Novel, cyclic heat dissipation method for the correction of natural temperature gradients in sap flow measurements. Part 2. Laboratory validation

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Sap flow measurements conducted with thermal dissipation probes (TDPs) are vulnerable to natural temperature gradient (NTG) bias. Few studies, however, attempted to explain the dynamics underlying the NTG formation and its influence on the sensors’ signal. This study focused on understanding how the TDP signals are affected by negative and positive temperature influences from NTG and tested the novel cyclic heat dissipation (CHD) method to filter out the NTG bias. A series of three experiments were performed in which gravity-driven water flow was enforced on freshly cut stem segments of Fagus sylvatica L., while an artificial temperature gradient (ATG) was induced. The first experiment sought to confirm the incidence of the ATG on sensors. The second experiment established the mis-estimations caused by the biasing effect of the ATG on standard TDP measurements. The third experiment tested the accuracy of the CHD method to account for the ATG biasing effect, as compared with other cyclic correction methods. During experiments, sap flow measured by TDP was assessed against gravimetric measurements. The results show that negative and positive ATGs were comparable in pattern but substantially larger than field NTGs. Second, the ATG bias caused an overestimation of the standard TDP sap flux density of ~17 cm$^3$ cm$^{-2}$ h$^{-1}$ by 76%, and the sap flux density of ~2 cm$^3$ cm$^{-2}$ h$^{-1}$ by over 800%. Finally, the proposed CHD method successfully reduced the max. ATG bias to 25% at ~11 cm$^3$ cm$^{-2}$ h$^{-1}$ and to 40% at ~1 cm$^3$ cm$^{-2}$ h$^{-1}$. We concluded that: (i) the TDP method is susceptible to NTG especially at low flows; (ii) the CHD method successfully corrected the TDP signal and resulted in generally more accurate sap flux density estimates (mean absolute percentage error ranging between 11 and 21%) than standard constant power TDP method and other cyclic power methods; and (iii) the ATG enforcing system is a suitable way of re-creating NTG for future tests.

Keywords: cyclic heat dissipation, Mariotte water-flow verification system, sap flow, temperature gradient, thermal dissipation probe.

Introduction

Thermal methods for measuring sap flow are widely used in hydrology, tree physiology, ecology and other fields to quantify and better understand the water use dynamics in the plant–atmosphere–soil continuum (Marshall 1958, Swanson 1994, Lubczynski 2000, Burgess et al. 2001, Nadezhdina et al. 2002, Lubczynski and Gurwin 2005, David et al. 2007, Dragoni et al. 2009). Such understanding is often related with achieving an accurate and precise estimation of whole-plant water use and how such water consumption can influence the environment (Williams et al. 2004, Hernández-Santana et al. 2008, Lubczynski 2009). In recent years, several studies have analysed the hydrological role of vegetation and its water use based on up-scaled measurements (Granier et al. 1996,
Wilson et al. 2001, Williams et al. 2004, Cavanaugh et al. 2011, Miller et al. 2010, Yaseef et al. 2010). However, many of such studies have not put enough attention to evaluate the accuracy of sap flow measurements and errors or biases that influence them. This is also the case when using the thermal dissipation probe (TDP) method of Granier (1985, 1987), one of the most widely used sap flow measuring methodologies, thanks to its low cost and ease of use. Nevertheless, the TDP has a series of drawbacks that, if not accounted for, can generate a large error while estimating plant water use (Do and Rocheteau 2002a, Lu et al. 2004, Steppe et al. 2010). Most of these drawbacks can be solved or minimized (Lu et al. 2004), but when the TDP method is used in semi-arid open forests or orchards, the bias caused by natural temperature gradients (NTGs) can be significant and difficult to deal with (Braun and Schmid 1999, Do and Rocheteau 2002a, Lubczynski et al. 2012), sometimes even creating errors larger than 100% (Do and Rocheteau 2002b, Reyes-Acosta and Lubczynski 2012).

The influence of NTG biases have been attributed to uneven temperature in the sapwood that affects one or both probes of the TDP sensor (Cermák and Kucera 1981, Lundblad et al. 2001, Do and Rocheteau 2002a, 2002b, Lu et al. 2004, Tatarinov et al. 2005). Several authors (Cermák and Kucera 1981, Köstner et al. 1998, Braun and Schmid 1999, Do and Rocheteau 2002a) attributed the NTG to differences in heat storage in the soil, stem and root tissues resulting in the lower temperature of the sap flowing from the roots early in the morning, as compared with the stem. Lu et al. (2004) demonstrated the role of direct solar radiation onto the stem as an additional source of NTG. Besides these explanations, there is no clear understanding of the exact dynamics generating the NTG influence. Field measurements using unpowered TDPs, to measure the temperature difference between probes installed 10 cm apart, have demonstrated that the NTG can be significant for several species and ranges between the following values: from −2.00 to +1.46 °C in Acacia tortilis (Forssk.) Hayne (Do and Rocheteau 2002a), from −2.45 to +1.38 °C in Quercus ilex ssp. ballota L. (Desf.) Samp, from −0.49 to +1.00 °C in Quercus pyrenaica Willd. (Reyes-Acosta and Lubczynski 2012), from −1.18 to +0.14 °C in Boscia albitrunca (Burch.) Gilg & Ben., from −5.20 to +0.97 °C in Dichrostachys Wight et Arn., from −1.65 to 0.98 °C in Acacia fleckii Schinz and from −1.69 to 0.68 °C in Acacia luedenitzi Engl. (Lubczynski et al. 2012).

Different methods have been proposed to avoid and/or correct the NTG bias, such as: (i) partial-to-full coverage of stem and superficial roots applying reflecting and isolating material (Braun and Schmid 1999, Lu et al. 2004); (ii) simultaneous monitoring of the variability of the NTG on neighbouring trees of the same species and similar size, to account for the NTG bias in the sap flow estimations (Köstner et al. 1998); (iii) measuring the NTG with another set of probes close to the TDP installation and subtracting its influence (Čermák and Kucera 1981); (iv) use of a new design of TDPs including an ‘extra’ pair of thermocouples (Goulden and Field 1994, Nourtier et al. 2011); and (v) use of a cyclic power schema (CPS), i.e., switching repeatedly power ON and OFF, to account for the variability of the NTG patterns on the sap flow measurements (Köstner et al. 1998, Do and Rocheteau 2002a, 2002b, Isarangkool Na Ayutthaya et al. 2010, Lubczynski et al. 2012).

The selection of the method to account for the NTG bias depends on the type of species, study objective, environmental conditions and capacity of the project carried out. However, when the NTG influence is large and a good accuracy and precision is needed, Lu et al. (2004) advised to use a CPS. They highlighted the advantages of cyclic power methodologies, in particular the transient thermal dissipation (TTD) method proposed by Do and Rocheteau (2002b). The TTD uses the same type of probes as designed by Granier but data are measured in short cycles (referred as transients in this paper), a signal rising part corresponding with the power ON (ON-transient) and a signal declining part corresponding with power OFF (OFF-transient). These transients are not long enough to reach thermal equilibrium as is required by the Granier method (Granier 1985), thus another empirical formula was developed and tested (Do and Rocheteau 2002a, 2002b). The TTD method has been validated on synthetic porous medium as to be insensitive to stable natural temperature gradients under laboratory conditions (Do and Rocheteau 2002b). Additionally, validation of the impact of an artificial temperature gradient (ATG) created by inserting an extra pair of heating probes upon the TDP signal was carried out (Do and Rocheteau 2002b). However, such a way of creating an ATG generates a disturbance of the wood matrix that might affect heat dissipation. Later, Isarangkool Na Ayutthaya et al. (2010) developed an improved multispecies calibration for the TTD method. This calibration was derived from tests in three species: Citrus maxima (Burm.) Merr., Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. et Arg., and Mangifera indica L. The tests concluded that the new multispecies calibration covered the variability of sap flux densities better than the calibration obtained in 2002, and thus it was species independent. These findings provided more confidence into the TTD method. However, the multispecies calibration of the TTD method is not as extensively tested as the original Granier calibration (1985) and it is not clear if it was tested for NTG.

