Analysis of sap flow dynamics in saplings with mini-HFD (heat field deformation) sensors

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Abstract

Sap flow dynamics of saplings, herbaceous species and plant parts with small dimensions has become an increasing point of interest in plant ecophysiological studies. At present, the most widely used sap flow sensors for these applications are the stem heat balance, the mini-HRM (heat ratio method) and the mini-HFD (heat field deformation). Each sensor has its own advantages and drawbacks, but when a high temporal resolution is needed in addition to measurements of positive and negative sap flow, only the mini-HFD suffices as the original heat balance method cannot distinguish between positive and negative sap flow, while the mini-HRM uses heat pulses rather than continuous heating partly sacrificing its temporal resolution. As such, the non-invasive mini-HFD is promising for studying hydraulic redistribution in saplings. However, when calibrating the mini-HFD by forcing water through cut branches problems arose during higher negative flows. The ratio used in the commercially available HFD was not optimal for the mini version. To resolve this shortcoming a new calculation method has been developed based on three temperature differences measured with the mini-HFD sensor. In this paper, the mini-HFD has been calibrated for flow rates ranging from -20 to 20 g.h^{-1}, whereby sensors were installed on beech (*Fagus sylvatica* L.) saplings. First results are promising and indicate a better calculation method for sap flow dynamics measured with the mini-HFD sensor.

Keywords: ecophysiology, miniature sap flow sensor, plant-water relations, hydraulic redistribution, reverse flow, bidirectional sap flow, beech (*Fagus sylvatica* L.)

INTRODUCTION

During development of the mini-HFD (heat field deformation) sensor, the main objective was to construct a non-invasive sensor capable of measuring bidirectional sap flow dynamics in plant parts with small dimensions (Hanssens et al., 2013). Sap flow and its dynamics are important factors in ecophysiological, ecological and agronomical studies as these measurements indicate changes in water use (Vandegehuchte et al., 2012) and the occurrence of drought stress (Epila et al., 2017). However, despite the wide range of sap flow (Smith and Allen, 1996) and sap flux density measurement methods (Vandegehuchte and Steppe, 2013), knowledge on sap flow dynamics in saplings is rather limited. When aiming at the assessment of sap flow dynamics through saplings, herbaceous species or plant parts with small dimensions, a limited number of non-invasive sap flow methods is available. The stem heat balance sensor can measure continuous sap flow, however, its major drawback is its inability to measure bidirectional sap flow when the original calculation method is used (Dynamax Inc, 2005). For most applications this is no limitation, but it makes the sensor less suitable for measurements of hydraulic redistribution resulting in a bidirectional flow. The mini-HRM (heat ratio method) sensor, on the other hand, is capable of measuring bidirectional sap flow, but uses heat pulses rather than continuous heating, partly sacrificing

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its temporal resolution, thus resulting in sap flow measurements typically every 10 minutes (Clearwater et al., 2009). This might not be a problem, but when sap flow is measured e.g. to measure how fast changes in sap flow dynamics occur, a higher temporal resolution is required. The mini-HFD (heat field deformation) sensor, based on the commercially available HFD sensor, can measure bidirectional sap flow, while maintaining a high temporal resolution (Hanssens et al., 2013), making this sensor the only one able to fit these criteria on such a small scale.

Calculations of sap flow dynamics measured with the mini-HFD are adopted from the sap flux density calculations used in the commercially available HFD sensor (Hanssens et al., 2013; Nadezhdina et al., 2012; Vandegehuchte and Steppe, 2012):

$$q_s = 3600. D \frac{K + dT_{s-a}}{dT_{as}} \frac{Z_{ax}}{Z_{tg}} \frac{1}{L_{sw}} \tag{1}$$

with q_s the sap flux density ($cm^3\ cm^{-2}\ h^{-1}$), 3600 a time conversion factor ($s.h^{-1}$), D the thermal diffusivity ($cm^2\ s^{-1}$), dT_{s-a} the temperature difference between upper axial and tangential needle (°C), K the absolute value of dT_{s-a} or dT_{as} during zero flow (°C), dT_{as} the temperature difference between the tangential and the lower axial needle (°C), Z_{ax} the axial distance between the heater and the axial needle (cm), Z_{tg} the tangential distance between the heater and the tangential needle (cm) and L_{sw} the sapwood depth at the location of the sensor (cm).

