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# Transient thermal dissipation method of xylem sap flow measurement: multi-species calibration and field evaluation

S. ISARANGKOOL NA AYUTTHAYA,<sup>1,2</sup> F.C. DO,<sup>3,4</sup> K. PANNENGPETCH,<sup>1</sup> J. JUNJITTAKARN,<sup>1</sup> J.-L. MAEGHT,<sup>5</sup> A. ROCHETEAU<sup>6</sup> and H. COCHARD<sup>2</sup>

<sup>1</sup> Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002 Thailand

<sup>2</sup> UMR547 PIAF, INRA, Université Blaise Pascal, 63100 Clermont-Ferrand, France

<sup>3</sup> Institute of Research for Development (IRD), Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

<sup>4</sup> Corresponding author (frederic.do@ird.fr)

<sup>5</sup> Institute of Research for Development (IRD), Land Development Department, Bangkok, Thailand

<sup>6</sup> Institute of Research for Development (IRD), Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), Montpellier, France

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Summary The transient thermal dissipation (TTD) method developed by Do and Rocheteau (2002b) is a close evolution of the original constant thermal dissipation (CTD) method of Granier (1985). The TTD method has the advantage of limiting the influence of passive natural temperature gradients and of yielding more stable zero-flux references at night. By analogy with the CTD method, the transient method was first calibrated on synthetic porous material (sawdust) on the assumption that the relationship was independent of the woody species. Here, our concern was to test the latter hypothesis with a 10-min heating time in three tropical species: Hevea brasiliensis Müll. Arg., Mangifera indica L. and Citrus maxima Merr. A complementary objective was to compare the field estimates of daily transpiration for mature rubber trees with estimates based on a simplified soil water balance in the dry season. The calibration experiments were carried out in the laboratory on cut stems using an HPFM device and gravimetric control of water flow up to 5 L dm<sup>-2</sup> h<sup>-1</sup>. Nineteen response curves were assessed on fully conductive xylem, combining 11 cut stems and two probes. The field evaluation comprised five periods from November 2007 to February 2008. Estimates of daily transpiration from the measurement of sap flow were based on the 41 sensors set up on 11 trees. Soil water depletion was monitored by neutron probe and 12 access tubes to a depth of 1.8 m. The calibrations confirmed that the response of the transient thermal index to flow density was independent of the woody species that were tested. The best fit was a simple linear response  $(R^2 = 0.88, n = 276 \text{ and}$ P < 0.0001). The previous calibration performed by Do and Rocheteau (2002b) on sawdust fell within the variability of the multi-species calibration; however, there were substantial differences with the average curve at extreme flow rates. Field comparison with soil water depletion in

the dry season validated to a reasonable extent the absolute estimates of transpiration acquired with the 10-min TTD method. In conclusion, evidence for the independence of calibration from woody species and the simple linear response of the thermal index strengthen the interest of the TTD method with 10-min heating.

*Keywords: Citrus maxima*, cut stem experiment, Granier's sensors, *Hevea brasiliensis*, *Mangifera indica*, soil water balance, tree transpiration, validation.

#### Introduction

During the last 20 years, the wide use of automatic thermal techniques to measure sap flow has been crucial in improving our understanding of the hydrological cycle, community ecology, whole-plant physiology and tradeoffs between water use and carbon acquisition (Granier et al. 1996, Burgess et al. 1998, Wullschleger et al. 1998, Eamus and Prior 2001, Meinzer et al. 2003, Roberts et al. 2005, Breda et al. 2006, Sevanto et al. 2008).

One of the most commonly used techniques to measure sap flow in trees is the constant thermal dissipation (CTD) method developed by Granier (1985, 1987). This method uses two needle sensors inserted radially into the sapwood. Each sensor contains a thermocouple, and the sensors are connected and yield a differential measurement of temperature. The downstream sensor is constantly heated, and the measured difference in temperature decreases when the sap flux density rises because the flow increases heat dissipation through a convective effect. Granier (1985) developed a flow index (K), which is the ratio between the difference in temperature at zero flow and at measured flow. The non-linear calibration between K and flow density holds for several woody species (Granier 1985) and for synthetic porous media (Granier unpublished data). Because of its empirical basis, Smith and Allen (1996) recommended checking the calibration for each new woody species. Applying the CTD method and Granier-type probes, several authors observed values close or identical to the original calibration: Cabibel and Do (1991) on Malus domestica Borkh., Lu and Chacko (1998) on Mangifera indica L., Braun and Schmid (1999) on Vitis vinifera L., Clearwater et al. (1999) on Eucalyptus deglupta Blume, Anacardium excelsum (Bert. & Balb.) Skeels and Bursera simaruba (L.) Sarg., Lu et al. (2002) on Musa 'cavendish', Do and Rocheteau (2002b) on a sawdust column, McCulloh et al. (2007) on Pseudobombax septenatum (Jacq.) Dugand and Calophyllum longifolium Willd., based on the cut stem experiments in the laboratory, pot or lysimeter experiments. A few authors found differences which they assumed to be due to the effect of woody species (de Oliveira Reis et al. 2006 on Carica papaya L.), or due to the configuration of the probe (Roupsard et al. 2006). Hence, calibration of the CTD method is mainly considered to be independent of the species even if some sort of validation is still recommended (Lu et al. 2004).