The cyclic heat dissipation (CHD) method for correcting the NTG bias of the TDP signal as presented by Lubczynski et al. (2012) in the companion paper constitutes an improved approach as compared with the TTD method because it: (i) provides physically based retrieval of the extrapolated signal to the steady-state required in the Granier method, which means it benefits from the widely tested Granier formula calibrated on many species by many authors; (ii) allows for a more
reliable definition of $\Delta T_{\text{max}}$; (iii) allows a better understanding on how the CPS can be used to correct the NTG bias in the signal. Even though this correction has been tested under field conditions (Lubczynski et al. 2012, Reyes-Acosta and Lubczynski 2012), a laboratory validation under controlled conditions is required to assess its reliability, accuracy, performance and precision.

Thus, the objectives of this study are: (i) to design and evaluate a heating–cooling system to realistically create ATGs comparable to the NTGs observed under field conditions and regulate its strength without affecting the wood tissue; (ii) to understand the influence of variable ATG upon the TDP signal under dissimilar sap flux densities and (iii) to validate sap flow estimations using the CHD correction method under varying ATG conditions and contrasting sap flux densities applying the Mariotte-based verification system of Steppe et al. (2010).

Materials and methods

Collection and processing of plant material

The plant material was collected from two 10–20-year-old European beech trees, *Fagus sylvatica* L., with stems of diameter at breast height (DBH) of ~8 cm located at the experimental forest ‘Aelmoeseneie’ in Gontrode, Belgium. In order to minimize evaporation from the stem tissues, the cut ends of the segments were covered with plastic bags and sealed with duct tape. The segments were transported immediately to the Laboratory of Plant Ecology, Ghent University, where they were stored and re-cut into smaller pieces. A summary of the plant material collected from the stems is given in Table 1. The stem segments were post-processed at the end of each experimental phase, time enough for the silicone layer to fully clogged sapwood vessels. If such vessels were found, and visually examined (using a stereomicroscope) to find even one segment that had 3.6 cm.

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Test 2</th>
<th>Segment 1</th>
<th>Test 2</th>
<th>Segment 2</th>
<th>Test 3</th>
<th>Segment 1</th>
<th>Test 3</th>
<th>Segment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
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<td>28.50</td>
<td>37.40</td>
<td>27.20</td>
<td>33.30</td>
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<tr>
<td>Stem diameter (cm)</td>
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<td>8.91</td>
<td>9.33</td>
<td>8.91</td>
<td>7.92</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sapwood area at reference needle (cm²)</td>
<td>N/A</td>
<td>50.24</td>
<td>56.82</td>
<td>46.66</td>
<td>48.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sapwood depth at reference needle (cm)</td>
<td>N/A</td>
<td>2.6</td>
<td>2.0</td>
<td>3.6</td>
<td>2.5</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 1. Biometric measurements of the stem segments of European beech (*F. sylvatica*) used in the tests.

Mariotte-based verification system

To provide a continuous, controlled water flow in the CHD validation experiment, the gravity-driven Mariotte-based verification system was used to enforce water flow in the collected stem segments, as proposed by Steppe et al. (2010) (Figure 1). This system consists of a Mariotte's bottle suitable to maintain a constant head of water (*McCarthy* 1934) during an experiment. The system was built using a 5 l Erlenmeyer flask and two glass tubes placed at the same depth in the flask. One of the glass tubes worked as an air inlet and the second one was connected through plastic tubing to a third one. The third tube acted as a siphon inside a plastic column filled with water and attached to the stem segments. The Erlenmeyer flask was placed on two platform jacks, so the water flow could be controlled by adjusting the height of the flask and, hence the bottom of the air inlet. The augmented pressure then delivered the necessary amount of water to the water column in the stem segment through the siphon tube and maintained equilibrium at the required water head h ($h$ = distance between the meniscus in the air inlet and the surface of the stem segment). The enforced water flow was continuously measured gravimetrically using an electronic balance (PS 4500/C1, Henk Maas Weegschalen BV, Veen, the Netherlands).

For a proper functioning of the water flow system, the stem segments required some processing in order to assure a continuous unbiased flow. First, the cut surfaces were rewetted and visually examined (using a stereomicroscope) to find eventually clogged sapwood vessels. If such vessels were found, the wood surface was trimmed using a scalpel until the vessels were completely opened. Second, a 2 cm wide bark-strip was removed from the upper side of the stem segments to firmly attach a plastic column directly on the sapwood. This assured that water was only conducted through the xylem. The plastic column was fixed with a double-sided adhesive tape and a thick silicone layer. Two stem segments were prepared ~18 h before each experimental phase, time enough for the silicone paste to harden properly. These and more details are given in Steppe et al. (2010).

Artificial thermal gradient enforcement

In order to induce ATGs in the stem segments, an experimental ATG enforcement system was designed to expose the segments to two contrasting temperatures: (i) air temperature and (ii) temperature enforced in the topside of the stem. The ATG enforcement system consists of three parts: (i) a temperature-regulated water circulation system, adjusting stem temperature; (ii) air temperature regulation; and (iii) a thermal infrared camera to monitor and verify the formation of the ATG on the segments (NEC, Thermal Imager R300 PANORAMA, Tokyo, Japan).

The temperature-regulated water circulation system is a flexible plastic tubing of 1 cm diameter and ~2 m length connected to a water thermostatic bath (20L GRANT GD120,
Cambridge, UK) with a cooling unit (GRANT C1G, Cambridge, UK). The tubing transports water kept by the bath at a constant temperature, at the following temperature range: 0–36 °C. The tubing was wrapped around the upper side of the stem to mimic the heat effect observed during the NTG formation on trees in the field (i.e., differences of storage and thermal resistance of the soil–wood continuum). The air temperature was regulated by an air-conditioning system inside a growth room, where the experiments took place. The room was ~5 m² and provided the possibility of swiftly changing the air temperature from 15 to 30 °C, a temperature range similar to night and day air temperatures in semi-arid regions.

The ATG enforcement system was operated in two contrasting modes simulating the most critical NTG field conditions, early in the morning and after sunset. In Mode I for simulating NTG after sunset, the circulating water from the top representing stem temperature was set at 36 °C and the air temperature at 15 °C, as observed in semi-arid open forests in Salamanca, Spain (Reyes-Acosta and Lubczynski 2012). In Mode II simulating early morning NTG, the circulating water temperature was set at 10 °C and the air temperature at 29 °C. The formation of the temperature gradients was monitored with the thermal camera by taking images every 10 min (Figure 2).

**Thermal dissipation probes for measuring sap flux densities**

We monitored the sap flux density passing through the stem segments in each of the experiments using 20 mm long, standard Granier-type (Granier 1985) TDP sensors (UP GmbH, Germany). The TDPs were installed radially in the stems, 10 cm apart and 2 cm deep, with the heating probe placed at the downstream water flow position, i.e., below the non-heated probe.