The dynamic part of Equation (1) correlates strongly with the sap flux density (Hanssens et al., 2013; Vandegehuchte and Steppe, 2012) and is used to calculate sap flow dynamics for the mini-HFD sensor (Hanssens et al., 2013), indicated as the HFD-ratio (-):

$$\mathrm{HFD} - \mathrm{ratio} = \frac{K + dT_{s-a}}{dT_{as}} \tag{2}$$

Sap flow dynamics are related to the asymmetry of the heat field originating from the central heater element. Development of this sensor has resulted in measurements of sap flow dynamics in tree seedlings to assess drought stress (Epila et al., 2017) and in the tomato peduncle to assess xylem and phloem influx into the tomato fruit (Hanssens et al., 2013, 2015). Although a good agreement was found between the HFD-ratio and the calculated water influx in the tomato fruit, based on fruit transpiration and changes in mass (Hanssens et al., 2013), an actual calibration of the sensor by forcing water through a cut plant part has not yet been performed. This paper investigates the reliability of adopting the ratio of the commercially available HFD to the mini sensor to investigate sap flow dynamics in saplings by performing a calibration using cut sapling stems.

MATERIALS AND METHODS

Experimental set-up

Five one-year-old beech (*Fagus sylvatica* L.) saplings (height ± 40 cm, diameter ± 6 mm) were planted on the 23 March 2016 into pots (diameter 30 cm, height 27 cm) containing seed soil for tree nurseries (VP308, Peltracom, Belgium) in a greenhouse at the Laboratory of Plant Ecology, Ghent, Belgium. One, one and four duo-sticks (Substral, Scotts Miracle Gro, USA) containing nutrition and pesticides were added to the soil of each pot on 11, 20 and 23 May 2016, respectively. Saplings were well watered by drip irrigation.

Sensor design

The mini-HFD sensor is a non-invasive sap flow sensor, based on the concept of the commercially available HFD (Hanssens et al., 2013; Nadezhdina, 2013). This sensor is ideal for measuring sap flow in saplings due to its small size, light weight and non-invasive characteristics. It is constructed by sewing a central heater element (surface-mounted device of 100 Ω) and three copper-constantan thermocouples (type T; two longitudinal, one tangential) onto rubber foam insulation tape (Armaflex) with nylon thread. The longitudinal thermocouples were spaced at 10 mm and the tangential thermocouple at 3 mm in reference to the centre of the heater element. The central heater element creates a heat field in the stem, which changes as a result of sap flow. Changes in this heat field are measured by the three thermocouples. A schematic of the sensor and the heat fields during positive, zero and negative flow are represented in (Figure 1). By using the measurements of these three thermocouples, three basic temperature differences can be calculated: the difference between the upper and the tangential thermocouple ($dT_{s\text{-}a} = T_1 - T_3$), the difference between the tangential and the lower thermocouple ($dT_{as} = T_3 - T_2$) and the difference between the upper and the lower thermocouple ($dT_{sym} = T_1 - T_2$).

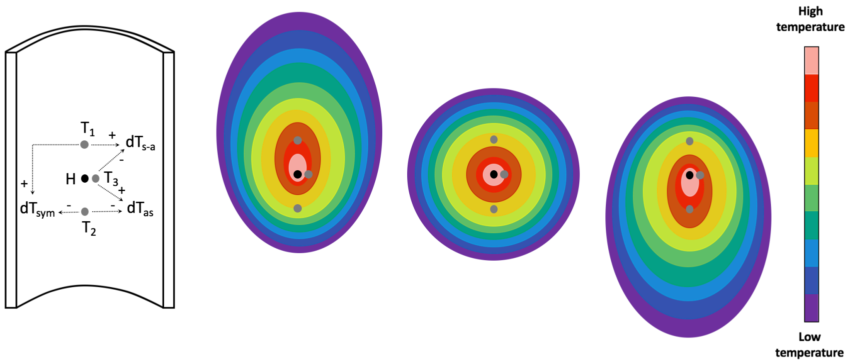

Figure 1. (i) Schematic of the mini-HFD sensor, consisting of a central heater element (H) and three thermocouples (T_1, T_2 and T_3) resulting in three temperature differences: $dT_{s\text{-}a} = T_1 - T_3$, $dT_{as} = T_3 - T_2$ and $dT_{sym} = T_1 - T_2$, (ii) schematic of heat field for positive sap flow, (iii) schematic of heat field for zero flow, and (iv) schematic of heat field for negative sap flow.