To avoid the influence of natural thermal gradients between the two probes and to obtain more stable zero-flux references, Do and Rocheteau (2002a, 2002b) introduced a non-continuous heating system with a cycle of heating and cooling. The temperature signal used became time-related: it was the difference between the temperature reached at the end of the heating period and the temperature reached after the cooling period. the final temperature reached after the cooling period. By analogy with the original CTD method, the flow index  $(K_a)$  was considered as the ratio of the signal at zero flow to the signal at measured flow. Due to its analogy with the original CTD method, the response of  $K_a$  was assumed to be independent of the woody species and the first calibrations were performed on a synthetic porous media (sawdust). The authors found a similar  $K_{\rm a}$  response to flow density with different cycles of heating and cooling: 45/15, 30/30, 15/15 and 10/10 min. The calibration was very different from the CTD method because of the transient conditions, i.e., after 10 min of heating, the temperature reached a slow kinetic phase, but for low and zero flow rates it was far from equilibrium and the equilibrium was not completely reached even after 30 min (Do and Rocheteau 2002a). This transient thermal dissipation (TTD) method has now been used on several tree species including Acacia tortilis (Forsk.) (Do and Rocheteau 2002b, Do et al. 2008), Adansonia sp. (Chapotin et al. 2006a, 2006b), Hevea brasiliensis Müll. Arg. (Isarangkool Na Ayutthaya et al. 2007, 2008) and Olea europaea L. (Abid-Karray et al. 2008). However, to our knowledge, no calibration study on these species has been published. Therefore, this work had two aims: the first was to test the hypothesis that calibration of the TTD method is independent of the woody species and porous media. The

response to flow density of the  $K_a$  index calculated with 10 min of heating was evaluated in three tropical species: *H. brasiliensis*, *M. indica* and *Citrus maxima* Merr. Several sets of cut stems were processed with *H. brasilienis* wood to assess the variability of response curves. We expected to find a calibration similar to the one obtained by Do and Rocheteau (2002b) on synthetic porous material (sawdust). The second related objective was to compare the estimates of daily transpiration between sap flow measurements and the soil water balance in the dry season in a mature stand of *H. brasiliensis*. We expected the values to be close if the hypothesis of tree transpiration estimates from the simplified soil water balance holds.

#### Materials and methods

#### TTD method of sap flow measurement

The TTD method (Do and Rocheteau 2002*a*, Do et al. 2008) is a close evolution of the original CTD method of Granier (1985, 1987). It is based on the change over time in the difference in temperature between two probes inserted radially into the xylem, one heated and other unheated, with a cyclic schedule of heating and cooling on the heated probe. Granier's probe of 2-mm diameter and 20-mm-long sensors were used (UP gmBh, Cottbus, Germany). When the heating power is adjusted to 0.200 W, it induces a maximum temperature difference of 8–12 °C after 10 min under zero flow conditions. Do and Rocheteau (2002*b*) found an empirical relationship between sap flux density ( $J_s$ ; L dm<sup>-2</sup> h<sup>-1</sup>) and an index of the change in the temperature difference, denoted alternate flow index ( $K_a$ ; dimensionless).

The calibration was performed on a synthetic porous media (sawdust in a plastic-glass cylinder).  $K_a$  was determined as below.

$$K_{\rm a} = 1/(1+11.3J_{\rm s}^{-1.414}), \text{ or}$$
  
 $J_{\rm c} = (11.3K_{\rm s}/(1-K_{\rm s}))^{0.707}$ 

A transient or alternate signal ( $\Delta T_a$ ) was defined as

$$\Delta T_{\rm a} = \Delta T_{\rm on} - T_{\rm off},$$

where  $\Delta T_{\rm on}$  is the temperature difference reached at the end of the period of heating and  $\Delta T_{\rm off}$  is the temperature difference after the period of cooling.

The alternate flow index was calculated as

$$K_{\rm a} = (\Delta T_{\rm 0a} - \Delta T_{\rm ua}) / \Delta T_{\rm ua},$$

where  $\Delta T_{0a}$  is the maximum alternate temperature difference obtained under zero flow conditions and  $\Delta T_{ua}$  is the measured alternate signal at a given  $J_s$ .

To measure  $J_s$  every half hour with a heating period of 10 min, a cycle of 10-min heating and 20-min cooling was applied and the temperature signals were recorded every