The TDP method uses an empirical calibration, which relates the temperature difference between the unheated and heated probe ($\Delta T$ in °C) to the sap flux density ($J_p$ in cm³ cm⁻² h⁻¹), as in Eq. (1) (Granier 1985)

\[
J_p = 0.119K_a^{1.231} \times 3600  
\]

(1a)

\[
K_a = \frac{\Delta T_{\text{max}} - \Delta T}{\Delta T}  
\]

(1b)

where $\Delta T_{\text{max}}$ is a reference temperature difference when $J_p$ is equal to zero, i.e., at no flow conditions. In this study the $\Delta T$
measurements for standard TDP (with constant power) were recorded every 30 s and stored at 10 min intervals in a Skye Datahog2 logger while \( \Delta T_{\text{max}} \) was determined at the end of each experiment at no-flow condition, by measuring \( \Delta T \) until its stabilization was achieved.

**Cyclic power scheme for correcting the influence of ATG in TDP**

The CHD method was applied using a cyclic power scheme (CPS) of \( \Delta T \) data acquisition, as suggested by Lubczynski et al. (2012). The CPS consisted of 30 s resolution measurements in consecutive cycle transients of 7.5 min power ON and 7.5 min power OFF, resulting in four cycles per hour. This time set for the ON–OFF transients was defined by optimization of the minimum number of samples per interval, still allowing an adequate extrapolation of the signal to asymptotic thermal equilibrium (Figure 3). The alternated ON–OFF cycles were controlled using a custom-specific, programmable power-relay regulator designed to count switching power intervals controlled by the logger.

The principle of the CHD method (Lubczynski et al. 2012) is to subtract the NTG bias \( \Delta T_{\text{NTG}}^E \) from the extrapolated, to steady-state, power ON signal \( \Delta T_{\text{ON}}^E \) (Eq. (2)).

\[
\Delta T_{\text{ON}}^E = \Delta T_{\text{ON}}^0 - \Delta T_{\text{NTG}}^E \tag{2}
\]

\[
\Delta T_{\text{NTG}}^E = 0.5(\Delta T_{\text{OFF},-1}^E + \Delta T_{\text{OFF},+1}^E) \tag{3}
\]

In Eq. (2), \( \Delta T_{\text{ON}}^0 \) is an NTG-corrected \( \Delta T \) (in this case ATG-corrected) in the \( i \)-th transient, \( \Delta T_{\text{NTG}}^E \) is the temperature bias caused by the NTG influence and \( \Delta T_{\text{ON}}^E \) is \( i \)-th cycle power ON signal extrapolated to steady state. In Eq. (3), \( \Delta T_{\text{NTG}}^E \) is calculated as an average of the two extrapolated to steady-state OFF-transients, the preceding \( \Delta T_{\text{OFF},-1}^E \) and succeeding \( \Delta T_{\text{OFF},+1}^E \) (Figure 3). The extrapolated to steady-state \( \Delta T_{\text{ON}}^E \),

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**Figure 2.** Infrared images monitoring the ATGs produced by the ATG enforcement system on the stem segments during the experiments: (a) shows the stem segments under Mode I and (b) under Mode II. The TDP reference needle is ~10 cm below the tubing and the heating probe another 10 cm below. The TDP sensors were intentionally left out of the images (they are in the backside of the stem), to avoid interferences with the observation of the temperature gradients.

**Figure 3.** Thermal dissipation probe alternated-power cycle (bold line) with its corresponding extrapolated ad-infinitum transients ON/OFF (dotted lines). Zoomed areas emphasize the position of the variables needed for Eqs. (2)–(6) in the NTG-bias correction.
\[ \Delta T_{ON}^E = \Delta T_{OFF}^E + A_1(1-e^{-t/T_1}) + A_2(1-e^{-t/T_2}) + A_3(1-e^{-t/T_3}) \]  

(4)

\[ \Delta T_{ON}^E = \Delta T_{OFF}^E - \left[ A_1(1-e^{-t/T_1}) + A_2(1-e^{-t/T_2}) \right] + A_3(1-e^{-t/T_3}) \]  

(5)

\[ \Delta T_{ON}^E = \Delta T_{OFF}^E - \left[ A_1(1-e^{-t/T_1}) + A_2(1-e^{-t/T_2}) \right] + A_3(1-e^{-t/T_3}) \]  

(6)

In Eqs. (4), (5) and (6) the time \( t = \infty \) and \( A_1, A_2, A_3 \) are parameters defining amplitudes of the following thermal components: 

(i) the heater filament and all the probe components; 
(ii) the aluminium tubes and silicon compound (inserted in the sapwood during installation of the probe); and 
(iii) the sapwood tissue. \( T_1, T_2 \) and \( T_3 \) are time constants defining the operating time of each thermal component. Fitting the amplitude and time parameters for the model to extrapolate the raw signals of each cycle (Eqs. (2), (4) and (5)) is a complex and time-consuming procedure. Thus, an automated MATLAB routine was used for: 

(i) fitting the signal by optimizing \( A_i \) and \( T_j \); 
(ii) calculating the NTG correction; and 
(iii) \( J_p \) calculation. In the optimization process \( T_1 \) and \( T_2 \) were set as time-independent constants as they refer to non-variable circuits (i.e., heat filament, probes and aluminium tube), as proposed by Lubczynski et al. (2012). However, parameters \( T_3, A_1, A_2 \) and \( A_3 \) were optimized for each transient, according to Eqs. (2) and (3), respectively.

**Experimental design**

The experiments were developed under laboratory conditions to: (i) provide a controlled environment in which it was possible to regulate the flowing water in fresh stem samples; and (ii) control external sources of heat affecting air and stem temperature to mimic NTG dynamics resulting from the contrasting and temporally variable day/night temperature variability. The water used for all tests was deionized and stored inside the growth room to maintain the same temperatures of the other elements and hence optimal for its use.

**Test 1: ATG formation monitoring**

The first test was designed to assess whether the ATG enforcement system was suitable to create temperature gradients comparable to those affecting the TDP signal in field conditions. Thus, the objective was to create, regulate and control the ATG simulating NTG. To monitor the ATG, an unpowered TDP sensor was installed in the stem segment exposed to the Mariotte-based verification system (Figure 1), set on zero water flow. The test started at 16:30 in Mode I (air temperature at 15 °C and circulating water temperature at 36 °C) and continued until 9:00 the next day when Mode II (air temperature at 29 °C and circulating water temperature at 10 °C) was started and lasted until 18:00 (Figure 4). The obtained \( \Delta T \) measurements were assessed according to the threshold value for non-negligible temperature gradients proposed by Do and Rocheteau (2002a). They proposed, as a first approximation, that if \( \Delta T \) values are \( >0.2 \) °C or \( <-0.2 \) °C, the impact of NTG upon the TDP signal generates a non-negligible ≥10% error in sap flow rate. Thus, \( \Delta T \) measurements crossing that

![Figure 4. Test 1: monitoring the ATG with un-powered TDP signal at zero flow. AT, air temperature; CW, circulating water temperature.](image-url)
threshold were considered as an indication of significant ATG influence upon the TDP signal.