The rubber foam insulation tape was fixed to the stem of the seedlings, just below the lowest leaves and branches. Subsequently, the sensor was insulated with bubble wrap, and a layer of aluminium foil to reflect incident radiation. A continuous voltage of 3 V was applied to the heater element. All sensor signals were recorded at 10 second intervals, averaged every 60 seconds with a Campbell data logger (CR6, Campbell Scientific Inc., Logan, USA) and stored using the PhytoSense cloud service (Phyto-IT, Belgium).

Calibration

A calibration is obligatory to obtain absolute sap flow data ($g.h^{-1}$) from the mini-HFD sensor. This can be achieved by putting the actual flow as a function of the HFD-ratio (Equation 2). The stem of each seedling equipped with a mini-HFD sensor was cut at both sides of the sensor and connected to a flow meter (Mini CORI-FLOW meter/controller, Bronckhorst, Ruurlo, Netherlands). The flow meter upstream controls and measures the actual flow, the flow meter downstream only measures the flow output. The data of the flow meter downstream were used for the calibration as these measurements indicate the actual flow that went through the stem, dismissing possible leakage before water entered the stem. While forcing water through the stems, the mini-HFD sensor was connected to a data logger (CR6, Campbell Scientific Inc., Logan, USA), which measured the temperature of the thermocouples and the flow rate entering and leaving the cut stem. Every flow setting was maintained for at least 30 minutes to allow stabilization of the flow. Different flow rates were imposed, starting from zero going up with discrete steps to 20 g.h^{-1}. After one calibration, the stem was connected in reverse to simulate negative sap flow using the same procedure.

The same calibration and data processing workflow was used for all five saplings, however, the data indicated in the results and discussion section of this paper are based on one individual sensor.

RESULTS AND DISCUSSION

HFD-ratio

In order to calculate the HFD-ratio using Equation (2), the K value needs to be determined. This value represents the absolute value of $dT_{s\text{-}a}$ or dT_{as} during zero flow, $dT_{0(s\text{-}a)}$ and dT_{0as}, respectively. When it is not possible to determine the point of zero flow and a calibration is absent, this value can be determined as the absolute value of the intersection with the $dT_{s\text{-}a}$ or dT_{as} axis by linear extrapolation of $dT_{s\text{-}a}$ or dT_{as} versus dT_{sym}/dT_{as} (Nadezhdina et al., 2012). Figure 2a illustrates the K value based on experimental and calibrated data. This dataset serves as proof of concept for the calculation of K with the mini-HFD sensor as $dT_{s\text{-}a}$ or dT_{as} derived from the experimental data result in the same value as the calibration dataset.