temperature difference at zero flow. Acronyms P1 and P2 indicate probe 1 and probe 2 when two probes were inserted into the same cut stem.										
Cut stem acronym	Length (cm)	Diameter (cm)	Dry density (g/cm <sup>3</sup> )	Stem water content (cm <sup>3</sup> /cm <sup>3</sup> )	$K_{\rm h}  [{\rm kg \ s}^{-1}  {\rm MPa}^{-1}  {\rm dm}^{-2}]$	Sapwood area (dm <sup>-2</sup> )		$\Delta T_0$ (°C)		
						P1	P2	P1	P2	
Hev. 1	55.20	3.93	0.49	0.54	$0.47 \times 10^{-5}$	0.115	0.105	7.9	9.3	
Hev. 2	53.75	4.38	0.52	0.51	$5.78 \times 10^{-5}$	0.123		9.1		
Hev. 3	55.30	4.30	0.48	0.56	$0.49 \times 10^{-5}$	0.108	0.095	9.4	10.3	
Hev. 4	55.00	4.90	0.50	0.58	$6.32 \times 10^{-5}$	0.143	0.139	7.9	7.9	
Hev. 5	38.55	4.88	0.52	0.58	$9.76 \times 10^{-5}$	0.151		7.9		
Hev. 6	37.75	5.08	0.48	0.55	$11.01 \times 10^{-5}$	0.166		10.3		
Hev. 7	52.10	5.33	0.49	0.51	$6.90 \times 10^{-5}$	0.153	0.151	10.5	9.6	
Hev. 8	56.00	4.71	0.47	0.54	$7.50 \times 10^{-5}$	0.145	0.141	9.3	10.0	
Man. 1	56.85	3.88	0.42	0.69	$5.19 \times 10^{-5}$	0.084	0.080	8.2	8.3	
Man. 2	53.30	4.82	0.41	0.63	$2.72 \times 10^{-5}$	0.115	0.109	8.8	9.6	
<i>Cit.</i> 1	54.00	5.14	0.80	0.72	$10.64 \times 10^{-5}$	0.168	0.144	9.6	10.2	

Table 1. Characteristics of experimental cut stems used for calibration of the TTD method on three species: *H. brasiliensis (Hev.)*, *M. indica (Man.)* and *C. maxima (Cit.)*. Parameter  $K_h$  is hydraulic conductance measured by HPFM and normalized by length and xylem area.  $\Delta T_0$  is the maximum temperature difference at zero flow. Acronyms P1 and P2 indicate probe 1 and probe 2 when two probes were inserted into the same cut stem.

10 min. According to Do and Rocheteau (2002b),  $\Delta T_{\text{off}}$  is the temperature after 10 min of cooling. Here, the calculation of  $\Delta T_{\text{off}}$  was slightly modified; the final temperature after 10 min of cooling was averaged with the initial temperature before heating, so after 20 min of cooling. Such modification yielded the same  $K_a$  but reduced the errors due to a quick change in natural thermal gradient, which may occur within 10 min in the early morning (Do unpublished).

#### Cut stem experiment in the laboratory

The characteristics of the cut stems used for calibration experiments are listed in Table 1. They comprised three species of particular interest for our laboratory: H. brasiliensis (rubber tree), M. indica (mango) and C. maxima (pummelo). The cut stems of *H. brasiliensis* received special treatment to avoid vessel blockage due to latex exudates. They were soaked for one full night in a water bath. For all species, after re-cutting each cut stem, a 2-cm-thick disc from both ends of stem was cut off and set up to enable capillary rise of a bromothymol blue solution. Only cut stems where wood staining was coarsely homogenous were retained for further analysis. The cut stems were connected to a high pressure flow meter (HPFM, Dynamax Co., Houston), which allowed the pressure,  $J_{\rm s}$  and conductance to be controlled. The reference measurement of  $J_s$  was obtained by weighing water flowing out of cut segments (0.01 g accuracy balance, Adventurer<sup>™</sup>, Ohaus, Pine Brook). Flow density ranged from 0.3 to 5.0 L dm<sup>-2</sup> h<sup>-1</sup>. Depending on the length of the cut segments and on the experiment, one or two sets of probes were inserted into the sapwood. Aluminum tubes were inserted into the stem before insertion of the probes. The distance between needles of the same probe was 10 cm, and the heated needle of probe 1 was separated from the reference needle of probe 2 by 10 cm too. Probe 1 was in upstream position. The same set of two probes was used for all tests and the probes were located at the same position. These were connected to a data logger (21X, Campbell Scientific, Leicester, UK). Data, such as the weight of water from the balance, were recorded every 10 min. To ensure best contact between the probes (especially the heated one) and the sapwood, only set-ups with  $\Delta T_{0a}$  below or equal to 10.5 °C were used (Table 1).

#### Sap flow measurements in the field

The field comparison of estimates of transpiration from the sap flow measurements and soil water balance was carried out in a mature stand of H. brasiliensis in northeast Thailand in a plantation of RRIM600 clones (15°16'23" N and 103°04' 51.3" E) that were located close to Khu-Muang, Buriram province. The spacing was  $2.5 \times 7.0$  m and the trees had been tapped for 4 years. The soil was a deep loamy sand. Mean contents of clay, loam and organic matter varied from 9.9, 24.2 and 0.78% in the top soil (0-20) to 20.2, 23.6 and 0.34% at a depth of 1.5 m, respectively. In this non-traditional rubber tree plantation area, the environmental conditions are water limited for H. brasiliensis. The dry season lasts for 6 months, from November to April, and the average annual rainfall is 1176 mm. In 2007, even drier climatic conditions occurred with an annual rainfall of 990 mm. Eleven trees were selected within the main classes of trunk girth. The trunk girth (measured at 1.5 m above the soil) ranged from 40 to 60 cm, yielding an average of 55 cm [standard deviation (SD) = 6.03 that corresponded to 17.5 cm in diameter.

