Non-destructive estimation of root pressure using sap flow, stem diameter measurements and mechanistic modelling

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INTRODUCTION

The process of water uptake and transpiration is generally explained by the cohesion–tension (C–T) theory (Dixon and Joly, 1894; Meinzer et al., 2001). According to this theory, the ascent of water is a passive process ultimately driven by the evaporation of water from leaves, which creates a water potential difference between the soil and the dry air (Steudle, 2001). Although this theory is still widely accepted, it has evoked some controversy (Meinzer et al., 2001; Angeles et al., 2004; Zimmermann et al., 2004) because of the meta-stable state of xylem water and the measuring methodologies of water potential. Canny (1995), for instance, proposed a mechanism of tissue pressure supporting the water column, in addition to C–T. Many others have reported the phenomenon of root pressure as a feature in water transport, in addition to C–T (e.g. Klepper and Kaufmann, 1966; Pickard 1981; Sperry et al., 1987; Tyree et al., 1987; Clearwater et al., 2007). In the absence of transpiration as the driving force of water flow across the soil–plant–atmosphere continuum (SPAC), root pressure could serve as the auxiliary engine for water supply to shoots under conditions of low transpiration (Steudle, 2001). At present, it is widely believed that root pressure arises from active loading of solutes into the xylem and subsequent osmotic uptake of water (Kramer and Boyer, 1995). More specifically, the endodermis retains solutes in a zone near the tracheary elements and allows root pressure to build up under conditions of low transpiration (Steudle and Peterson, 1998). Under these conditions, water flow is coupled to solute flow, whereas interactions between solute and water flows are of minor importance in a transpiring plant because the hydrostatic gradient will usually be the dominating force (Pickard, 2003).

Phenomena attributed to root pressure in horticulture

The best known phenomenon attributed to root pressure is guttation: due to a positive pressure in the xylem, as a result of root pressure, excess water is pushed out of the leaf ends via hydathodes (Kramer and Boyer, 1995). If plants are unable to dispose of this excess water, cells might leak or burst. Several physiological disorders are attributed to excessive root pressure such as watery fruits in tomato (Dorais et al., 2001) and glassiness in lettuce (Maaswinkel and Welles, 1986). In addition, bursting of cells allows pathogens such as Botrytis and Mycosphaerella to infect the damaged tissue and spread around in the plant. Many studies have demonstrated that the water relations in tomato (Solanum lycopersicum L.) have crucial implications for fruit production and fruit quality (e.g. Mitchell et al., 1991; Johnson et al., 1992; Dorais et al., 2001; De Swaef et al., 2012), but few deal with the effects of root pressure. However, current efforts to
reduce energy use in horticulture have led to modified glasshouse climate conditions (i.e. more humid) that might promote the occurrence of excess root pressure (Heuvelink et al., 2008).

Beside the above-mentioned disorders, root pressure is expected to play a beneficial role in the distribution of calcium to the leaves of cabbage and lettuce (Palzkill and Tibbitts, 1977), root-to-shoot water transport in trees during spring (e.g. Sperry et al., 1987; Clearwater et al., 2007) and in refilling embolized xylem vessels (e.g. Tyree et al., 1987; Zwieniecki and Holbrook, 2009). Moreover, Clearwater et al. (2007) recently uncovered a relationship between the rootstock ability to build up root pressure and the scion vigour for kiwi.

The above-mentioned physiological effects attributed to root pressure indicate the need to investigate this intriguing phenomenon further. Currently, a ‘bottle-neck’ in root pressure research is the lack of a system that allows continuous and non-destructive measurements, especially for herbaceous plants. Clearwater et al. (2007) measured root pressure using pressure transducers that were installed inside the xylem of kiwi roots. To our knowledge, this is the only existing non-destructive continuous method to measure root pressure. However, the application of this method on herbaceous plants with limited root and stem diameters such as tomato may be difficult, if not impossible, because this method requires installation of the system 10–15 mm inside the xylem. Other methods are mainly destructive, such as installing manometers on excised stems (Grossenbacher, 1938) or root pressure probes in excised roots (Steudle et al., 1993).

To allow non-destructive and non-invasive estimation of root pressure, we present a new approach using continuous measurements of sap flow and stem diameter variations in tomato combined with a mechanistic flow and storage model, based on C–T principles. Estimated values of root pressure, under conditions of low transpiration rates, were compared with destructive measurements using a manometer installed on an excised stem.