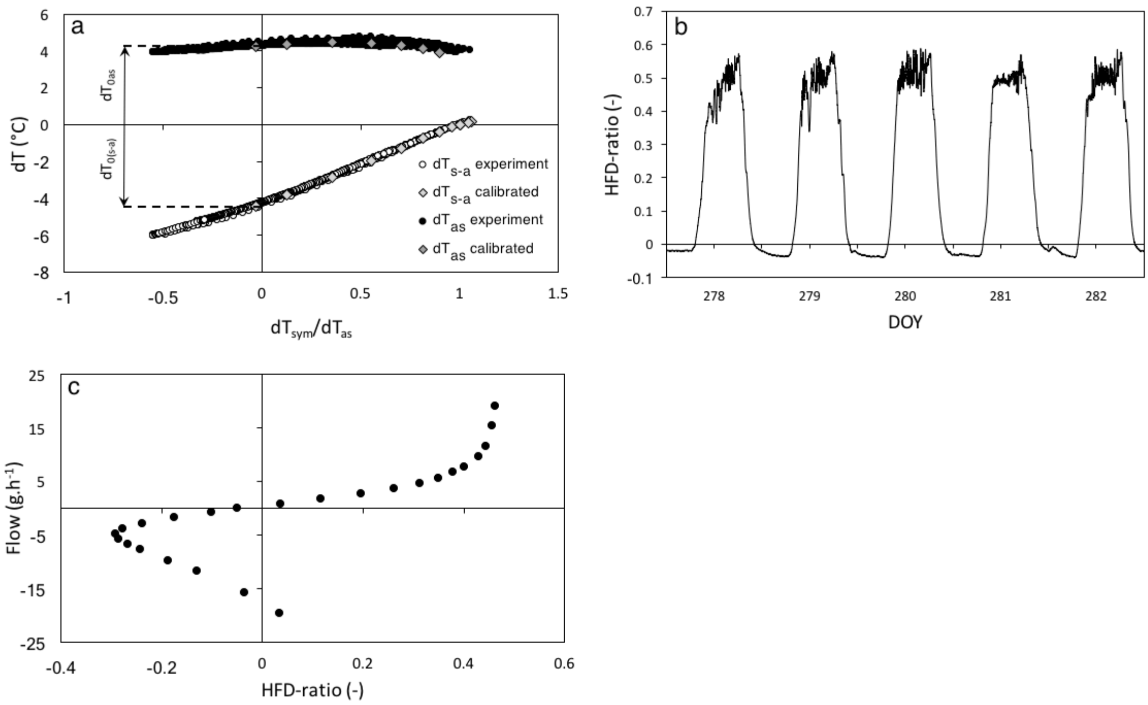

Figure 2. (a) Temperature differences $dT_{s\text{-}a}$ and dT_{as} as a function of dT_{sym}/dT_{as} from an experimental and a calibrated dataset, both measured in beech (*Fagus sylvatica* L.) saplings. (b) Sap flow dynamics of the beech sapling calculated with the HFD-ratio, and (c) the actual flow as a function of the HFD-ratio measured in the beech sapling.

Subsequently, the HFD-ratio can be calculated, resulting in sap flow dynamics (Figure 2b), illustrating a typical hump-shaped pattern (Steppe et al., 2015). Whereas sap flow dynamics are useful as an indicator for plant stress or to assess negative sap flow as a result of hydraulic redistribution, actual sap flow data are more valuable for quantification purposes.

A sensor calibration was performed to obtain actual sap flow data from the HFD-ratio by plotting the actual flow as a function of the HFD-ratio (Figure 2c). When performing this calibration, it became clear that different negative sap flow rates resulted in the same HFD-ratio. As such, the HFD-ratio can distinguish sap flow from -5 to 20 $g.h^{-1}$, but fails to univocally separate larger negative sap flow rates.

HFD-revised

To resolve the problem of two different flow rates resulting in the same HFD-ratio the three temperature differences were analysed. Nadezhdina et al. (2012) stated that, for the commercial HFD, dT_{sym} is a good indicator for bidirectional low flows, failing to characterize sap flux densities during larger flows by a fading linear correlation between dT_{sym} and the sap flux density, while dT_{as} is important to distinguish high from low flows. For the mini-HFD dT_{s-a} was a good indicator for positive flow, while dT_{as} was a good indicator for negative flow, and dT_{sym} was good at distinguishing low flows, either positive or negative, and zero flow (Figure 3a). These findings correspond with the statement of Nadezhdina et al. (2012), however, a combination of dT_{s-a} and dT_{as} was needed to assess high positive and negative flows.