The xylem area was estimated from the observations of cores and whole sections of freshly cut trees or branches from the stand for a wide range of girths. There was a strong relationship between bark thickness (*B*) and total radius ( $R_t$ ), which allowed the deduction of xylem radius ( $R^2 = 0.93$ ,  $B = 0.0822 R_t - 0.0287$  and n = 30). Dying experiments with bromothymol blue showed that xylem was completely conductive, except at a pith of an almost



Figure 1. Radial pattern of relative sap flux density  $(J_s)$  in *H. brasiliensis*. The percentage of xylem radius starts at the center of the trunk. Bold points represent the average of four radial profiles (three depths) of daily maximum  $J_s$  recorded for 7 days in four representative trees taken within 11 instrumented trees. Vertical bars are SDs. Horizontal bars represent the average location of 2-cm sensors along the profile. Xylem radius averaged 7.25 cm, SD = 0.74. Maximum  $J_s$  in the outer ring averaged 2.36 L dm<sup>-2</sup> h<sup>-1</sup>, SD = 0.45.

constant radius of 0.3 cm. However, an area with slightly less staining was observed toward the pith. Probes were inserted into the trunks at a height of 1.8 m above the soil. At this height, the average xylem area was estimated at  $2.04 \text{ dm}^2$  (SD = 0.47). The set-up took into account the circumferential and radial variability of  $J_s$  within the sapwood (Granier et al. 1996). Xylem area was schematically divided into four rings: the outer ring comprised between 100% and 60% of xylem radius, the intermediate ring between 60% and 30%, the inner low-conducting ring between 30% and 5% and the non-conductive pith. Standard equipment corresponded to three probes inserted into the outer ring of each trunk to a depth of 0.5-2.5 cm after removal of the bark. In four representative trees within the same sample of 11 trees, sensors were inserted at two complementary depths: 5 and 7 cm beneath cambium (xylem radius ranging from 6 to 8 cm and average = 7.33 cm). The exposed parts of the needles were coated with silicone. The trunk area containing the probes was protected from direct solar radiation and from rainfall with a waterproof deflector. The 41 probes were connected to a data logger (CR10X, Campbell Scientific, Leicester, UK). The zero-flux signal was determined every night, assuming that sap flow was negligible at the end of the night. Toward the dry season, nocturnal vapour pressure deficit of the air (VPD) (at the same time of  $\Delta T_{\text{max}}$ recording) differed from zero, and reached a maximum of 0.6 KPa. However, our assumption relied upon the facts that  $\Delta T_{\rm max}$  of probes was quite stable over the 4-month period (variation coefficient = 1.8% on average), and overall there was no relationship between these small variations and the progressive increase of nocturnal VPD toward the dry season. The explanation of negligible night-time sap flows despite substantial VPD was likely due to several features: the

dramatic decrease in the leaf area toward the dry season, the night-time stomatal behavior of the species and the low soil water availability. In a representative record of 7 days, the radial profile of  $J_s$  showed the classical shape of diffuse porous species (Figure 1). Midday  $J_s$  was maximum in the outer ring (2.36 L dm<sup>-2</sup> h<sup>-1</sup> and SD = 0.45).  $J_s$  decreased by 20% in the intermediate ring and by 70% in the low-conducting wood close to the center. The final calculation of total flow ( $F_{tree}$ ) gave

$$F_{\text{tree}} = A_{\text{sw}} \times J_{\text{s\_ut}} \\ \times (0.64\text{C1} + 0.27\text{C2} + 0.0875\text{C3}),$$

where  $A_{sw}$  is the total cross-sectional area of xylem at the level of the heating probe,  $J_{s\_out}$  is the  $J_s$  measured in the outermost ring and  $C_i$  is the ratio of  $J_s$  to  $J_{s_{out}}$  in the inner rings. The latter formula showed that due to the relative areas, the measurements of  $J_s$  close to the center were of little importance. The ratio of  $J_s$  applied to the successive rings from the outside toward the center was 1, 0.79 and 0.27. The result was the application of a reduction coefficient of 0.874 to the  $J_s$  measured in the outer ring of conducting xylem. Hourly total flow over a period of 24 h was cumulated to calculate daily total flow. Additionally, daily total flow was an estimate of daily tree transpiration, ignoring the changes in tree water storage. Daily transpiration of an individual tree over a period of time ( $\Delta t$ ) was computed as the sum of daily transpiration divided by the period of time. The final estimate of daily transpiration was the mean of the average daily transpiration of the 11 trees.

#### Simplified soil water balance

Tree transpiration can be estimated from the depletion of the soil water content (SWC) profile alone when the following conditions are assumed in the soil water balance: (i) zero water input (rainfall and irrigation), (ii) negligible soil evaporation, (iii) negligible lateral and deep water transfers and (iv) root water uptakes limited to the measured soil profile. Under these circumstances, the tree water uptake (*E*) equals the soil water depletion ( $\Delta S$ ) from the root zone for a period of time ( $\Delta t$ ) according to the simple formula

$$E = \Delta S / \Delta t = [S(t) - S(t + \Delta t)] / \Delta t,$$

where *E* represents tree water uptake or transpiration in mm day<sup>-1</sup>,  $\Delta S$  is the soil water depletion expressed in mm, *S*(*t*) is the soil water storage in mm at the initial date *t* and S(*t* +  $\Delta t$ ) is the soil water storage in mm after a period  $\Delta t$  expressed in days.