MATERIALS AND METHODS

Plant material and experimental set-up for the development of the technique (expt 1)

Tomato plants (Solanum lycopersicum ‘Admiro’) were grown in a greenhouse compartment measuring 16 × 12 × 5 m at the Research Centre Hoogstraten, Belgium (51°27′N, 4°47′E). Plants were sown on 5 November 2009, grafted on the rootstock Emperador and planted on rockwool substrate (Master, Grodan, Hedehusene, Denmark) on 5 January 2010. Plants were provided with a nutrient solution of approx. 2-6 ds m⁻¹ (i.e. osmotic potential of approx. −0.08 MPa) using a drip irrigation system, based on sums of solar radiation, to attain a daily drainage of approx. 30–50%. Leaves under the lowest ripe truss were removed and the lowest part of the stem was horizontal, as is common practice in tomato. Three plants were continuously monitored during six consecutive days, starting on 17 September 2010.

Continuous measurements

Heat balance sensors (Model SGA13-WS, Dynamax Inc., Houston, TX, USA; accuracy approx. 10%) were used to measure sap flow (F_H2O) below the lowest leaf on three plants and were installed according to the operation manual (van Bavel and van Bavel, 1990). At the start of this experiment, all plants were approx. 8 m long and the sensors were installed at about 0.5 m from the roots.

The sensors were thermally insulated by wrapping them in several layers of aluminium and bubble foil. Stem diameter (D) variations were measured just below the sap flow sensors using linear variable displacement transducers (LVDTs; model 2-5 DF, Solartron Metrology, Bognor Regis, UK; accuracy approx. 1 μm). The LVDT sensors were attached to the stem by custom-made stainless steel holders. Tests with a 12 mm aluminium rod showed that no temperature correction was required (Steppe and Lemeur, 2004). Absolute stem diameter was measured once before the start of the experiment using an electronic calliper.

Relative humidity (RH) of the air was measured with an aspirated capacitive sensor (HMP50, Vaisala, Helsinki, Finland). Air temperature (T) was measured using a copper–constantan thermocouple (Type T, Omega, Amstelveen, The Netherlands). Both sensors were installed at 1.5 m height and were shielded from direct radiation. Vapour pressure deficit (VPD) was calculated from RH and T according to Jones (1992).

Data from all sensors were logged (CR1000, Campbell Scientific Inc., Logan, UT, USA) at 30 s intervals and averaged every 5 min.

Plant material and experimental set-up for validation of the technique (expt 2)

Tomato plants (S. lycopersicum L. ‘Admiro’) were grown in a greenhouse compartment measuring 2 × 2.5 × 4 m at the faculty of Bioscience Engineering, Ghent, Belgium (51°03′N, 3°43′E). Plants were sown on 4 September 2011 and transplanted on rockwool substrate (Master, Grodan, Hedehusene, Denmark) on 21 November 2011.

To generate high night-time RH, an air humidifier (type Boneco 7135, Plaston, Widnau, Switzerland) was switched on between 1600 and 0900 h. The above-mentioned sap flow and LVDT sensors were installed on one plant. For three measurement periods (31 January–3 February 2012; 14–16 February 2012; 21–25 February 2012), root pressure was continuously recorded using a manometer (type 401002, Jumo Scientific Inc., Logan, UT, USA) at 30 s intervals and averaged every 5 min.

Modelling, simulations, calibrations

In this study, the principles of a mechanistic flow and storage model, originally developed for trees (Steppe et al., 2006), were used. These equations allow simulation of D, stem water potential in the xylem (Ψx), and total (Ψs), osmotic (Ψo,s) and hydrostatic potential in the surrounding

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storage tissues ($\Psi_{p,s}$). The model uses $F_{H2O}$ as an input variable, which is directly linked to $\Psi_s$ via the flow resistance concept (van den Honert, 1948):

$$\Psi_s = \Psi_m - F_{H2O}R_s$$

(1)

where $\Psi_m$ is the water potential in the growing medium (MPa) and $R_s$ is the hydraulic resistance between the growing medium and the point of interest in the plant stem (MPa h g$^{-1}$). The model simulates variations in water content of the storage tissue ($W_s$) as a result of the water potential gradient driving radial water transport between the xylem and the storage tissue:

$$\frac{dW_s}{dt} = \frac{\Psi_x - \Psi_s}{r}$$

(2)

where $r$ is the hydraulic resistance between xylem and storage (MPa h g$^{-1}$).

