumetric elastic modulus, $\varepsilon_p$, of the tissue beneath the clamp (Philip 1958).

$$\left(\frac{\delta P_c}{\delta V}\right)_T = \frac{\varepsilon_p}{V} \quad (3)$$

$\varepsilon_p$ is a very complex parameter and will depend *inter alia* on the magnitude of the turgor pressure. For a first approximation, we assume that $\varepsilon_p$ increases linearly with $P_c$ (support for this assumption is given by Zimmermann & Steudle 1978; Zimmermann & Hüskens 1980; Wendler et al. 1983):

$$\varepsilon_p = aP_c + b \quad (4)$$

where $a$ and $b$ are constants for an individual leaf. Because of the viscoelastic properties of the cell walls, the magnitude of the constants depends on the duration of pressure application (Zimmermann & Hüskens 1980). The constants are relatively large if rapid turgor pressure changes are induced (e.g. by using the cell turgor pressure probe), whereas slow turgor pressure changes (e.g. induced by transpiration) result in small values.

Combining Equations (2)–(4) leads to Equation (5):

$$\frac{dT_f}{T_f} = -\frac{dP_c}{aP_c + b} \quad (5)$$

Equation (5) can be integrated if we assume for a first approximation that at $P_c = 0$ $T_f = 1$ and that the internal osmotic pressure of the cells remains nearly constant in the range of cell turgescence. After appropriate re-arrangements Equation (6) is obtained:

$$T_f = \left(\frac{b}{aP_c + b}\right)^{\frac{1}{2}} \quad (6)$$

Introducing Equation (6) into Equation (1) yields a relationship between the parameters $P_p$ and $P_{in}$.
Equation (7) can experimentally be proved. Inspection of the equation shows that the output patch clamp pressure, $P_p$, is a power function of the turgor pressure $P_c$. The exponent of the function is equal to or smaller than unity. If $a = 1$ and $b << P_c$, Equation (7) goes over into $P_p = b P_{in}/P_c$, i.e. both parameters are directly recipro-
cally coupled with each other. Thus, $T_f$ assumes low 
values if $P_c$ is high and vice versa, a value close to unity if
$P_c$ is close to zero. Using appropriate values for $a$ and $b$
for a given leaf (see below) it can be shown that below
$P_c = 100$ kPa, $P_p$ must increase dramatically.

RESULTS
Evaluation of the leaf patch clamp pressure probe

One problem with semiconductor strain gauges is that they
are frequently somewhat sensitive to temperature variations
and tend to change resistance as they age, which, in turn,
affects the attenuation factor $F_a = P_{in}/P_{clamp}$. Zero drifts
can also occur. For measurements at constant temperature
(e.g. under laboratory conditions or in clinical settings),
this may not be a serious concern (Citerio et al. 2004), but
under field conditions rapid temperature changes of 25 $^\circ$C
and more were quite common. According to the specifica-
tions of the manufacturer, the silicone-embedded sensors
used here were temperature- and baseline drift-compen-
sated. To prove this, the magnetic clamp probes were sub-
jected to temperature regimes ranging from 10 $^\circ$C to
35 $^\circ$C. Measurements were performed in accessible climate
chambers. Probes were only used that exhibited a system-
atic error smaller than 2 kPa. Changes of the leaf patch
clamp pressure induced by temperature-dependent changes
of the material properties of the clamp assemblies could
also be excluded. This was verified by cooling, selectively
and temporarily, components of the clamps (except the sili-
cone sensor) using cooling spray. Pressure responses were
only observed if the sensors exhibited relatively large tem-
perature sensitivity.

Leaf movements induced by strong or gusting winds
and/or rain did not affect the contact between the probe
and the leaf surface. Finding of an optimum $P_{clamp}$ that
allowed monitoring over the range of leaf turgescence was
performed empirically and depended on the compressibil-
ity and deformability properties of the individual leaves,
which may vary considerably due to age, morphology,
abiotic factors etc. Optimum $P_{clamp}$ could easily be
adjusted with the magnetic clamp probe. The $P_{clamp}$ pres-
sure was considered to be optimal if the output pressure,
$P_p$, ranged between 10 and 25 kPa, after clamping in the
early morning hours, i.e. at full turgescence of the leaf
cells, or between 50 and 70 kPa after clamping around
noon, when turgor pressure assumed minimum values.