**Test 2: ATG effect upon the standard TDP signal and water flow estimations**

In the second test, the effect of ATG on the standard TDP signal and the sap flux density estimation was monitored and compared with the gravimetric sap flux density. Two stem segments with plastic columns were used and one set of TDP sensors with continuous power supply was installed in each stem segment as described before. Then, each stem column was filled with ~7 cm³ of water to verify that it was flowing through the stem segments and that there were no leaks in the columns. The obtained sap flux densities were different in each segment due to their unique wood-tissue characteristics, even with the same water levels in the column. As a final step, the plastic tubing from the ATG enforcement system was wrapped around the segments.

At the beginning of the test, from 11:30 to 13:15 h, the ATG enforcement system was at the same temperature as the room, i.e., without ATG formation, to guarantee that the temperature from the TDP signal and the flowing water achieved thermal equilibrium. This period was called ‘stabilization’. At 13:15 h, the ATG enforcement system was switched to Mode I (air temperature at 15 °C and circulating water temperature at 36 °C) and then after 2 h and 15 min (at 15:30 h) to Mode II (air temperature at 29 °C and circulating water temperature at 10 °C) and let run for 2 h and 30 min, until 18:00 h (Figure 5).

The sapwood area of both segments was measured by adding a dye (commercial food colorant) to the water column following Steppe et al. (2010). The chemical was let run for >7 h to properly stain the whole sapwood area of the segment. Several discs were cut after staining and verified that the installations of the TDP sensors properly covered the sapwood tissue (~2 cm depth) and were not in contact with the heartwood. Additionally, to guarantee low circumferential \( J_p \) variability in the selected segments, microscopic investigations of the uniformity of the colouring dye across the conductive sapwood area were carried out in the cut discs. If a segment presented a non-uniform dying pattern of sapwood area, the results were considered as uncertain and excluded from further analysis.

This procedure followed the findings of Cermák et al. (2000) and Saveyn et al. (2008), who pointed out the relation between the uniformity of the conductive sapwood structure and low circumferential \( J_p \) variability.

The measured sapwood areas were used to convert the gravitational flow into gravitational flux densities (Grav-Flux in Figure 5) in order to compare them with \( J_p \) obtained with standard TDP (presented as Standard-TDP in Figure 5). The gravitational flux densities followed low (0.5–2 cm³ cm⁻² h⁻¹) and mean values (11–14 cm³ cm⁻² h⁻¹) of the \( J_p \) range observed in field conditions for *F. sylvatica*. This range corresponds to the lower \( J_p \) value range for the species, ensuring low radial variability (Steppe et al. 2010), and low circumferential variability (Saveyn et al. 2008).

**Test 3: Validation of the CHD method for NTG correction**

The third test was designed to validate the efficiency of the CHD method to correct CPS TDP measurements biased by ATG. This was done by comparing the sap flux density estimations obtained from the CHD method with the gravimetric sap flux densities.

Following the same procedure as in Tests 1 and 2, new stem segments were prepared and one set of TDP sensors installed in each segment. During this test, the two segments produced different sap flux densities (11 cm³ cm⁻² h⁻¹ for segment 1 and 1 cm³ cm⁻² h⁻¹ for segment 2), despite the use of same water levels in the columns (Figure 6), as in Test 2. The test started at 12:30, and first the system was stabilized for 30 min. At 13:30 the ATG enforcement system was set in Mode I (air temperature at 15 °C and circulating water temperature at 36 °C), and run for 2 h and 15 min. After this time period the ATG enforcement system was set in Mode II (air temperature at 29 °C and circulating water temperature at 10 °C), and let run for 2 h and 15 min. During the whole test, one set of TDP sensors per segment was used to acquire CHD ATG data at 7.5 min powered and unpowered transients (ON/OFF) with the ATG sampling and storage resolution of 30 s. Simultaneously the actual gravitational flux density was obtained by dividing the gravimetric flow by the sapwood area of the stems. The sapwood area was measured following the same procedures as for Test 2, and the stem segments were verified for uniformity of the sapwood structure to ensure low circumferential variability.

Additionally, two extra repetitions were performed, to verify the reproducibility of the CHD method in NTG correction following the same procedure as in Test 3.

**Comparing CHD and TTD methods**

The CHD sap flux densities \( J_p \) were compared with \( J_p \) estimates of the TTD method corresponding to different CPS: (i) CPS 10 min ON and 20 min OFF with the calibration of Do and Rocheteau (2002b)—data acquired by CHD extrapolation from 7.5 min raw data; (ii) CPS 10 min ON and 20 min OFF with the calibration of Isarangkool Na Ayutthaya et al. (2010)—data acquired by CHD extrapolation from 7.5 min raw data. Additionally, for assessing the fit of the extrapolated ON–OFF cycles, TTD was applied using the raw data (7.5 min ON and 7.5 min OFF) for both TTD calibrations. For each calibration, the raw and extrapolated TTD \( J_p \) estimates were consistently similar (10% difference). Thus, in order to follow the most updated CPS recommendation for both TTD calibrations (Isarangkool Na Ayutthaya et al. 2010), the TTD estimates extrapolated to 10 and 20 min were also used for comparison with the CHD \( J_p \) and with the reference gravimetric sap flux densities.
To calculate the TTD calibration of Do and Rocheteau (2002b) (TTD-2002) the following equation was used on the extrapolated ΔT data obtained from Eq. (6):

\[ J_p = \frac{11.3K_a}{(1 - K_a)^{0.707}} \]  

where \( J_p \) is the sap flux density and \( K_a \) is a transient flow index. \( K_a \) was calculated from

\[ K_a = \frac{(\Delta T_{oa} - \Delta T_s)}{\Delta T_s} \]

where \( \Delta T_{oa} \) is the maximum alternated temperature difference under zero flow and \( \Delta T_s \) is the transient or alternate signal. The alternated power \( \Delta T_{oa} \) value necessary for applying the TTD method was defined from 10–15 min extrapolation of an ON cycle under zero flow conditions, using the alternated data collected from both segments. The \( \Delta T_s \) was defined as

\[ \Delta T_s = \Delta T_{on} - \Delta T_{off} \]

where \( \Delta T_{on} \) is the temperature difference reached at the end of a powered cycle and the \( \Delta T_{off} \) is the temperature difference at

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Figure 5. Test 2: impact of ATG upon the Standard-TDP measurements for two stem segments with different sap flux densities (Segment 1 with \( J_p \sim 17 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1} \) and Segment 2 with \( J_p \sim 2 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1} \)): (a) raw TDP measurements of ΔT for both stem segments; (b) comparison of Standard-TDP \( J_p \) vs. gravimetric control (Grav-Flux). AT, air temperature; CW, circulating water temperature.
the end of an unpowered cycle. The $\Delta T_{\text{off}}$ was calculated by averaging the OFF cycles before and after an ON cycle.

