Calibration of thermal dissipation sap flow probes for ring- and diffuse-porous trees

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Summary Thermal dissipation probes (the Granier method) are routinely used in forest ecology and water balance studies to estimate whole-tree transpiration. This method utilizes an empirically derived equation to measure sap flux density, which has been reported as independent of wood characteristics. However, errors in calculated sap flux density may occur when large gradients in sap velocity occur along the sensor length or when sensors are inserted into non-conducting wood. These may be conditions routinely associated with ring-porous species, yet there are few cases in which the original calibration has been validated for ring-porous species. We report results from laboratory calibration measurements conducted on excised stems of four ring-porous species and two diffuse-porous species. Our calibration results for ring-porous species were considerably different compared with the original calibration equation. Calibration equation coefficients obtained in this study differed by as much as two to almost three orders of magnitude when compared with the original equation of Granier. Coefficients also differed between ring-porous species across all pressure gradient conditions considered; however, no differences between calibration slopes were observed for data collected within the range of expected in situ pressure gradients. In addition, dye perfusions showed that in three of the four ring-porous species considered, active sapwood was limited to the outermost growth ring. In contrast, our calibration results for diffuse-porous species showed generally good agreement with the empirically derived Granier calibration, and dye perfusions showed that active sapwood was associated with many annual growth rings. Our results suggest that the original calibration of Granier is not universally applicable to all species and xylem types and that previous estimates of absolute rates of water use for ring-porous species obtained using the original calibration coefficients may be associated with substantial error.

Keywords: Elaeagnus angustifolia, Gleditsia triacanthos, Granier, Populus fremontii, Quercus gambelii, sap flux, Sophora japonica, Tilia cordata, transpiration, validation.

Introduction

Thermal dissipation probes (the Granier method) are routinely used in forest ecology and water balance studies to estimate whole-tree transpiration. The method involves measuring the temperature difference between a heated and a reference sensor, which are inserted radially into sapwood. Sap flux density is calculated according to an empirically derived equation originally validated for two gymnosperm species (*Pseudotsuga menziesii* (Mirb.) Franco, Douglas fir and *Pinus nigra* Arnold, Austrian pine) and one ring-porous angiosperm species (*Quercus pedunculata* Ehrh., English oak) (Granier 1985). The calibration has since been validated with measurements showing generally good agreement with the original calibration for many other woody species (Cabibel and Do 1991, Lu and Chacko 1998, Braun and Schmid 1999, Clearwater et al. 1999, Catovsky et al. 2002, Lu et al. 2002, McCulloh et al. 2007). Goulden and Field (1994) reported calibration data for excised stems of *Quercus agrifolia* Nee (California live oak) that had calibration slopes that differed from the original calibration, where the use of the Granier equation would have resulted in underestimated sap flux density. However, the original design had been modified such that the heated sensor received only a fraction of the power compared with the original design. The validity of the original calibration has also been supported by results from studies showing generally good quantitative agreement between water flux estimates obtained with the Granier method compared with other sap flow methods, water absorption, branch bag measurements, eddy covariance measurements and catchment-scale water balance, as well as between sap flux-derived canopy stomatal conductance and porometry-derived stomatal conductance (Granier et al. 1990, 1994, Kostner et al. 1996, Saugier et al. 1997, Tournebize and Boistard 1998, Ewers et al. 2007, Ford et al. 2007). However, results of calibration measurements conducted on excised stems of *Quercus gambelii* Nutt. (Gambel oak) and *Acer grandidentatum* Nutt. (Bigtooth maple) differed substantially relative to the original calibration of Granier

(Taneda and Sperry 2008), in which differences were attributed at least in part to sensor contact with non-conducting wood. More recently, Steppe et al. (2010) and Hultine et al. (2010b) reported calibration results that differed from the original calibration for excised stems of *Fagus grandifolia* and *Tamarix ramosissima* × *chinensis*, respectively. In all three aforementioned studies, the Granier equation underestimated sap flux density.

Large errors in calculated sap flux density can occur in cases in which steep gradients in sap velocity occur along the sensor length or in which a portion of the sensor is inserted into non-conducting wood (Clearwater et al. 1999). Although the original calibration of Granier has been reported to be independent of wood characteristics (Granier et al. 1990), steep velocity gradients along the sensor length and sensor contact with non-conducting wood are conditions that may be encountered more often in ring-porous species relative to species with diffuse porous or tracheid wood anatomy. This is because ring-porous wood has a vessel diameter distribution that is bimodal, with high conducting capacity, large diameter, early season vessels and lower conducting capacity, smaller diameter, late season vessels, leading to potentially very steep gradients in sap velocity across the conducting sapwood in a single growth ring (Tyree and Zimmermann 2002). In fact, previous studies have documented large gradients in sap velocity that are associated with depths equivalent to the typical length of a Granier sensor (20 mm) and cases in which most of the fluid flow through the xylem of ring-porous species was associated with current year vessels (Ellmore and Ewers 1986, Cermak et al. 1992, Granier et al. 1994, Cermak and Nadezhdina 1998, Jaquish and Ewers 2001, Gebauer et al. 2008).

Despite these considerations, most of the reports validating the initial calibration equation are associated with diffuse-porous, agricultural cultivars or tropical species. Validation of the initial calibration for ring-porous taxa is largely absent in the literature and, to our knowledge, is limited to the ring-porous genera *Quercus* and the *Castanea sativa* species. In this article, we report results from laboratory calibration experiments conducted on four ring- and two diffuse-porous tree species. Our objective was to assess the applicability of the original empirical calibration equation reported by Granier to ring-porous trees representing different genera. In addition, because we expected that the potential errors mentioned above were less likely to be associated with diffuse-porous or tracheid-only species, we also report here the results of calibration measurements for two diffuse-porous species.