The soil water storage in the root zone S(t) was derived from soil moisture measurement at different depths following the formula:

$$S(t) = \int_0^z \theta(z,t) \times dz,$$

where  $\theta$  is the volumetric SWC and z is the depth of the root zone.



Figure 2. Values of the flow index ( $K_a$ ) versus flux density based on the cut stem experiment with the 10-min TTD method: (A) *H. brasiliensis* (*Hev.*), numbers related to acronyms indicate the set of cut stems and probes (presented in Table 1) ( $K_a = 0.0778 J_s$ ;  $R^2 = 0.89$  and n = 155); (B) *M. indica* (*Man.*) and *C. maxima* (*Cit.*) ( $K_a = 0.0749 J_s$ ;  $R^2 = 0.84$  and n = 121). The lines indicate the respective linear regressions.

The soil water balance was assumed to fulfill these requirements during the dry season, long enough after the last rainfall and considering the soil profile to a depth of 1.8 m. Rubber tree roots are mainly concentrated within the first meter but a few roots are observed at a greater depth (Devakumar et al. 1999). Soil moisture was measured with a neutron probe (3322, Troxler, Research Triangle Park, NC) every 0.2 m to a depth of 1.8 m. Two gravimetric calibrations were applied, separating the top layer from the other layers. The SWC was not uniform in the horizontal direction due to run-off towards the inter-row in the rainy season. Therefore, we set up 12 tubes by couples; for each couple, 1 tube along the planting line between two trees, approximately 1.75 m from each, and 1 tube in the middle of the inter-row, approximately 3.5 m from each planting line. An average soil water profile was determined by couple of tube and statistics showed the variability related to the six repetitions. Tree water use was estimated from soil water depletion for five periods starting 15 days after the last rainfall (October 30) to February 21, at leaf fall peak. Average soil water depletion was computed as the mean of measurements made on six pairs of tubes. Soil moistures at 'field capacity' (-0.03 MPa) and 'permanent wilting point' (-1.5 MPa) were deduced from soil water retention curves modeled from soil properties (% clay, % silt, % OM, medium size of sand and bulk density) of the four main layers of the soil profile (0-0.2, 0.2-0.4, 0.4-1.0 and 1.0-1.8 m) using the van Genuchten-Mualem model adapted by Wosten et al. (1998).

#### Data analysis

Regression analysis and other statistics were performed using Sigmaplot Version 10.0 and SPSS Version 11.5. Linear slopes were compared using their confidence intervals at 95%. Curve fits and estimates were compared using the root mean

Table 2. Details of regression curves from cut stem experiments with the TTD method: (*Hev\_L*), linear regression of *H. brasiliensis* data; (Oth\_L), linear regression of data from other species, *M. indica* and *C. maxima*; (MS\_L), multi-species linear regression including all the data; (MS\_sig), multi-species sigmoid regression and (D&R\_sig) sawdust sigmoid regression of Do and Rocheteau 2002b. The form of the sigmoid function is

$$K_a = \frac{a}{1 + \left(\frac{J_s}{X_o}\right)^b}.$$

The parameters of the columns are *n*, number of data; *a*, slope of linear curve;  $X_0$ , constant value of sigmoid curves; *b*, power of sigmoid curve; SE, standard error of regression parameters (related to  $X_0$  for MS\_sig); CL\_95, confidence interval at 95% of linear slopes; ( $R^2$  and P), statistics related to each regression; Total RMSE, Total root mean square error (n = 276) and RMSE<sub>rel</sub>, relative root mean square error at several ranges of flow rates.

	n	а	$X_0$	b	SE	CL_95	$R^2$	Р	Total RMSE	% RMSE <sub>rel</sub> range of $J_{\rm s}$ (L dm <sup>-2</sup> h <sup>-1</sup> )			
_										0-0.5	0.5-1.0	1.0-3.0	3.0-5.0
Hev_L	155	0.0778	_	-	0.0022	0.0045	0.89	< 0.0001					
Oth_L	121	0.0749	-	-	0.0030	0.0060	0.84	< 0.0001					
MS_L	276	0.0772	-	-	0.0018	0.0035	0.88	< 0.0001	0.52	41.4	30.6	26.3	20.5
MS_sig	276	1	6.8986	1.414	2.8519		0.87	< 0.0001	0.57	58.6	27.3	24.1	23.1
D&R_sig	-	1	5.5557	1.414									

square error (RMSE) according to the following formula:

RMSE = 
$$\sqrt{\frac{\sum_{i=1}^{n} (x_{1,i} - x_{2,i})^2}{n}},$$

where in the analysis of cut stem experiments,  $x_{1,i}$  and  $x_{2,i}$  are sap flow densities from weighed and calculated values at the same  $K_a$ , respectively, and n is the number of estimated values. In the field comparison,  $x_{1,i}$  and  $x_{2,i}$  are soil water depletion and tree transpiration for the same period of time, respectively and n is the number of tree transpiration values.

The comparison of curve fitting, which separated the ranges of flow rates in cut stem experiments, was made using relative root mean square error ( $RMSE_{rel}$ ) according to the following formula:

RMSE<sub>rel</sub> = 
$$\sqrt{\frac{\sum_{i=1}^{n} \left(\frac{x_{1,i}-x_{2,i}}{x_{1,i}}\right)^2}{n}}$$
,

where  $x_{1,i}$  and  $x_{2,i}$  are sap flux densities from weighed and estimated values at the same  $K_a$ , respectively and n is the number of values.