Measurements showed that very reproducible results
were obtained when the sensor-containing pad faced the
abaxial side of the leaf. The reason for this was presum-
bly because the abaxial side of the leaves was not covered
by a dust layer or other dirt and its more elastic surface
optimised the uniform contact between the leaf and the
silicone membrane. However, it is important to note that
adaxial clamping yielded similar results, excluding the
possibility that the stomatal density plays a role. Viewing
of the leaf patches under the microscope after removal of
the probes showed that the area beneath the pads were
practically as green as the surrounding leaf tissue. However,
it is important to note that adaxial clamping yielded similar results, excluding the possibility that the stomatal density plays a role. Viewing of the leaf patches under the microscope after removal of the probes showed that the area beneath the pads were practically as green as the surrounding leaf tissue. Only after 3 months of clamping (i.e. after harvest of the grapevines at the end of September) was the leaf patch a little lighter in colour (see Fig. 2), indicating that the chlorophyll concentration had somewhat decreased. Occasionally a very slight impression of the pads on the leaf

![Fig. 2. Appearance of a leaf of a grapevine in the vineyard at Gedera, Israel, after removal of a probe clamped for an extremely long period (about 3 months). Leaves were taken after grape harvest at the end of September. Note that the area beneath the pads was somewhat lighter on both the adaxial (a) and abaxial side (b) than the surrounding leaf tissue, suggesting some decrease in chlorophyll concentration. It is evident that cells were still turgescent. Necroses or lesions were not observed, even after this long time of clamping. Note further that a decrease in chlorophyll concentration was not observed after about 2 months of clamping.](image-url)
surface was found. Necroses or lesions on the leaves were never observed.

Clamping of several nearby leaves exposed to the same local environmental conditions demonstrated that the clamp pressure, $P_{\text{clamp}}$, has no effect on the diurnal profiles of the patch clamp pressure, $P_p$. The relative changes in the $P_p$ values in response to changes in environmental conditions were always completely identical (data not shown). Similarly, using different-sized pads (from 20 mm$^2$ up to an area of 119 mm$^2$) resulted in comparable-shaped diurnal $P_p$ curves. Problems only occurred if the diameter of the circular pads exceeded the spacing between the leaf veins. This could prevent a uniform contact between the leaf and the pads. Non-uniform contact resulted in a reduced pressure response of the probe or even, at high non-uniformity, in a reversal of $P_p$, i.e. $P_p$ decreased with increasing transpirational water loss. Under these conditions, the probe is measuring changes in leaf thickness rather than turgor pressure-dependent pressure propagation through the leaf, as demonstrated by control experiments in which a force sensor with non-uniform pressure transfer was used (data not shown). Therefore, throughout the experiments, pads of 20 mm$^2$ area were used that allowed the placing between relatively small veins of the leaves.

Diurnal leaf patch clamp pressure profiles

Because of nearly constant climate conditions in June and September 2007, the diurnal changes in the $P_p$ values of grapevines in the vineyard at Quiryat-Gat and irrigated regularly were very similar. At noon, peak temperatures of around 37 °C were usually measured. Minimum temperatures of 20 °C or slightly less were recorded at about 04:00 h (EET = Eastern European Time) in the morning. Conversely, RH dropped down to values of about 20% at

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**Fig. 3.** Leaf patch clamp pressure ($P_p$) recordings on a sun-exposed leaf (area = 41 cm$^2$; a) and on a shaded leaf (area = 61 cm$^2$; b) of a grapevine in the ‘lysimeter row’ and irrigated daily (Quiryat-Gat, Israel). c: The corresponding diurnal changes in ambient temperature ($T$; black line) and relative humidity (RH; dotted line) measured in the neighbourhood of the sun-exposed leaf. Measurements were performed between September 20th and 22nd 2007. Note that the shaded leaf was exposed to sunlight between 15:15 h and 16:30 h, resulting in a second peak of the $P_p$ values during afternoon. For further details, see text.
noon, whereas towards the early morning RH reached 100%, frequently leading to slight drizzle before predawn. Figure 3 represents $P_p$ recordings performed on a grapevine in the ‘lysimeter row’ between September 20th and 22nd 2007. This grapevine was located towards the middle of the row. Figure 3 shows typical diurnal changes in the $P_p$ values of a fully sun-exposed leaf (Fig. 3a; leaf area about 41 cm$^2$) and of a shaded leaf (Fig. 3b; leaf area about 61 cm$^2$). The shaded leaf was only exposed to direct sunlight between 15:15 h and 16:30 h. The corresponding diurnal changes in ambient T and RH measured in the neighbourhood of the sun-exposed leaf are given in Fig. 3c. The diurnal profiles of ambient T and RH in the neighbourhood of the shaded leaf were similar, except that T around noon was by about 2–3 °C lower than at the sun-exposed sites. Fig. 3a and b demonstrate that the diurnal changes in the $P_p$ values were very reproducible over the experimental period. Consistent with the theory [see Equation (7) above], at noon, when the cell turgor pressures, $P_c$, usually assume minimum values, $P_p$ values peaked, whereas during the night, when $P_c$ assumed maximum values, the $P_p$ values dropped to a minimum. A close examination of Fig. 3 further shows that the magnitude of the $P_p$ values of the sun-exposed and shaded leaves was closely related to the diurnal changes in RH and T. Temporary changes in ambient RH and T were reflected in immediate changes in the $P_p$ values. Changes in temperature and RH were more or less closely related to each other. Thus, it was not possible to unambiguously separate the effects of these parameters on the $P_p$ values.