The relationship between $W_s$ and $D$ is defined by:

$$W_s = \frac{D^2(1-a^2)^2L \rho_{H2O}}{4}$$

(3)

where $a$ is the proportion of inelastic tissue (dimensionless), $L$ is the length of the stem element (m) and $\rho_{H2O}$ is the density of water (g m$^{-3}$). Assuming a fixed volume of inelastic tissue, variations in $W_s$ and $D$ are related as follows:

$$\frac{dD}{dt} = \frac{2}{\pi L \rho_{H2O}D} \frac{dW_s}{dt}$$

(4)

Water transport in and out of the surrounding tissues of the stem induces changes in $\Psi_{p,s}$. If $\Psi_{p,s}$ is smaller than the threshold pressure at which wall yielding occurs ($\Gamma$), then variations in $D$ only reflect reversible swelling/shrinking:

$$\frac{d\Psi_{p,s}}{dt} = \frac{\epsilon}{W_s} \frac{dW_s}{dt}$$

(5)

where $\epsilon$ is the elastic modulus of the storage tissue (MPa).

If $\Psi_{p,s}$ is larger than $\Gamma$, then irreversible radial growth occurs in addition to shrinkage/swelling (Steppe et al., 2006):

$$\frac{d\Psi_{p,s}}{dt} = \frac{\epsilon}{W_s} \frac{dW_s}{dt} - \epsilon \varphi (\Psi_{p,s} - \Gamma)$$

(6)

where $\varphi$ is the cell wall extensibility of the storage tissue (MPa$^{-1}$ h$^{-1}$).

In accordance with De Schepper and Steppe (2010), $\Psi_{\pi,s}$ was calculated using the van ’t Hoff equation (Jones, 1992):

$$\Psi_{\pi,s} = -\frac{R T M_s}{MM_{\text{sucrose}}} W_s$$

(7)

where $R$ is the universal gas constant (8.31 MPa g mol$^{-1}$ K$^{-1}$), $T$ the temperature (K), $M_s$ the sugar content of the storage tissue (g) and $MM_{\text{sucrose}}$ the molar mass of sucrose (342.3 g mol$^{-1}$). $\Psi_{p,s}$ is calculated as the sum of $\Psi_{\pi,s}$ and $\Psi_{p,s}$. Initial $\Psi_{p,s}$ is determined by assuming equilibrium between $\Psi_x$ and $\Psi_s$ at the start of the simulation period and estimation of the initial $\Psi_{\pi,s}$:

$$\Psi_{\pi,s}(0) = -\frac{R T C(0)}{MM_{\text{sucrose}}}$$

(8)

where $C(0)$ is the initial sugar concentration in the storage tissue (g g$^{-1}$).

A summary of the model equations, used in the present study, is presented in Fig. 1A.

Model development, simulations, calibrations and identifiability analyses were conducted with PhytoSim (Phyto-IT BVBA, Mariaikerke, Belgium), a software developed for plant modelling and simulation. For model calibration, the simplex method, originally developed by Nelder and Mead (1965) and available in PhytoSim, was used to minimize the weighted sum of squared errors for the variable $D$. Identifiability analysis was done as described by De Pauw et al. (2008).

Methodology

Four steps are involved in the presented approach for non-destructive estimation of root pressure:

Step 1: measurement of sap flow and stem diameter variations; and Step 2: forward simulation and calibration.

Using the model, described in Fig. 1A, $\Psi_s$ and $D$ were simulated for three plants. Estimation of the parameters $\Psi_m$ and $R_s$ was done based on previous measurements of $\Psi_s$, as described by Begg and Turner (1970), on enclosed leaves using the pressure chamber (PMS Instruments Co., Corvallis, OR, USA; $n = 28$; data not shown) and concurrent $F_{H2O}$. These were inserted in eqn (1) and both parameters could be estimated independently. Parameter $\phi$ (cell wall extensibility) was assumed to be very small ($10^{-5}$ MPa$^{-1}$ h$^{-1}$) and other model parameters were adopted from a previous modellng study on tomato (De Swaef and Steppe, 2010) or estimated via model calibration based on daytime $D$ measurements between 1000 h and 2000 h on the first day (Table 1). Parameter $S$ (rate of change of sugar concentration in the storage tissue) was recalibrated daily (Table 2).