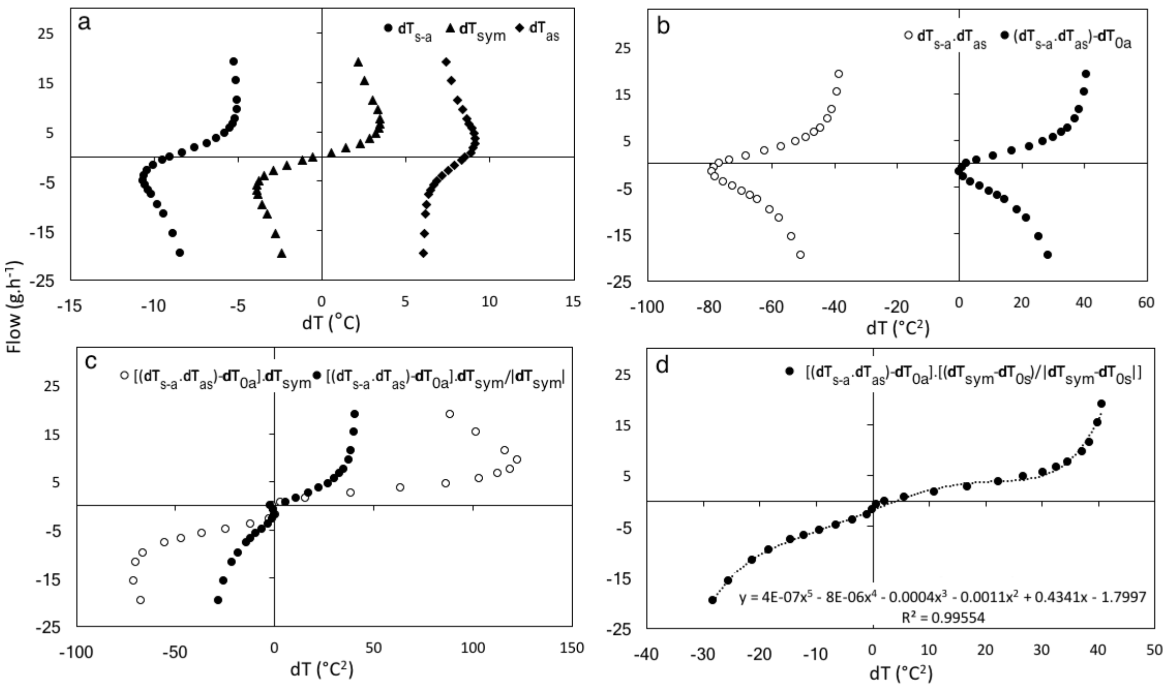

Figure 3. (a) Actual flow as a function of the three temperature differences for the mini-HFD sensor, measured in a beech (*Fagus sylvatica* L.) sapling, and procedure of HFD-revised for the mini-HFD sensor with (b) actual flow as a function of the multiplication of the asymmetrical temperature differences (dT_{s-a} and dT_{as}), with and without linear translation by subtracting dT_{0a}, (c) actual flow as a function of the multiplication with linear translation from panel "b" multiplied with the symmetrical temperature difference (dT_{sym}) and divided by the absolute value of the symmetrical temperature difference, and (d) actual flow as a function of the multiplication with linear translation multiplied with the symmetrical temperature difference and divided by the absolute value of the symmetrical temperature difference from panel "c" with an additional correction factor for the symmetrical temperature difference in both the denominator and the nominator, henceforth referred to as HFD-revised.

Based on these observations an asymmetrical product was calculated as $dT_{s-a}.dT_{as}$ (Figure 3b). This product is translated by subtracting a correction factor dT_{0a}, resulting in a good indicator for positive and negative flows. However, this product cannot distinguish between the two. By multiplying the asymmetrical product with a dummy variable based on

dT_{sym}, a proxy for sap flow was obtained. When multiplying the previous indicator with the symmetrical temperature difference (dT_{sym}), characteristics of the symmetrical temperature difference were reintroduced, i.e. a good indicator for low positive and negative flows was obtained, which fails to distinguish the higher flows (Figure 3c). When dividing dT_{sym} by its absolute value a good indicator across the entire flow range (-20 to 20 $g.h^{-1}$) was obtained. In essence, the asymmetrical product is multiplied with 1 or -1 depending on the flow direction. However, a discrepancy between theory and practice of introducing this dummy variable exists around zero flow. This discrepancy typically results from a thermocouple, which has moved during installation, i.e. an imperfect sensor. This can be solved by subtracting both dT_{sym} in the denominator and the nominator with a correction factor dT_{0s} (Figure 3d). This final result will henceforth be referred to as HFD-revised (°C^2):

$$\mathrm{HFD-revised} = (\mathrm{dT_{s-a}.dT_{as}} - \mathrm{dT_{0a}})\frac{\mathrm{dT_{sym}} - \mathrm{dT_{0s}}}{|\mathrm{dT_{sym}} - \mathrm{dT_{0s}}|} \quad (3)$$