To determine if sap flow measurements and soil water balance resulted in similar estimates of daily transpiration, a paired t test was performed for each period of time.



Figure 3. Multi-species calibration of the flow index ( $K_a$ ) versus flux density with the 10-min TTD method including three species: *H. brasiliensis*, *M. indica* and *C. maxima*. The dotted line indicates the original sigmoid calibration performed by Do and Rocheteau on sawdust (2002b, D&R\_sig), the bold dotted line indicates the multi-species sigmoid calibration (MS\_sig) and the continuous line indicates the multi-species linear calibration (MS\_L:  $K_a = 0.0772 J_s$ ,  $R^2 = 0.88$  and n = 276).



Figure 4. Sap flux density ( $J_s$ ) estimated from the equation of Do and Rocheteau (2002*b*) (open circles) versus  $J_s$  estimated from the new multi-species linear equation (presented in Figure 3). The closed circles related to the second *Y*-axis indicate the differences between the two estimates relative to the estimates from the multi-species equation.

#### Results

#### Cut stem experiments

Firstly, for *H. brasiliensis* wood, the responses of the  $K_a$  index to  $J_s$  showed a strong linear relationship (Figure 2A; Table 2,  $R^2 = 0.89$ ). A substantial discrepancy was observed over 13 response curves. Figure 2A shows that variability originated from the differences in response both between cut stems and between probes within the same cut stem. For example, between cut stems, the global response of Hev. 1, at the top end of the scatter, contrasts with that of Hev. 7 at the low end. And within the same cut stem, the response of Hev. 8\_2 contrasts with that of Hev. 8\_1. The data recorded using M. indica and C. maxima woods (six sets) displayed a linear relationship close to the one assessed using *H. brasiliensis* wood (Figure 2B; Table 2,  $R^2 = 0.84$ ). The slope of M. indica and C. maxima data did not differ from that of H. brasiliensis (Table 2). Similarly to H. brasiliensis data, a substantial discrepancy was observed within the scatter of points coming from the differences in response both between cut stems and between probes within the same cut stems.



Figure 5. Tree transpiration estimated from sap flow measurements versus estimates from soil water depletion for five periods in the dry season. Estimates from sap flow measurement are the average of 11 mature trees (three probes per tree) of *H. brasiliensis*. Estimates from soil water depletion are the average of neutron probe measurements made on six pairs of tubes inserted to a depth of 1.8 m. The closed circles indicate tree transpiration estimates from the multi-species linear calibration (presented in Figure 3). Open squares are tree transpiration estimates from Do and Rocheteau's calibration (2002*b*). Vertical and horizontal bars indicate standard errors. \* and \*\* indicate when estimates differ significantly from each other, which was the case during the first period at the onset of the dry season (November, P < 0.05) and during the last period (leaf fall peak, February, P < 0.01).

The whole data set was fitted to a linear function passing through 0 (Figure 3; Table 2):

$$K_a = 0.0772 J_s$$
, or  $J_s = 12.95 K_a$ ,

where  $r^2$  equals 0.88 and *n* equals 276.

The fit to a sigmoid function similar to the form used by Do and Rocheteau (2002b) was very close to the previous linear function (Figure 3) and proved to be no better (Table 2). The RMSE (absolute and relative) were even slightly lower for the linear function. The calibration curve obtained with sawdust by Do and Rocheteau (2002b) is located at the upper end of the scatter of points (Figure 3). The comparison of the two sigmoid functions (MS\_sig and D&R\_sig in Table 2) showed that the uncertainty on parameter  $(X_0)$  in the multi-species calibration included the response curve assessed with sawdust by Do and Rocheteau (2002b). However, the estimates of  $J_s$  differed substantially between the average calibrations at very low flow and high flow rates. Compared to the multi-species calibration, the values from the Do and Rocheteau (2002b) calibration were overestimated at low  $J_s$  (< 0.8 L dm<sup>-2</sup> h<sup>-1</sup>); e.g., +30% at 0.5 L dm<sup>-2</sup> h<sup>-1</sup>, and were underestimated at high  $J_s$ , e.g., -20% at 4.0 L dm<sup>-2</sup> h<sup>-1</sup> (Figure 4).

#### Estimates of H. brasiliensis transpiration in the field

Tree transpiration estimated from sap flow measurements with the multi-species linear calibration decreased from  $1.6 \text{ mm day}^{-1}$  in November to 0.4 mm day<sup>-1</sup> in February

at leaf fall peak (Figure 5). Comparison with estimates from the depletion of soil water profile to a depth of 1.8 m showed a strong correlation (R = 0.97) and RMSE equal to 0.47 mm day<sup>-1</sup>. However, there were large discrepancies at the borders, and for these two extreme points, the estimates differed significantly from each other (P < 0.05). In November, at the start of the dry season, the transpiration estimated from soil water depletion was two-fold higher (2.5 mm day<sup>-1</sup>). Compared to the previous equation of Do and Rocheteau (2002b), the multi-species calibration has little effect on the transpiration estimates, it slightly lowered RMSE, from 0.55 to 0.47 mm day<sup>-1</sup>, mainly through the differences at low  $J_s$  (Figure 5). The modest difference of transpiration estimates between the two calibrations was related to the measured range of medium to low  $J_s$  (< 2.0), and was foreseeable from the previous analysis of Figure 4.