Step 3: inverse simulation.

After the forward simulation, the model equations were rearranged in such a way that the measured $D$ was the input variable (Fig. 1B). $\Psi_s$ was then simulated based on these $D$ data, using the model parameter values estimated in step 2.

Step 4: comparison of step 2 and step 3.

The night-time difference between $\Psi_s$ simulated in step 2, and $\Psi_s$ simulated in step 3 resulted in a positive pressure component ($P_s$). Differences following a drop to zero in $P_s$ were small and inconsistent, and were therefore attributed to simplifications inherent to models. These differences were filtered out and set to zero.

RESULTS

Figure 2 shows photosynthetically active radiation (PAR; Fig. 2A), air temperature ($T$; Fig. 2B) and vapour pressure deficit (VPD; Fig. 2C) in the direct vicinity of the monitored plants for a period of six consecutive days starting on 17 September 2010. Pre-dawn VPD in the glasshouse
compartment approximated zero for the first 5 d, as a result of high RH (Fig. 2C). On the last day of the period, pre-dawn VPD remained a little higher compared with the previous 5 d.

Step 1: sap flow and stem diameter variations measurements.

Figure 3 displays sap flow ($F_{H2O}$) and stem diameter variation ($D$) measurements for 6 d for three plants. Profiles of both $F_{H2O}$ and $D$ corresponded well for all plants, indicating the interplant similarity of the response to prevailing environmental conditions as previously reported by De Swaef et al. (2009).

Step 2: forward simulation and calibration.

Using the model, described in Fig. 1A, $\Psi_s$ (Fig. 4A–C) and $D$ (Fig. 4D–F) were simulated for all plants. The simulation of $D$ corresponded well with the measurements during the day,
Table 2. The optimized parameter value of $S$ (rate of change of sugar content in the storage tissue) in g h$^{-1}$ in each time frame and for every plant for expt 1

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plant 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>-0.0009</td>
<td>-0.0005</td>
<td>-0.0029</td>
</tr>
<tr>
<td>24–48</td>
<td>-0.0011</td>
<td>-0.0047</td>
<td>-0.0029</td>
</tr>
<tr>
<td>48–72</td>
<td>-0.0009</td>
<td>0.0055</td>
<td>0.0010</td>
</tr>
<tr>
<td>72–96</td>
<td>0.0045</td>
<td>0.0135</td>
<td>0.0010</td>
</tr>
<tr>
<td>96–120</td>
<td>-0.0017</td>
<td>0.0008</td>
<td>-0.0007</td>
</tr>
<tr>
<td>120–144</td>
<td>-0.0017</td>
<td>-0.0057</td>
<td>-0.0063</td>
</tr>
</tbody>
</table>

but differed explicitly during the nights following day 1, 2, 3 and 4 (Fig. 4D–F), reaching a maximal discrepancy at the end of the night. During the night following day 5, this discrepancy was not visible.

Step 3: inverse simulation.

In step 3, $\Psi_3$ was simulated (Fig. 5A, C, E) based on $D$ data (Fig. 3D–F), using the model parameter values estimated in step 2 and the model equations presented in Fig. 1B. This shows an increase in $\Psi_3$, during the nights following day 1, 2, 3 and 4 which is not visible in $\Psi_3$ simulated in step 2. In the night following day 5, $\Psi_3$ simulations for step 2 and 3 were not different.

Step 4: comparison of step 2 and step 3.

The night-time difference between $\Psi_3$ simulated in step 2, and $\Psi_3$ simulated in step 3 (Fig. 5A, C and E) resulted in a positive pressure component ($P$, Fig. 5B, D and F) for the nights following day 1, 2, 3 and 4.

Manometer measurements of root pressure

The approach described above was validated in an extra experiment on tomato plants in which RH of the air was manipulated to be high during the night. Figure 6A–C shows PAR and VPD for three measurement periods (A, 31 January–3 February 2012; B, 14–16 February 2012; C, 21–25 February 2012). The same model was used for the forward simulation using sap flow as input variable (Fig. 6D–F). Figure 6G–I shows comparison between measured and simulated $D$ for the forward simulation. Parameter values are given in Table 3.

The resulting estimates of root pressure were then compared with actual measurements of root pressure on excised shoots (Fig. 7).