To calculate the TTD of Isarangkool Na Ayutthaya et al. (2010) (TTD-2010), the same set of equations from TTD-2002 was used (Eqs. (7)–(9)), but the multi-species calibration for calculating $J_p$ (Eq. (10)) was used instead.

$$J_p = 12.95K_s \quad (10)$$

**Error calculations**

Three measurements of sap flux density error were used to report the results of the laboratory validation in terms of the disagreement between the gravimetric reference and the estimated sap flux densities. The relative percentage error (%Error) provided information on the under–overestimations during the experiments in proportion to the magnitude of the gravimetric reference and in time. Such events were indicated as a negative error for overestimations and positive for underestimations. The %Error is calculated as follows:

$$\%\text{Error} = \frac{\text{Gravimetric} - \text{Estimated}}{\text{Gravimetric}} \times 100 \quad (11)$$

The second measurement of error, the 'residual', is a straightforward measurement of deviation of the estimated data in the same units of measurements (in this study °C); however, it does not take into consideration the magnitude of the gravimetric flux density. The 'residual' is calculated as follows:

$$\text{Residual} = \text{Gravimetric} - \text{Estimated} \quad (12)$$

Finally, the mean absolute percentage error (MAPE) was calculated to determine the overall error. The MAPE indicated the level of accuracy for each correction methodology avoiding miscalculations due to self-cancellation of under–overestimations in the whole time series. The MAPE provided a way to objectively compare the assessed methods and was calculated as follows:

$$\%\text{MAPE} = 100 \sum_{i=1}^{n} \left| \frac{\text{Gravimetric}_i - \text{Estimated}_i}{\text{Gravimetric}_i} \right| \quad (13)$$
Results and discussion

Characteristics of the plant material

Table 1 presents the biometric characteristics of the stem segments used during the experiments. From this information it is clear that the TDPs were completely surrounded by xylem tissue and not in touch with the heartwood (≥ 2 cm for all segments). All the stems had regular shape, the stem diameter was on average 8.5 cm, with low variations (std. dev. = 0.6 cm), and all the probe installations covered between 50 and 90% of the cross-sectional sapwood area.

This, together with the low \( I_p \) radial variability obtained with cut segments under laboratory conditions at \( I_p < 17 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1} \) (Steppe et al. 2010), reduced the probability of \( I_p \) miss-estimation due to radial water flow variability as pointed out by Clearwater et al. (1999). They concluded that radial variability within 2 cm of the TDP is more consequential for steep sap flux gradients, which was not the case in our measurements.

Test 1: confirming ATG formation

The infrared images confirmed that the ATG enforcement system in Mode I and Mode II was able to generate substantial ATG on stem segments (Figure 2a and b). This can be observed in the colour gradient that stretched from the plastic tubing towards the stems and indicating the direction of conducted heat. In Mode I the ATG enforcement system increased the stem temperature (warmer colours in close contact with plastic tubing in Figure 2a), while in Mode II it decreased the stem temperature (cooler colours in close contact with plastic tubing in Figure 2b). In both modes, most of the stem surface was primarily affected by air temperature, which was warmer in Figure 2b than in 2a. The difference between circulating water temperature on the stem and the air temperature forms the ATG.

The enforced temperature conditions of Mode I created an ATG influence revealed by negative \( \Delta T \) in Test 1 under no-flow conditions (Figure 4). Such influence corresponds to the warm gradient in the infrared image in Figure 2a. A negative \( \Delta T \) means that the upper probe located closest to the tubing (lower probe in the standard TDP installation) had a higher temperature than the lower probe closer to the stem bottom outlet (the one normally heated probe in the standard TDP installation). Such gradient decreases the magnitude of the recorded, biased by ATG, \( \Delta T \) values. The Mode I was started at 16:30 with immediate influence of the heat of the ATG enforcement tubing. After 3 h, i.e., at ~19:30, the \( \Delta T \) reached a point of quasi-stabilization lasting for another 13.5 h until 09:00 of the next day when \( \Delta T \) reached nearly −3 °C. This covered the whole spectrum of negative NTG (\( \Delta T \) biased by temperature gradient) reported so far in field conditions (Do and Rocheteau 2002a, Lubczynski et al. 2012, Reyes-Acosta and Lubczynski 2012).

The ATG enforcement of Mode II resulted in an ATG revealed by positive \( \Delta T \) in Figure 4 and in the cold blue colours in the infrared image in Figure 2b. A positive \( \Delta T \) means that the upper probe located closest to the tubing was more influenced by the low temperature than the probe closest to the stem bottom outlet (the one normally heated probe in the standard TDP installation). Such a gradient increases the magnitude of the recorded, biased by ATG, \( \Delta T \) values. The Mode II started at 09:00 immediately after termination of Mode I (Figure 4). Transition to Mode II took place as an abrupt change in ATG due to the reversing of the ATG enforcement conditions. This resulted in a sharp rise of \( \Delta T \) from approximately −3 °C to 0 °C in 1.5 h and further to 1.5 °C within another 1.5 h, i.e., at 12:00, when the semi-stabilization started. The final stabilization at \( \Delta T = +2.6 \text{ °C} \) was achieved after another 6 h, i.e., at 18:00 when the Test 1 was concluded. This covered the whole spectrum of positive NTG (\( \Delta T \) biased by temperature gradient) reported in field conditions (Do and Rocheteau 2002a, Lubczynski et al. 2012, Reyes-Acosta and Lubczynski 2012).

Test 1 showed that the proposed laboratory ATG enforcement system was able to generate large ATG influences on the TDP signal, −1.5 times larger than typical field observations of NTGs that approximately vary between −2.5 and 1.5 °C (Reyes-Acosta and Lubczynski 2012). Thus, the ATG enforcement system can be considered as a suitable laboratory method to create thermal gradients that bias the \( \Delta T \) signals of TDP, and hence it is adequate for validating the CHD method.

Test 2: understanding the biasing dynamics of ATGs

Test 2 presented in Figure 5 followed three sequential stages: (i) stabilization; (ii) Mode I; and (iii) Mode II. During the initial stage of stabilization the TDP signal reached thermal equilibrium after ~45 min, i.e., at 12:15 (Figure 5a) in both segments and continued for 1 h until 13:15. During this 1 h period, the \( I_p \) Standard-TDP\(_1\) and Standard-TDP\(_2\) in Figure 5b reached comparable values to the \( I_p \) gravimetric measurements Grav-Flux\(_1\) and Grav-Flux\(_2\), showing a mean underestimation of 3% for Segment 1 (16 cm\(^3\) cm\(^{-2}\) h\(^{-1}\)) with high flow and 27% for Segment 2 (2 cm\(^3\) cm\(^{-2}\) h\(^{-1}\)) with low flow. These results show a better agreement with the gravimetric measurements than the 60% underestimation found by Steppe et al. (2010) for American beech across a range of sap flux densities between 5 and 80 cm\(^3\) cm\(^{-2}\) h\(^{-1}\), using the same validation methodology. This disagreement might be attributed to the type of TDP sensor used by Steppe et al. (2010), a modified version of the original design (30 mm long instead of 20 mm) with a probe spacing of 40 mm (Dynamax Inc., Houston, TX, USA), which does not follow the original design of Granier (1985). In this study, the UP sensors were used for all the tests, which are built following the original Granier design. The good agreement between \( I_p \) Standard-TDP and \( I_p \) Grav-Flux without ATG bias proves appropriate TDP performance in optimal conditions.
Mode I was activated at 13:15. In the first 15 min ΔT in both segments started to decrease (Figure 5a) due to the fast effect of the heat released by the tubing, influencing the upper reference probe, thus continuously decreasing the measured ΔT. A similar fast effect was also observed at the beginning of Test 1 under Mode 1. At about 13:30 ΔT of Segment 1 (Standard-TDP1, with high \( J_p \sim 17 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1} \)) sharply changed its pattern starting to increase. This was likely attributed to the moment when the heat front arrived to the lower heated probe, a process dominated by conduction enhanced by a fast water flow. After 30 min, i.e., at ~14:00, the ΔT pattern changed again sharply, decreasing for another 1 h. This ΔT reduction was likely attributed to the arrival of the convective heat ‘dragged’ along the stem that gradually homogenized the temperature along the reference and heating probes. This is indicated by ΔT gradually reaching a quasi-stabilization at about 11 °C, observed after 15:00 for 30 min. This stabilization was possible only because: (i) the water flow was fast and constant; and (ii) the temperature conditions for simulating ATG were constant as well (air and stem temperature). Under field conditions the air temperature and \( J_p \) are highly variable, so NTG is always present but changing.