#### Discussion

#### TTD calibration independent of woody species

By analogy with the CTD method of Granier (1985), the TTD method developed by Do and Rocheteau (2002b) was first calibrated on synthetic porous material (sawdust), assuming that the relationship was independent of the woody species and porous media. Our results demonstrated that the response of the  $K_{\rm a}$  index to flux density, calculated with 10 min of heating, was similar for three tropical woody species: H. brasiliensis (with a large sample of cut stems and two probes), M. indica and C. maxima. All the species tested had diffusive-porous wood. So, if ring-porous wood had been used, would the responses have been different? The previous calibration performed by Do and Rocheteau (2002b) was done using sawdust. This is very different porous media than cut stems and likely induced heterogeneous  $J_s$ . However, it fell within the variability of the multi-species calibration. These results strongly support the hypothesis that the TTD empirical calibration is independent of the woody species, although it is recommended to check results by some sort of validation before interpreting any absolute values obtained from different species of wood and in different environmental conditions (Lu et al. 2004). Hence, the TTD method appears to share the property of universal calibration with the original CTD method. What is the explanation? Even concerning the CTD method, this point has not been much discussed, but this independence is likely due to Dr. Granier's design of the thermal index (K), which is also used in the TTD method. It is the ratio of signal response at zero flow to signal response at actual flow. Unlike most physical methods of measurement, each probe has no particular calibration coefficient. As it works, it appears that zero-flux response of the set-up system acts as a calibration coefficient for the whole probe-porous media system.

#### Limitations of cut stem experiment with TTD methods

Response curves showed an intra-species discrepancy for the different species. The discrepancy came from both differ-



Figure 6. Changes in the volumetric SWC profile during the dry season from October 2007 to February 2008. Soil moisture at field capacity is indicated by the profile at -0.03 MPa, and soil moisture at wilting point is indicated by the profile at -1.5 MPa. Horizontal bars indicate standard errors (n = 6).

ences between the cut stem samples and the probe set-ups within the same segment of cut stem. Similarly to the CTD method, the discrepancy in the TTD method may come in particular from (i) probe contact with non-conductive sapwood, (ii) poor contact between the probe and the sapwood, (iii) heterogeneous flow density within the cut sample and poor representativeness of the probe position, (iv) heterogeneous flow density along the length of the probe and (v) the existence of transient natural thermal gradients. Firstly, contact between the probe and the conductive sapwood only has been a known requirement of TD methods since the start (Granier 1985, Braun and Schmid 1999, Clearwater et al. 1999). The response curves assessed for the multi-species calibration corresponded to fully conductive sapwood confirmed by staining. We did some tests on the cut stems of Longan (Dimocarpus longan Lour.) and observed a large non-stained area around the pith, implying that the 2 cm tip of the probe was in partial contact with non-conductive wood. The final results were a much lower response curve than the range presented in Figure 3 (data not shown). Secondly, correct contact between the probe and the sapwood is also a basic requirement of TD methods (Lu et al. 2004). This is particularly true when dealing with dead wood in cut stem experiments because there is no progressive tightening of wood as occurs in living trees.  $\Delta T_0$  is a good indicator of such contact. In this experiment,  $\Delta T_0$  below 10.5 °C insured a good contact and heat dissipation between probe and wood.

However, there was still a substantial variability of  $\Delta T_0$ , between 10.5 and 7.5 °C (Table 1), despite an accurate regulation of the power of each probe heating at 0.2 W. The data do not support a difference related to the probe itself because within the same stems, the mean and variation coefficient of  $\Delta T_0$  were similar, 9.0 °C (SD = 0.9) and 9.4 °C (SD = 0.9), for probes 1 and 2, respectively. Hence, the variability of  $\Delta T_0$ , particularly within the same cut stem, reflects mainly the variations of heat dissipation by conduction due to variation in the quality of the contact between probe and wood. This could explain the substantial differences observed in probe responses within the same cut stem and between cut stems.

Thirdly, heterogeneous flows within cut samples are naturally expected. They could be due to the existence of tension wood counteracting the effect of wind or weight. The probe may have been inserted into a sector that was not representative of average flow. Moreover, Table 1 shows that conductance could vary considerably in similar cut segments of the same species, and such differences may also exist between sectors within the same sample. This could explain some differences in the response of a probe within the same cut sample. Fourthly, Clearwater et al. (1999) showed that with the CTD method, heterogeneous flow density along the probe may lead to underestimation of sap flow due to the non-linear response of  $J_s$  to the temperature signal. Sap flow along the length of the probe was likely heterogeneous in our cut stem experiments at a magnitude that varied with stem samples. This feature may have induced some discrepancy between responses. However, the more linear response of the TTD thermal index to flow density should decrease sensitivity to this effect. Finally, by contrast with the CTD method, the TTD method is insensitive to a stable natural thermal gradient (Do and Rocheteau 2002b). However, a change in the natural thermal gradient over the 10-min period of measurement may induce a large error in the measurement  $(\Delta T_{\rm on} - \Delta T_{\rm off})$  as it was noticed in the field in the early morning when  $\Delta T_{\rm off}$  was not interpolated between values before and after heating. Even in the laboratory experiments, the use of an interpolated  $\Delta T_{\rm off}$  decreased the overall discrepancy (data not shown). There are certainly other unknown factors that contribute to the uncertainty and to the difficulty of laboratory calibration on cut stems. Nevertheless, the sources of variability are surely emphasized in the field with large trees. Even with small trees in containers, McCulloh et al. (2007) observed large individual errors, (up to 50%), when comparing the responses of the CTD method with gravimetric measurements. Consequently when working in the field, as in cut stem experiments, it appears important to estimate the mean response using a large number of set-ups and repetitions.