DISCUSSION

Model parameter values

Parameters $\Psi_m$ (substrate water potential) and $R_s$ (hydraulic resistance between soil and stem) were estimated in advance using destructive measurements of stem water potential ($\Psi_s$) (data not shown). Such an initial model calibration has proven its importance to allow unambiguous estimation of the model parameters $\Psi_m$ and $R_s$ (De Pauw et al., 2008; Steppe et al., 2008a). Different estimates for $R_s$ in expt 1 (0.0035 MPa h g$^{-1}$) and 2 (0.0025 MPa h g$^{-1}$) and an earlier study on tomato (0.0057 MPa h g$^{-1}$; De Swaef and Steppe, 2010) may result from plant age, root development, cultivar and presence/absence of a rootstock. Parameter values for $r$ (radial hydraulic resistance), $\Gamma$ (threshold hydrostatic water potential at which wall yielding occurs) and $a$ (proportion of inelastic tissue in the total stem diameter) were adopted from an earlier modelling study on tomato (De Swaef and Steppe, 2010) and $C(0)$ (initial sugar concentration in the storage tissue) was taken from Liu et al. (2007). Cell wall extensibility ($\phi$) is known to decrease as the tissue ages (Liu et al., 2007). Because the measured stem element was rather old, it can be assumed that $\phi$ was close to zero ($1 \times 10^{-5}$ MPa$^{-1}$ h$^{-1}$). For expt 2, $\phi$ was set to 0.001 MPa$^{-1}$ h$^{-1}$, which is an acceptable value for young tomato stems (De Swaef and Steppe, 2010). The subset of parameters $b_0$ (proportionality constant for the elastic modulus) and $S$ (rate of change of sugar concentration in the storage tissue) was found to be identifiable. Consequently, these parameters could be estimated independently using automated calibration in which the sum of squared errors between daytime (between 1000 and 2000 h) simulations and measurements of $D$ was minimized. For $b_0$, this was done once on the first day and the resulting parameter value was used from then onwards. Differences in estimated values for $b_0$ between different plants originate from differences in elasticity, which is affected by the stem tissue age ($\epsilon_0$) increases with age for
Parameter $S$ is determined by loading and unloading mechanisms in the plant, and is therefore influenced by factors such as climatic conditions and crop load. The value of $S$ can be negative or positive, as the sugar content in the storage tissue can decrease or increase. Because climatic conditions were variable among the different days in expt 1, parameter $S$ could not be considered constant for the entire period, but was recalibrated every day (Table 2). In expt 2, parameter $S$ was kept constant for each measurement period, because the climate was similar for the different days within each period.

Parameters $S$ and $\phi$ predominantly determine the simulated overall stem growth rate and could not be estimated independently. Therefore, we opted to attribute a plausible value to parameter $\phi$ (1 × 10$^{-5}$ MPa$^{-1}$ h$^{-1}$ in expt 1 and 0.001 MPa$^{-1}$ h$^{-1}$ in expt 2) and to estimate parameter $S$ automatically. The strong correlation between both parameters is, however, of limited importance in our approach of root pressure estimation: a test with different values of $\phi$ (and corresponding optimized values for $S$) showed no difference in estimated root pressure (data not shown).

**Fig. 3.** Measurements of sap flow ($F_{H2O}$) of plants 1, 2 and 3 (A, B, C, respectively) and stem diameter ($D$) of plants 1, 2 and 3 (D, E, F, respectively) for a period of 6 d starting on 17 September 2010 at midnight. The shaded bands indicate night-time.
Parameters $e_0$ and $R_x$ predominantly determine the diurnal amplitude of variations in $D$, and could also not be estimated independently from each other. The correlation between both parameters could, however, be broken, because $R_x$ was estimated using previous measurements of stem water potential. The independent estimation of $R_x$ is needed, because the value of $R_x$ has an important effect on the estimated values of root pressure.

**General discussion of the method**

Daytime $F_{H_2O}$ and $D$, shown in Fig. 3, demonstrated inverse dynamics in accordance with previous results (De Swaef and Steppe, 2010). Because $F_{H_2O}$ results from a water potential gradient ($\Delta \Psi$) between the substrate and the stem xylem, higher $F_{H_2O}$ rates typically correspond to more negative $\Psi$ values under well-watered conditions [eqn (1)]. Due to the
between xylem and surrounding tissues and is therefore influenced instantaneously by variations in transpiration. Models that are based on the C–T theory, such as our original model (Fig. 1A), can simulate these transpiration-driven variations well (Fig. 4). However, if a mechanism other than transpiration affects \( \Psi_c \), \( \Psi_s \) or the radial hydraulic resistance between xylem and surrounding tissues, the response of \( D \) cannot longer be simulated accurately, because this affecting mechanism is not included in the model.