The second peak of the $P_p$ values of the shaded leaf, which occurred regularly in the afternoon, obviously resulted from the short-term exposure of the leaf and of the adjacent leaves to sunlight. Direct exposure to sunlight presumably changed the RH and T close to the leaf surfaces. Similar results (Fig. 3) were found for other leaves of regularly irrigated grapevines measured between June and September 2007.

Figure 4a shows a typical 1-week patch clamp pressure recording on a grapevine leaf in the vineyard at Quiryat-Gat after irrigation was stopped on June 19th 2007. The grapevine was in the middle of the fifth row. The probe was clamped on a leaf (area about 24 cm$^2$) at the top of the grapevine. The leaf was fully exposed to sunlight during the day. The concomitant online measurements of T and RH measured close to the site of the leaf patch clamp pressure probe are given in Fig. 4b. Inspection of the figure shows that during the night $P_p$ assumed a low, nearly constant value over the entire period of non-irrigation (June 19th to 24th 2007). Peaking of the $P_p$ values always occurred around noon. Interestingly, peaking of the $P_p$ values (and thus turgor pressure loss) increased concomitantly with the proceeding non-irrigation, but dropped dramatically upon the onset of irrigation on June 24th. Irrigation also significantly lowered the $P_p$ values during the night, suggesting improved water uptake and, in turn, turgescence of the cells. Data collection of recordings of $P_p$ values on other leaves gave the same results, provided that sun-exposed leaves from the top of the grapevines were used (data not shown).

![Fig. 4.](image)

Long-term leaf patch clamp pressure, $P_p$, recordings (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) performed on a non-irrigated/irrigated grapevine in the vineyard close to Quiryat-Gat, Israel, during June 2007. Measurements were performed on a sun-exposed leaf (area = 24 cm$^2$). Irrigation was stopped at the beginning of the measurements (June 19th, downwardly directed arrow) and switched on again on June 24th (horizontally directed arrow). Note the effect of irrigation on the peak of the patch clamp pressure around noon and on the minimum value during the night. For further details, see text. Note that continuous irrigation leads to a permanent decrease of the peak $P_p$ value.
Figure 5a and b represent typical patch clamp pressure recordings together with the ambient T and RH profiles recorded on a grapevine leaf (area = 113 cm²) in the vineyard at Gedera between July 7th and July 27th 2008. A leaf of the plant at a height of 1.5 m was clamped. Days were also hot, but ambient changes in T and RH during the days and nights were more variable than during the experiments on grapevines in Quiryat-Gat. Despite this, it is obvious

Fig. 5. Typical patch clamp pressure recordings taken from a 3-month long experiment (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) recorded on a grapevine leaf (area = 113 cm²) in the vineyard of Gedera between July 7th and July 27th 2008; the experiment ended at the end of September. Irrigation is indicated with open columns. Even though the diurnal changes in T and RH were more variable than during the experiments on grapevines in Quiryat-Gat (Fig. 4), the irrigation effects can clearly be distinguished from microclimate effects on the $P_p$ values. Note that irrigation resulted in an immediate, temporary decrease of the peak $P_p$ value at noon.

Figure 6a and b represent typical patch clamp pressure recordings during the three-week experiment performed on a non-irrigated grapevine leaf (area = 121 cm²) in the vineyard close to Würzburg, Germany, between August 1st and 20th 2007. Between August 7th and 9th it was very rainy (extremely heavy rainfall on August 8th: 38 l per m²). Note that rainfall had a similar effect on $P_p$ peaks at noon and on the $P_p$ night values during the following days as the onset of irrigation in Figs 4 and 5.