#### *Comparison between current calibration and that of Do and Rocheteau (2002b)*

The sawdust calibration of Do and Rocheteau (2002b) was included in the variability of response curves observed in

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cut stems of the three tropical species (Figure 3). However, the estimates using the average multi-species calibration differed substantially from the previous calibration at high flow and very low flow rates. The multi-species calibration was based on the 19 response curves mixing 15 wood samples and two probes. The calibration of Do and Rocheteau (2002b) on sawdust was based on two sawdust columns and one probe. The data basis of the multi-species calibration likely maximized variability and provided a more reliable average response curve. We assume that a larger sample of sawdust columns and probe set-ups would have yielded a mean response closer to our multi-species calibration.

#### *Field evaluation of transpiration estimate in mature H. brasiliensis*

The field evaluation compared the estimates of transpiration from sap flow measurements with the estimates from a simplified soil water balance. The comparison validated the magnitude of transpiration estimates from sap flow measurements with the multi-species calibration when considering the middle of the dry season. The estimate of 1.6 mm day<sup>-1</sup> may appear quite low after only 1 month of dry season, when trees are still in full foliage and evaporative demand is relatively high. However, Isarangkool Na Ayutthaya et al. (2008) showed that at this time both  $J_s$  and whole-tree hydraulic conductance had already decreased about 40% compared to values measured at the end of the rainy season in October  $(2.5 \text{ mm day}^{-1})$ . At the start and at the end of the soil drying period used for comparison, we found significant discrepancies between transpiration estimates from sap flow measurements and from the soil water balance. For the first period of comparison, starting 15 days after the last rainfall, the change in soil water profiles to a depth of 1.8 m did not support the hypothesis of negligible soil surface evaporation and water transfers outside the soil profile (Figure 6). There was still a large amount of water available in the soil surface layer, and at the lower end, the SWC had substantially decreased. Moreover, at the end of the dry season, the hypothesis of negligible root water uptake outside the measured profile was doubtful. At this time of leaf fall peak, mean transpiration estimated from the sap flow measurements was 0.41 mm day<sup>-1</sup> while the 1.8 m soil water profile showed almost no change over a period of 30 days, yielding 0.1 mm of depletion. Figure 6 shows that the soil water profiles were not far from the reference profile at -1.5 MPa. In the same plantation, Gonkhamdee et al. (2008) reported root activity at a depth of 4 m in the dry season, so it is fairly possible that at this time the trees tap water from deeper soil layers. Additionally, in this study, the transpiration estimate of 0.4 mm day<sup>-1</sup> corresponded to very low hourly  $J_s$ , below 0.5 L dm<sup>-2</sup> h<sup>-1</sup>. At this rate, the accuracy of absolute measurement with the TTD method is poor because of the calibration uncertainty (see RMSE<sub>rel</sub> in Table 2) and because of the relative influence of any inaccuracy in the measurement of  $\Delta T_0$  in the field. The relative inaccuracy of  $\Delta T_0$  and its assumed correspondence to zero flow are a common weakness of all thermal methods (Do and Rocheteau 2002*a*, Lu et al. 2004, Regalado and Ritter 2007). The CTD and more recently the TTD (Chapotin et al. 2006*a*, 2006*b*, Abid Karray et al. 2008, Do et al. 2008) methods have demonstrated their ability to accurately record the changes in hourly and seasonal transpiration. The accuracy of absolute estimates of transpiration is another step, which obviously requires a large number of probes and cross-validation with an independent method such as soil water balance or gas exchange measurements.

#### Conclusion

In conclusion, the evidence of non-species specific calibration strengthens the interest of the 10-min TTD method developed by Do and Rocheteau (2002b). In addition, the field comparison with the soil water balance method validated to a reasonable extent the absolute estimates of transpiration from the new multi-species calibration. Compared to the CTD method, the current advantages of the TTD method are (i) a more stable night-time reference (Do and Rocheteau 2002a) and insensitivity to a stable natural thermal gradient (Do and Rocheteau 2002b) and (ii) the simple linear response of the transient  $K_a$  index. Obvious drawbacks are (i) a low resolution time of measurement - with 10 min of heating and cooling: one measurement every 30 min or every 20 min at the best and (ii) a little more complex electronic circuitry required including a relay box (Lu et al. 2004). Finally, one promising new advantage of the TTD method is the applicability of its time-related signal to a single probe using exactly the same  $K_a$  index (Do unpublished) or a more sophisticated index (Mahjoub et al. 2009). The use of a single probe will further reduce problems associated with thermal gradients and make instrumentation simpler and cheaper, which may increase the current advantages of thermal dissipation methods over other thermal methods.

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