As such, \( \Psi_s \) could increase under conditions of low transpiration as a direct result of root pressure. An increase in \( \Psi_s \) theoretically will promote water transport from the xylem towards the surrounding tissues, ultimately enhancing \( D \) growth. Our \( D \) measurements show such a night-time increase (Fig. 3D–F), that could not be explained by the original model, based on C–T. The simulations conducted with the model, displayed in Fig. 1A, showed a linear \( D \) growth, whereas the measurements of \( D \) showed an explicit increase in the second half of the night (Fig. 4D–F), except for the night following day 5. During the second part of the first nights, the microclimatic conditions were very humid (Fig. 2C), favouring the build-up of root pressure. Therefore, we suggest that the deviating \( D \) pattern, which was observed between measurements and model simulations, was caused by root pressure. In the night following day 5, VPD remained slightly higher, enabling enough transpiration to prevent the build-up of root pressure.

Root pressure always started to build up at night when VPD approximated zero (Figs 2C and 5). Interestingly, estimated root pressure continued during a significant part of the day, especially on day 5 (Figs 5 and 8). When investigating this day in detail, root pressure continued to increase until 1000 h, and remained constant until 1200 h, whereas sunrise was at 0630 h. Only when the sap flow rate rose sharply at 1200 h did root pressure disappear. Apparently, water was highly available in this period to meet the low rates of transpiration between 0630 and 1200 h. The abundant water in the leaves may have contributed to transpiration, postponing the effects at the stem level. In contrast, root pressure in the 2012 experiment always declined at sunrise (Fig. 7). Because transpiration increased faster after sunrise (Fig. 6D–F), compared with 2010, root pressure probably disappeared immediately. Therefore, further research should take into account these contributions from the leaves using continuous measurements of leaf thickness (Burquez, 1987) or leaf patch clamp pressure probes (Zimmermann et al., 2008; Lee et al., 2012).

In addition to \( \Psi_s \), both sugar content of the storage tissues and stem age are known to affect \( D \) variations (De Swaef and Steppe, 2010). However, it is unlikely that these features have played an important role in the reported night-time \( D \) pattern. First, it is unlikely that the night-time diameter increase could have evolved from an increased sugar content of the storage tissues, because sugar loading generally decreases during the night and plants seem to maintain a constant sugar supply to the sinks (Geiger et al., 2000; Komor, 2000). Secondly, stem ageing is a gradual process, which could be taken into account in the model by assigning a time-dependent function to the cell wall extensibility (\( \phi \)) (e.g. Liu et al., 2007; De Swaef and Steppe, 2010). However, because our work focuses on a period at the end of the growing season and measurements were done on an older part of the stem, \( \phi \) was assumed to be close to zero (1 ×
It was unnecessary to include this dependency in the model. Furthermore, stem ageing is a process that limits $D$ growth (Steppe et al., 2008a, 2008b), whereas the measured $D$ in this study showed a sudden increase.

The magnitude of the destructively measured root pressure using a manometer installed on excised tomato stems agreed well with the model-based estimations (Fig. 7). However, destructively measured root pressure showed a decrease during

![Graph showing PAR and VPD over time]

**Table 3. Model parameters for the three periods in expt 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_x$ (MPa h g$^{-1}$))</td>
<td>0.0025</td>
<td>0.0025</td>
<td>0.0025</td>
<td>Initial calibration</td>
</tr>
<tr>
<td>$\Psi_p$ (MPa)</td>
<td>-0.1</td>
<td>-0.1</td>
<td>-0.1</td>
<td>Initial calibration</td>
</tr>
<tr>
<td>$\phi$ (MPa$^{-1}$ h$^{-1}$)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>Assumption</td>
</tr>
<tr>
<td>$r$ (MPa h g$^{-1}$)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>Initial calibration</td>
</tr>
<tr>
<td>$l$ (MPa)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>De Swaef and Steppe (2010)</td>
</tr>
<tr>
<td>$\alpha$ (dimensionless)</td>
<td>0.8137</td>
<td>0.8137</td>
<td>0.8137</td>
<td>De Swaef and Steppe (2010)</td>
</tr>
<tr>
<td>$C(0)$ (g g$^{-1}$)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>Liu et al. (2007)</td>
</tr>
<tr>
<td>$e_0$ (m$^{-1}$)</td>
<td>478</td>
<td>690</td>
<td>770</td>
<td>Model calibration (step 2)</td>
</tr>
<tr>
<td>$S$ (g h$^{-1}$)</td>
<td>0.000817</td>
<td>-0.000355</td>
<td>0.0035</td>
<td>Model calibration (step 2)</td>
</tr>
</tbody>
</table>