In contrast, ΔT of Segment 2 (Standard-TDP2, with low \( J_p \sim 2 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1} \)) continuously decreased during Mode I due to the effect of the heat released by the tubing, influencing the upper reference probe. A gentle inflection point was observed around 14:00, likely indicating the moment when the moving heat front reached the lower heated probe. From that moment the reduction of the ΔT was less distinct, although it did not reach the stabilization within the time frame of Mode 1, due to the low flow and therefore low speed of heat redistribution. At 15:30 the ATG enforcement system was changed to Mode II. A similar pattern was observed as for Mode I but with inverse magnitudes of ΔT for both segments, and likely the same explanation of heat transfer but with cooling instead of heating released from the tubing (Figure 5b). During the first 15 min ΔT followed an increasing effect for both segments. At 15:45 ΔT of the Segment 1 (Standard-TDP1 in Figure 5) started to decrease until it reached a minimum at 17:25 when it suddenly started to increase towards thermal equilibrium. From 16:30 to 17:30 a series of peaks can be observed, but this was likely due to external disturbances caused by entering and exiting the growth room where the experiments took place. In the case of Segment 2 (Standard-TDP2), after a time lag of about 15 min, ΔT started to increase continuously. As of 17:00, this increasing effect slightly diminished.

The maximum overestimations of \( J_p \) as compared with the gravimetric controls (Grav-Flux1 and Grav-Flux2) during Test 2 were (Figure 5b) 1.76 times for Standard-TDP1 and >8 times for Standard-TDP2. The minimum underestimations of \( J_p \) were 0.4 times for Standard-TDP1 and 0.6 times for Standard-TDP2 (Table 2). Do and Rocheteau (2002a) calculated the overestimating effect of NTG upon TDP as >100%. Lubczynski et al. (2012) found that for low flows (1–2 cm\(^3\) cm\(^{-2}\) h\(^{-1}\)) the NTG bias created overestimations of 6 to 8 times higher than the control.

The results of Test 2 showed that: (i) the ATG enforcement system was able to create ATG biases of \( J_p \) even higher than those affecting TDP sensors in field conditions; (ii) the TDP sap flux density estimates had good agreement with the gravimetric measurements for both segments in the absence of ATG influence; (iii) the ATG bias upon the TDP signal depends on how strong the temperature gradient is and on the magnitude of \( J_p \) (stronger bias at low \( J_p \) than at high \( J_p \)); (iv) with higher \( J_p \) the time to reach thermal stabilization is shorter; and (v) the effect of the created ATG upon the measured ΔT was detected quite early in the experiment, due to the combined effect of heat convection and conduction.

### Test 3: assessing the accuracy of CHD to correct the ATG bias

The CPS data needed to calculate the CHD correction were processed with an automatic routine in which each ON/OFF interval was extrapolated to steady-state equilibrium according to a physical model (Lubczynski et al. 2012) optimized with a least-squared method algorithm. The optimization provided a set of calibrated \( A \) and \( T \) parameters, for Eqs. (4)–(6). The statistical residuals of the extrapolations (measurement of deviation between the simulated and measured signals) were carefully verified to avoid sub-optimal or ill-posed calibrations. The processed CHD data consist of three outputs: (i) the ATG obtained by extrapolation of the OFF intervals according to Eqs. (5)–(6) averaged with Eq. (3); (ii) ATG biased ΔT obtained by extrapolation of intervals ON as per Eq. (4), corresponding to the signal of constantly powered scheme (i.e., Standard-TDP); and (iii) ATG-corrected ΔT according to Eq. (2). Outputs (ii) and (iii) were further processed using Granier’s calibration to obtain the \( J_p \) estimates of ATG biased (Standard-TDP) and ATG corrected (CHD-Flux), respectively (Figure 6).

The ATG-biased ΔT signal of both stem segments (ΔT Extr.-ON1 in Figure 6a and ΔT Extr.-ON2 in Figure 6b) followed similar patterns as in Test 2 for Mode I and II: (i) Segment 1

**Table 2. Basic statistics of sap flux density measurements and estimates for Test 2 excluding stabilization period.**

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Grav-Flux in cm(^3) cm(^{-2}) h(^{-1})</th>
<th>Standard-TDP flux in cm(^3) cm(^{-2}) h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segment 1</td>
<td></td>
<td>Segment 2</td>
</tr>
<tr>
<td>Max</td>
<td>18.30</td>
<td>32.16</td>
</tr>
<tr>
<td>Min</td>
<td>15.23</td>
<td>9.04</td>
</tr>
<tr>
<td>Mean</td>
<td>16.32</td>
<td>15.82</td>
</tr>
<tr>
<td>Std.</td>
<td>0.79</td>
<td>4.61</td>
</tr>
</tbody>
</table>

Tree Physiology Online at http://www.treephys.oxfordjournals.org
in Test 3 showed a double peak that started in Mode I and reversed in Mode II, and (ii) for Segment 2 (faster water flow) a parabolic shape. These results indicated that the combined effect of (i) the heat conduction due to the ATG enforcement, and (ii) the heat convection due to the flowing water took place during Test 3 as well.

After the stabilization period $\Delta T_{\text{Extr.-ON}_1}$ during Mode I showed an initial decrement for 1 h, between 13:00 and 14:00, similar to $\Delta T_{\text{Standard-TDP}_2}$ in Test 2 but less steep, between 13:00 and 13:30 in Figure 5b. It corresponds to the increase of the temperature of the upper reference probe, thus a decrease in $\Delta T$. At 14:00, the $\Delta T_{\text{Extr.-ON}_1}$ reversed and started to increase as a result of the arrival of the heat dragged from the tubing to the lower probe. After about 1 h a quasi-stabilization at 9°C was achieved due to the gradually homogenized temperature along the distance between the reference and heating probes. The stabilization period lasted for another 0.5 h after which Mode I was terminated. In Mode II of Test 3, the Segment 1 had similar behaviour but opposite pattern, which was explained by similar heat transfer mechanisms but with a cooling effect of the tubing instead of warming and opposite air temperature.

For Segment 2 in Mode I and II, $\Delta T_{\text{Extr.-ON}_2}$ followed a similar pattern as $\Delta T_{\text{Standard-TDP}_2}$ in Test 2 but the range of magnitudes of $\Delta T$ was different (Test 2 ranged between 7 and 11°C and Test 3 between 8.5 and 9.5°C). This difference could be attributed to differences in $J_p$ (−1 cm$^3$ cm$^{-2}$ h$^{-1}$ in Test 1 and −2 cm$^3$ cm$^{-2}$ h$^{-1}$ in Test 2) and the differences in the length and the sapwood tissue arrangement of both segments. Segment 2 used in Test 3 had a considerably larger sapwood depth (50% larger) than the segment of Test 2 (see Table 1).