In general, HFD-revised is a good indicator for the actual sap flow when both correction factors dT_{0a} and dT_{0s} are determined correctly. When plotting dT_{sym} as a function of $dT_{s\text{-}a}.dT_{as}$, an elliptic dataset is generated (Figure 4). The correction factors can be found by determining the lower intersection between the elliptic dataset and the long axis of the ellipse encircling the dataset. For a perfect sensor, this results in a dT_{0s} value of zero and a dT_{0a} value dependant of the stem moisture content, dry wood density, thermal conductivity and possible wound effects. In this case, both dT_{0s} and dT_{0a} correspond to the values of dT_{sym} and $dT_{s\text{-}a}.dT_{as}$ during zero flow, respectively (Figure 4a). For an imperfect sensor, the elliptic dataset undergoes a translation and rotation (Figure 4b). The correction factors are found by the same intersection as for a perfect sensor, however, both correction factors do not necessarily correspond to the values of dT_{sym} and $dT_{s\text{-}a}.dT_{as}$ during zero flow as these correction factors inverse the translation and rotation of the ellipse. Noteworthy is that a sufficiently wide range of data is needed to be able to complete the ellipse encircling the dataset, preferably from -3 to 3 $g.h^{-1}$.

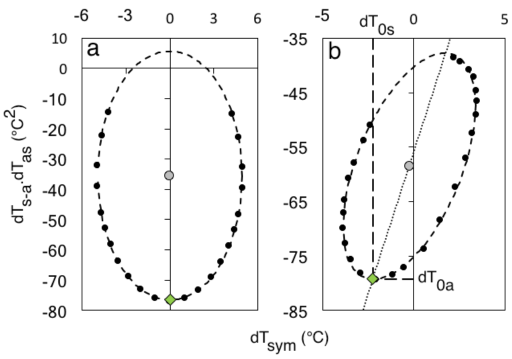

Figure 4. Asymmetrical multiplication ($dT_{s\text{-}a}.dT_{as}$) as a function of the symmetrical temperature difference (dT_{sym}) to determine the correction factors dT_{0a} and dT_{0s}. Black filled circles indicate the dataset, grey filled circle the centre of the ellipse describing the dataset (dashed line). Green filled diamond indicates the lower intersection between the elliptic dataset and the long axis of the ellipse, from which the correction factors can be derived. Dotted line indicates the long axis of the ellipse and is an indicator of the tilt of the elliptic dataset. (a) Data for a perfect sensor. (b) Data for an imperfect sensor.

HFD-revised solved the problem during negative flows, while maintaining a similar sensitivity during positive flows as for the HFD-ratio. This new calculation is strongly correlated with the actual flow by a fifth-degree polynomial equation, however, a calibration is needed to determine the actual sap flow. An inherent property of this calculation is its incapability to measure flows when dT_{0s} equals dT_{sym}, resulting in a division by zero. This can be resolved by (1) making the dummy variable provisory when dT_{0s} does not equal dT_{sym}, or (2) by removing these values as they only correspond to one specific flow, dependent on the construction of the sensor.

CONCLUSIONS

HFD-revised can univocally determine a wider range of sap flows (-20 to 20 $g.h^{-1}$) for the mini-HFD sensor compared to the HFD-ratio (-5 to 20 $g.h^{-1}$). A calibration is needed to determine the actual flow, either by the HFD-ratio or HFD-revised. From our analysis, we would not recommend using the HFD-ratio of the commercially available HFD sensor for the mini-HFD sensor, and showed that the HFD-revised is a good proxy for sap flow dynamics measured with the mini-HFD. Nonetheless, for positive and low negative flows the HFD-ratio may suffice to assess sap flow dynamics.

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