Fig. 6. Three-week leaf patch clamp pressure ($P_p$) recordings (a) together with the corresponding ambient T (solid line) and RH (dotted line) profiles (b) performed on a non-irrigated grapevine (leaf area = 121 cm²) in the vineyard close to Würzburg, Germany, between August 1st and 20th 2007. Between August 7th and 9th it was very rainy (extremely heavy rainfall on August 8th: 38 l per m²). Note that rainfall had a similar effect on $P_p$ peaks at noon and on the $P_p$ night values during the following days as the onset of irrigation in Figs 4 and 5.
from the figure that irrigation effects on the $P_p$ values can clearly be distinguished from microclimate effects. Irrigation on July 7th, 14th, 21st and 23rd led to an immediate reduction of the peak $P_p$ values at noon. In the following days after irrigation the peak values increased continuously, as observed under the irrigation and climate conditions of Quiryat-Gat (Fig. 4).

Fig. 6 represents typical measurements on grapevines in the non-irrigated vineyard close to Würzburg recorded between August 1st and 20th 2007 (CET = Central European Time), together with the corresponding T and RH profiles, as well as rainfall events. As indicated by the weather data, the days before August 7th were partly sunny, but were generally very cloudy, whereas the days between August 7th and August 9th were characterised by strong rainfall (38 l per m$^2$ on August 8th). The following days were partly cloudy, interrupted only occasionally by short duration rainfall. During the days of strong rainfall, the temperature did not exceed 20°C, whereas the temperature usually reached 30°C in the preceding and following days. Interestingly, in contrast to the environmental conditions at Quiryat-Gat and Gedera, RH dropped down to extremely low values (<10%) at noon, but usually reached 100% during the early morning.

Before the days of strong rainfall, the $P_p$ peaks recorded at noon increased continuously from day to day, whereas during the night the $P_p$ values assumed a nearly constant low value. During the rainy days, the $P_p$ values remained at this low level, even during the day. The small changes in $P_p$ values were in the range of accuracy of the sensor. The onset of significant diurnal changes in $P_p$ values occurred immediately after rainfall stopped. Furthermore, on the following day the level of $P_p$ values during the night dropped considerably further and remained nearly constant over the next few days. Similar findings were measured with other probes, indicating that shifts in 'night levels' of $P_p$ values were not induced by baseline drift effects. Rather, the results are consistent with the findings on grapevines in vineyards at Quiryat-Gat and Gedera subjected to a non-irrigation/irrigation regime. Also, similar to the findings on grapevines in the vineyards at Quiryat-Gat and Gedera, magnification of the diurnal changes in $P_p$ values demonstrated (not shown) that changes in T and particularly in RH were immediately reflected in changes in the magnitude of $P_p$ values before and after the heavy rainfall.

**Relationships between patch clamp pressure, balancing pressure and cell turgor pressure**

Figure 7a and b represent diurnal changes in the leaf patch clamp pressure, $P_p$, and balancing pressure, $P_b$, values measured on leaves detached from vines close to those on which the leaf patch clamp pressure measurements were performed. The balancing pressure experiments in Quiryat-Gat were performed between June 19th and June 27th 2007 and at Würzburg between August 1st and August 7th 2007. Results are shown for grapevines at Quiryat-Gat (Fig. 7a) and Würzburg (Fig. 7b). It is obvious that diurnal changes in the $P_b$ values coincided with diurnal changes in the $P_p$ values if the limited accuracy of the spot measurements of the balancing pressure values is taken into account. The number of $P_b$ data measured on grapevines at Würzburg was large enough to correlate the