Initial calibration was done via measurements of stem water potential ($n = 48$ in total) before the experiment. Model calibration was done on measurements of stem diameter every 5 min between 1000 and 2000 h on the first day of each period ($n = 120$ per plant).
the night, presumably as a result of decreasing substrate temperature, whereas estimated root pressure in intact leafed plants showed an increasing pattern towards the end of the night. It is therefore hard to relate diurnal dynamics of destructively measured root pressure to diurnal dynamics of transpiring plants: because the excised stems did not transpire during the day, root pressure enhanced during the day because of the higher temperature in the greenhouse, whereas root pressure is not allowed to develop in transpiring plants.

**Critical considerations**

Concurrent with the root pressure-induced night-time increase in $D$, it might be expected that $F_{H,O}$ must increase. This was not always visible in our $F_{H,O}$ data (Fig. 3A–C), but could be clarified by the model calculations. The measured night-time diameter increase corresponded with a calculated maximum water mass inflow of approx. 250 mg h$^{-1}$ for an 8 m long tomato stem, which is 400 times smaller compared with daytime $F_{H,O}$ rates. Because of the low VPD at the end of these nights, plant water loss via transpiration could be neglected. Therefore, nearly all of the upward pushed water flow in the xylem was stored in the plant itself and thus resulted in the observed increase in $D$. However, the sensitivity of the heat balance sensors used in our study did not always allow detection of these low amounts of water flow (van Bavel and van Bavel, 1990).

**Significance**

In the last decades, the reduction of energy use has become an important issue in glasshouse cultivation. This has led to a modified crop management and new energy-efficient climate strategies that may create conditions favouring the build up of root pressure. Because the occurrence of excessive root pressure may cause considerable damage to the harvestable product (Dorais et al., 2001), our approach could be of direct significance to growers, as an indicator when root pressure-associated problems might occur. Because these new energy-efficient technologies allow better glasshouse climate control, potentially unfavourable conditions could be avoided using an intelligently managed compromise between energy saving and optimal growing conditions.

In our study, no instant visible effects of excess root pressure were noticeable, probably indicating that root pressure had not yet reached a harmful level. Because some important physiological problems in horticulture are currently attributed to excess root pressure, a critical level at which root pressure becomes undesired should be determined. To date, the main problem for investigating this critical level, as well as the environmental factors driving root pressure, is the lack of appropriate systems for determining root pressure. Therefore, our non-destructive technique could allow root pressure to be estimated continuously, while imposing a wide range of growing conditions.

Recently, Clearwater et al. (2007) unravelled a correlation between the rootstock affinity to build up root pressure and the scion vigour after grafting in kiwi. For tomato, it is known in practice that rootstocks have an important effect on scion vigour and on the occurrence of root pressure-related problems. Our approach could be used to select rootstocks
quantitatively with a different affinity for root pressure. As such, better combinations of rootstocks and productive grafts could be made.

Because our model and method rely on physiologically sound mechanisms which are valid for a broad range of vascular plants, our approach is perfectly applicable for many other plant species. Our technique to estimate root pressure in a continuous and non-destructive way is therefore a potential breakthrough for fundamental research in plant water relations. As the role of root pressure in general plant functioning is still not well understood, many plant physiological research topics such as cavitation or nutrient translocation could benefit from using this approach.

Conclusions

The combination of continuous measurements of $F_{H2O}$ and $D$ and the mechanistic water flow and storage model allowed elucidation of the effects on $D$ of mechanisms other than the C–T theory, such as root pressure, which is as yet difficult to measure. Therefore, the presented method could have important contributions both in practice and in future root pressure research. As such we believe that our approach can contribute to a future solution of the ‘riddle of root pressure’.

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LITERATURE CITED