The $\Delta T_{\text{Extr.-ON}}$ measurements extrapolated to steady-state thermal equilibrium reflecting the ATG bias in Mode I and Mode II ($\Delta T_{\text{Extr.-OFF}_1}$ and $\Delta T_{\text{Extr.-OFF}_2}$) in general followed the pattern observed for the $\Delta T_{\text{OFF}}$ ATG-biased in Test 1, but the magnitudes were different (Test 1 was between −3 and 2.3°C whereas Test 3 was between −1.5 and 1.5°C). This difference could be explained by three possible reasons: (i) for Test 1 the gravimetric $J_p$ was kept at zero thus the heat convection was minimal, limiting heat exchange and therefore enlarging ATG; (ii) the length of the stem segment in Test 1 was 10 cm shorter than the two segments of Tests 2 and 3 (see Table 1), implying that the tubing was ~3 cm closer to the reference probe; and (iii) as a previous example indicated different thermal properties of the stem segments is another possible reason.

By comparing $\Delta T_{\text{Extr.-ON}}$ (with ATG bias) and $\Delta T_{\text{Corrected}}$ (without ATG bias) versus $\Delta T_{\text{Extr.-OFF}}$ (representing the ATG bias), it can be seen that the CHD correction removed the parabolic ATG bias patterns resulting in an unbiased nearly straight line of the corrected signal (Figure 6). This $\Delta T_{\text{Corrected}}$ signal resulted in $J_p$ estimates (CHD-Flux) that were in good agreement with the gravimetric measurements (Grav-Flux$_1$ and Grav-Flux$_2$) as seen in Figure 6, the boxplot in Figure 7 and the accumulated water calculations in Figure 8. The CHD-Flux$_1$ and CHD-Flux$_2$ covered the maximum and minimum values of the actual sap flux densities satisfactorily (Grav-Flux$_1$ and Grav-Flux$_2$) as can be observed in Table 3 and Figure 7. The results for Test 3 showed that the CHD method works better when the heat convection is high, as is the case at high sap flux densities. The repetitions of Test 3 showed the same patterns described for Mode I and Mode II during Test 3.

An important consideration for the results obtained in Test 3 is that a single set of TDP sensors is not sufficient for field measurements in trees with significant circumferential variability. In such trees it is necessary to use at least three sets of TDP sensors but optimally four TDP sets in four cardinal directions, i.e., north, west, south and east. Moreover, each individual measurement must be corrected for NTG, which varies circumferentially as well. Unfortunately, this multi-sensor setup was difficult to implement because there was not enough room to install more TDP sensors. The surface of the stem segments was covered by the following components: (i) the space required for the installation of Mariotte system, (ii) the space for the cable ties holding the stem segment to the Mariotte structure, (iii) the space required for plastic tubes circulating the water, and (iv) a wide-enough ‘free’ area in which we could monitor the formation of the ATG with the infrared camera. Because of these constrains only one set of TDP sensors was used. However, to minimize the eventual $J_p$ circumferential variability two procedures were used. First, a test was performed without ATG to verify the agreement between the $J_p$ measured by the TDP and the $J_p$ of the entire segment obtained by the gravimetric method. Second, after the test, the uniformity of the xylem was verified by microscopic inspections of stained segments following Čermáková et al. (2000) and Savery et al. (2008) who related homogeneous distribution of sapwood with lower circumferential variability. The staining of the sapwood was carried out after the test to avoid the risk of clogging the conducting vessels. The stem segments used in Test 3 yielded a gravimetric $J_p$ in good agreement with the $J_p$ of TDP and had uniform sapwood structure, thus suggesting low circumferential variability and confidence in the obtained results.

Test 3 concluded that the CHD method satisfactorily corrected the TDP signal biased by ATG, producing a sap flux density estimate in good agreement with the gravimetric measurements. This confirms the adequacy and usefulness of CHD to accurately correct NTG biases of the standard TDP signals acquired in field conditions.

**Comparing cyclic power correction methods**

By observing the results of the cyclic power correction methods in Figures 6 and 7, it was clear that TTD-2002 (Do and Rocheteau 2002a, 2002b) and TTD-2010 (Isarangkool Na Ayutthaya et al. 2010) methods reduced the biasing effect of the ATGs upon the
Figure 7. Boxplot for the corrections obtained during Test 3 vs. the gravimetric reference (Grav-Flux) and uncorrected data (Standard-TDP), for (a) Segment 1 and (b) Segment 2.

Figure 8. Accumulated water estimates, calculated for Standard-TDP (constant power), CHD, TTD-2002 and TTD-2010 for both segments (upper figures = Segment 1 and bottom figures = Segment 2) in ATG Mode I and II, vs. gravimetric reference measurements (Grav-Flux). AT = air temperature and CW = circulating water temperature.
Table 3. Basic statistics of the sap flux density measurements and estimates for Test 3, using different correction methods: CHD-Flux 7.5 min; TTD 7.5 min; and TTD 10-20 min. Segment 1 = 11 cm$^3$ cm$^{-2}$ h$^{-1}$ and Segment 2 = 1 cm$^3$ cm$^{-2}$ h$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Grav-Flux</th>
<th>Standard-TDP Flux</th>
<th>CHD-Flux 7.5 min</th>
<th>TTD 2002 7.5/7.5 min</th>
<th>TTD 2010 7.5/7.5 min</th>
<th>TTD 2002 10/20 min$^1$</th>
<th>TTD 2010 10/20 min$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segment 1</td>
<td>Max</td>
<td>13.12</td>
<td>15.60</td>
<td>11.85</td>
<td>16.46</td>
<td>13.94</td>
<td>16.46</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>10.26</td>
<td>5.27</td>
<td>9.09</td>
<td>6.13</td>
<td>6.75</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>11.72</td>
<td>11.70</td>
<td>10.42</td>
<td>10.25</td>
<td>10.25</td>
<td>10.29</td>
</tr>
<tr>
<td></td>
<td>Std.</td>
<td>0.93</td>
<td>0.96</td>
<td>0.60</td>
<td>0.85</td>
<td>0.79</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>MAPE$^2$</td>
<td>N/A</td>
<td>183%</td>
<td>21%</td>
<td>176%</td>
<td>14%</td>
<td>16%</td>
</tr>
</tbody>
</table>

$^1$These corrections were calculated from extrapolations of the original CPS, 7.5 min ON/OFF transients, to 10–20 min ON/OFF transients.

$^2$The MAPE was calculated using the Grav-Flux as reference.

The comparison of the CHD with the TTD methods shows that the CHD had the most accurate (Figures 7 and 8 and lowest MAPE in Table 3) and smoothest (Figure 6) sap flux density estimates as compared with the gravimetric reference, likely because: (i) the CPS routine is extrapolated to thermal equilibrium while the TTD method uses thermally non-equilibrated cycle endpoints prone to error (Nourtier et al. 2011); and (ii) TTD calibrations (Do and Rocheteau 2002b, Isarangkool Na Ayutthaya et al. 2010) have not been tested on the species used in this study (F. sylvatica). In terms of accumulated water flow, the TTD methods showed comparable results as the CHD for Segment 1 (11 cm$^3$ cm$^{-2}$ h$^{-1}$), but the TTD largely overestimated accumulated water in Segment 2 at low flows (1 cm$^3$ cm$^{-2}$ h$^{-1}$) in both ATG enforcement modes (Figure 8).