**Fig. 7.** Correlation between balancing pressure values ($P_b$; open circles) and leaf patch clamp pressures ($P_p$; solid lines). Measurements were performed on grapevines in the vineyard at Quiryat-Gat (a; probe was clamped on a leaf of area = 41 cm$^2$) and at Würzburg (b; probe was clamped on a leaf of area = 121 cm$^2$). c: Plot of the $P_p$ values against the corresponding $P_b$ values; data were taken from (b). Note that the sigmoid-shaped relationship between the $P_p$ and $P_b$ values can be approximated by a straight line up to a balancing pressure of about 600 kPa (least square method, $r = 0.996$). For further details, see text. [New figure added on 26 February 2009, after first online publication.]
low turgor pressure, the increase in \( P_c \) and the decrease after the afternoon, when all cells throughout the leaves exhibit record values. Consistent with this explanation, towards the end of the day, cell turgor pressure \( P_c \) was approximated a straight line. The above theoretical considerations have shown that the transfer function of a defined leaf area depends only on turgor pressure if the structural elements do not contribute or constantly contribute to the pressure signal transfer of the external input pressure to the pressure sensor. In order to prove the theory, parallel measurements of leaf patch clamp pressure \( (P_p) \) and cell turgor pressure \( (P_c) \) were performed on grapevines of the second row in the vineyard at Quiryat-Gat that was irrigated daily (Fig. 8a). For technical reasons, the patch clamp pressure measurement was performed on the leaf that was closest to the leaf on which the cell turgor pressure was determined. Data recorded by the patch clamp pressure probe and the cell turgor pressure probe are given for September 19th 2007. During the morning hours the leaves were in the shade; they became sun-exposed around noon. As indicated in the figure, cell turgor pressure assumed values of about 500 kPa during the night and dropped to about 70 kPa around 14:00 h then increased continuously again during the afternoon (note the reverse scaling of the \( P_p \) ordinate in Fig. 8a). The diurnal changes in \( P_c \) correlated surprisingly well with the diurnal changes in \( P_p \). Interestingly, during the morning hours the drop in turgor pressure lagged by about 20 min behind the corresponding increase in \( P_p \). The delay in the response of \( P_p \) resulted most likely from the distance between the different measuring sites on the leaves. After the onset of transpiration, loss of turgor pressure of cells located at the periphery of the leaves (where \( P_p \) is measured) will immediately be compensated by water shifting from the xylem and the cells located close to the main vein (where \( P_c \) is recorded). Consistent with this explanation, towards the afternoon, when all cells throughout the leaves exhibit low turgor pressure, the increase in \( P_p \) and the decrease in \( P_p \) occurred nearly concomitantly. In Fig. 8b the \( P_c \) values are plotted against the corresponding \( P_p \) values measured during the morning hours (by neglecting the delayed response of \( P_p \)). As indicated in the figure, the data could be fitted quite well to Equation (7), particularly if the limited accuracy of the spot turgor pressure measurements at low values is taken into account. The relatively high temperatures measured at this time of day may also affect the elastic properties of the leaf and thus the constants a and b in Equation (4). Plots of the \( P_p \) values against the corresponding \( P_c \) data measured during the afternoon could also be fitted to Equation (7) (data not shown). A similar dependency of \( P_p \) on \( P_c \) was also obtained if the \( P_c \) values were plotted against \( P_p \) values recorded simultaneously on leaves located on the same branch or on a parallel branch up to 1.5 m away from the site of the turgor pressure measurements (Fig. 8b), indicating a hydraulic continuum between the measuring sites.

Concomitant measurements of \( P_p \) and \( P_c \) on grapevines in the vineyard at Gedera, which were irrigated once a week, gave similar results (data not shown).
DISCUSSION

As shown here, the leaf patch clamp pressure probe provides very precise information about the supply of the leaves with water, independent of the size (i.e. age) of the leaves and knowledge of the transfer function of the leaves, which dictates the output patch clamp pressure, $P_p$. The patch clamp pressure also responded closely to the wetting and drying of the soil. This was demonstrated by modulation of the irrigation scheduling in the vineyards at Quiryat-Gat and Gedera, Israel (Figs 4 and 5) as well as by measurements on grapevines in the vineyard at Würzburg, Germany (Fig. 6), where the plants were exposed to very unsettled weather conditions.

For application of the leaf patch clamp pressure probes in the field, knowledge of the dependency of the transfer function on turgor pressure changes (and osmotic pressure changes which will be reflected in corresponding turgor pressure changes) is not required. It is only of relevance to know that the transfer function assumes small values at full turgescence and reaches nearly unity at low turgescence. These boundary values are easily found empirically under field conditions, particularly if the magnetic leaf patch clamp pressure probe is used. However, introduction of new methods requires calibration against currently used, physically sound methods, including the elucidation of their limits. Concomitant leaf patch clamp pressure and cell turgor pressure measurements have qualitatively demonstrated (Fig. 8) that the patch clamp pressure is inversely coupled with the turgor pressure, $P_c$, of the leaf cells. The theoretical analysis of the transfer function of the leaf cells yielded a relationship between clamp pressure, $P_{clamp}$, and the attenuated output pressure, $P_p$, which could explain quantitatively the balance between clamp pressure, $P_{clamp}$, and the attenuated output pressure, $P_p$. The theoretical part of the study and to S. Nieft and B. Eberhardt for skilful technical assistance. We are also very grateful to G. Kunze, Raumedic, for supplying some pressure sensor chips.

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