The CHD method has the following main advantages over the TTD method: (i) the extrapolation to steady state permitted coping better with the ATG influence and fast temporal variations (Figure 6); (ii) the CHD uses the original Granier calibration that applies stable steady state as principle and is widely verified on various species; and (iii) more reliable $J_p$ estimates, particularly for low flows. In contrast, the disadvantage of the CHD is the complex and large amount of data to be acquired.

Currently, the choice of using TTD or CHD depends on the objectives and environments where the sap flow measurements are taking place. If the objective is to measure accurately species with highly variable sap flow and located in areas where high NTGs are likely (e.g., arid, semi-arid, very-dry and open forests), CHD is the optimal choice. Conversely, if the measurements are taking place in areas with lower NTGs with species of low sap flow variability and the accuracy of the measurements is not a priority, the TTD method might be easier to apply (e.g., closed forest with considerable water availability). Nevertheless, future improved applications applying powerful automatic parameter optimization techniques used in hydrological modelling (Vrugt et al. 2003a, Vrugt and Robinson 2007), such as the SCEM-UA (Vrugt et al. 2003b) or DREAM (Laloy et al. 2010), would decrease the processing time substantially making the CHD method more user friendly and simple enough to be used effectively in any areas, including those with lower NTGs as well.

**Limitations of the CHD method for correcting ATGs**

The results showed that the CHD method performed better at correcting ATG biases than other cyclic-power methods. However, the tests also highlighted two situations in which the effectiveness of CHD decreased and the percentage of error was higher than an acceptable range of 15% as compared with gravimetric measurements (Figure 9): (i) when ATG $\Delta T > 1.0$ °C and $J_p = 11$ cm$^3$ cm$^{-2}$ h$^{-1}$ (Segment 1) during the moment of change from maximum ATG in Mode I towards maximum ATG of Mode II (16:30 to 18:00 in the error time series of Figure 9a); and (ii) when ATG $\Delta T < -0.5$ °C at a low $J_p = -1$–2 cm$^3$ cm$^{-2}$ h$^{-1}$ (Figure 9b).

The error measurements during Mode I and Mode 2 followed in general the patterns described for Test 2 and Test 3, increasing and decreasing according to the ATG bias, but were especially sensitive during $\Delta T$ changes. For example, at about 14:15 (Figure 9a), when ATG $< -1.0$ °C (Mode I) and $J_p = 11$ cm$^3$ cm$^{-2}$ h$^{-1}$ (Segment 1), the CHD correction reduced the bias but still the error was >10%. This period corresponds to the moment of thermal change, and continued until quasi-stabilization at 15:15 (Figure 9a) when the error decreased to...
<10% during 15 min. At 15:30, when ATG > 1.0 °C (Mode II) and \(J_p = 11 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}\) (Segment 1), the error increased again >10% and reached a maximum of 25% at 16:30. After this point the error started to decrease and at 17:00 it stabilized at ~10% for the rest of the test. To explain the precise reason for these two peaks of error is difficult, but a likely explanation is that the CHD method could not keep up with the speed of the temperature change in the stem caused by the ATG enforcing system in both modes, because: (i) the time resolution of the cycle power scheme (7.5 min ON–OFF) was not high enough to account for this fast change, and/or (ii) the optimization of the \(A\) and \(T\) parameters of the CHD produced poorer fits in those specific time periods of fast temperature change. These patterns were observed in the repetitions as well, but they do not constitute a drawback on the method for field implementation. In natural conditions the differences of temperature are more gradual than with the ATG enforcing system, and thus the CPS resolution is more likely to cover the temporal variability of the temperature gradients and produce better fits for the \(A\) and \(T\) parameters.

For Segment 2, with a sap flux density of 1 \(\text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}\), the CHD correction did clearly decrease the overestimation error, but when the ATG was ≤−0.5 °C (ATG enforcement set in Mode II), the error increased and reached a maximum of −40% at 15:30 (Figure 9b). After this point the error started to decrease and stabilized towards ∼20% for the rest of the test. This implies that despite much lower residual error in Segment 2 as compared with Segment 1, its relative percentage error was much larger (Figure 9). The described situations when the error was >15% and <−15% are particularly relevant if the objective of the study requires accurate high temporal resolution sap flux density measurements (e.g., minutes to hours), but

![Figure 9. Relative percentage error calculated from gravimetric measurements versus ATG (above) and relative percentage error/residuals in time (below) influencing \(J_p\) from CHD-Flux, in Segment 1 (a) and Segment 2 (b). The error obtained from the Standard-TDP is displayed as an example of a biased signal to highlight the performance of the CHD. AT = air temperature and CW = circulating water temperature.](image-url)
for a longer time water flow integration (e.g., daily variation), small under- and overestimation errors can cancel out as mentioned by Braun and Schmid (1999) and Köstner et al. (1998).

The MAPE for Test 3 showed that CHD-Flux had an 11% error, whereas the constantly powered Standard-TDP1 had 33% error. In the case of Segment 2, the CHD-Flux had 21% error and the constantly powered Standard-Flux2 183% error. The higher error for CHD-Flux in Segment 2 as compared with Segment 1 does not indicate that the CHD failed in correcting the ATG influence, but that at high \( J_p \) values the correction is more accurate. Thus, the difference between the MAPE calculations of both segments might be explained by the difference in sap flux densities. This is the case, because the thermal gradients are likely to stabilize faster at higher sap flux densities than at low densities. Hence, the error at low sap flux densities after CHD correction could be caused by: (i) at lower flow there is lower convection so the ON/OFF extrapolations require longer time for stabilization and thus fitting of the curves is more difficult and prone to error; (ii) the stem segment for these tests having a deeper sapwood depth (3.6 cm) than the length of the TDP probe; thus, that section of the sapwood area, albeit smaller than the initial section, was unmeasured; and (iii) inherited error due to the poorer accuracy of TDP probes at low flows as discussed by Braun and Schmid (1999).

At the end of Test 3, the CHD-Flux showed a poorer performance at correcting the NTG bias in Mode II as compared with Mode I (Figure 9a), so as a result the \( J_p \) was underestimated (Figure 6a). However, the lower performance in such situations would not importantly affect the overall sap flux density estimation because at night the flow is normally zero or very low (Lu et al. 2004, Regalado and Ritter 2007). This means that the CHD method corrects the ATG bias the best before sunrise (when the \( \Delta T_{\text{max}} \) is normally defined) and during the peak of water flow, when the heat convected by water flow is more important.

In spite of the appointed limitations, the CHD methodology was confirmed to be the most appropriate method to reduce the bias caused by the ATGs.

**Conclusions**

Accounting for NTG constitutes one of the most important biasing aspects to consider when applying TDP in semi-arid environments with open vegetation. Thus, the results gathered with this study are an important contribution towards the understanding of NTG biases and appropriate ways to account for it in semi-arid vegetation. It was concluded that: (i) the ATG enforcement system is appropriate to generate ATG biases on the TDP signal under controlled laboratory conditions at various sap flux densities (similar to NTGs observed under field conditions); (ii) TDP is highly sensitive to the ATG bias; (iii) the assessed bias is dependent on the sap flux density and the strength of the applied ATG; and (iv) the CHD method constitutes a satisfactory method to remove the influence caused by varying and contrasting temperature gradients at different sap flux densities and resulted in generally more accurate estimates than other cyclic power methods. Finally, this study verified the relation of the magnitude of the sap flux densities to the NTG bias. Such information provided a better understanding on the dynamics governing the NTG influence and susceptibility upon the TDP signal.

**Conflict of interest**

None declared.

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**References**