Materials and methods

Plant material

Calibration measurements were made during the 2008 and 2009 growing seasons using excised branch segments from four ring-porous species and two diffuse-porous species.

The ring-porous species included *Elaeagnus angustifolia* L. (Russian olive, $n = 7$), *Gleditsia triacanthos* L. (Honey locust, $n = 6$), *Q. gambelii* Nutt. (Gambel oak, $n = 6$) and *Sophora japonica* L. (Japanese pagoda, $n = 6$), and the two diffuse-porous species included *Populus fremontii* S. Watson (Fremont cottonwood, $n = 6$) and *Tilia cordata* Mill. (Littleleaf linden, $n = 5$). Plant material was collected from five sites located either in or near Salt Lake Valley, UT, USA (latitude 40°66′; longitude 111°55′; elevation 1275–1550 m). The Salt Lake Valley is a metropolitan area, with a semi-arid climate. Mean annual temperature and precipitation are 11.1 °C and 411 mm, respectively (Alder et al. 1998). The sites included a riparian area associated with the Jordan river corridor, which runs south to north across the center of Salt Lake Valley (*E. angustifolia*), three irrigated, landscaped sites including a Salt Lake City park (*T. cordata*), the University of Utah campus (*G. triacanthos*, *S. japonica*), a site at the base of Red Butte Canyon Research Natural Area (*Q. gambelii*) and, lastly, a site located inside Red Butte Canyon Research Natural Area, just east of Salt Lake City (*P. fremontii*).

Material preparation and experimental apparatus

Stem segments ∼3 m in length and 4–6 cm in diameter at the center were cut in air, bagged after foliage removal and taken back to the laboratory, where they were re-cut underwater to remove 1 m lengths on either side of the final measurement segment. Table 1 provides characteristics of the final measurement segments for each species. In the laboratory, both ends of each measurement segment were shaved with a sharp razor blade underwater. Stem segments were then fastened upright to a ring stand using clamps. The lower end of each stem segment was submerged in a beaker of filtered 20 mM KCl solution that rested on a balance (LP34000P, Sartorius, Goettingen, Germany), such that the stem segment did not touch any part of the beaker. The balance was used to make gravimetric measurements of water flow through the stem segments concurrently with sap flow measurements using Granier sensors (described below). The top (downstream end) of each stem segment

Table 1. Characteristics of branch segments used to collect calibration data. Length and diameter are given as mean ± standard error.

Species	Wood type	Length (cm)	Diameter (cm)	N
E. angustifolia	Ring-porous	57.5 ± 10.0	4.36 ± 0.30	7
G. triacanthos	Ring-porous	74.1 ± 5.0	5.06 ± 0.26	6
Q. gambelii	Ring-porous	79.6 ± 8.3	4.37 ± 0.08	6
S. japonica	Ring-porous	76.1 ± 1.5	4.47 ± 0.22	6
P. fremontii	Diffuse-porous	76.8 ± 1.9	5.08 ± 0.15	6
T. cordata	Diffuse-porous	80.6 ± 2.2	4.83 ± 0.15	5

The sample size represents the number of segments per species, where segments were collected from independent trees.

was stripped of bark and connected with tubing to a vacuum pump via a 4 l Erlenmeyer vacuum flask.

Sap flow measurements

Stem segments were instrumented with heat dissipation probes to measure sap flux density according to Granier:

$$F_d = ak^b \qquad (1)$$

where F_d is sap flux density in g cm^{-2} s^{-1}, a the coefficient (0.0119), b the scaling exponent (1.23) and k a dimensionless quantity related to the temperature difference between a heated and reference probe:

$$k = \frac{\Delta T_{max}}{\Delta T} - 1 \qquad (2)$$

where ΔT is the temperature difference between the sensor probes and ΔT_{max} is the temperature difference between the sensor probes under zero flow conditions. A constant 0.2 W of power was delivered to the heated probes via a coiled constantan heating element. In order to calculate sap flux density, conducting sapwood area was obtained from dye perfusions (see below). Both 10 mm (*S. japonica*) and 20 mm (*E. angustifolia*, *G. triacanthos*, *P. fremontii*, *Q. gambelii* and *T. cordata*) length probes were used for calibration measurements in order to be consistent with the size of sensors used previously in field installations (Bush et al. 2008, Hultine et al. 2010*a*). Our goal was not to assess the differences between the use of 10 and 20 mm probes, but rather to assess whether either type yielded calibration results consistent with the widely used empirical calibration equation. Sensor pairs were inserted radially into each stem segment, with a vertical separation of 15 cm. The temperature difference associated with each sensor pair was measured every 5 s, and 5 min averages were stored using a datalogger (CR23X, Campbell Scientific Inc., Logan, UT, USA).

A range of sap flux density values was obtained for each stem segment by changing the vacuum pressure of the system. The applied pressure gradient ranged from 0.001 to 0.14 MPa m^{-1}. This bracketed the expected range of in situ frictional gradients. Trunks and large branches are generally thought to experience a frictional pressure gradient of the order of one or two times the hydrostatic slope (∼0.01 MPa m^{-1}), with greater gradients in smaller branches (Tyree and Zimmermann 2002). Following each change in vacuum pressure, the pressure and flow rate were kept constant for a minimum of 20 min before gravimetric and thermal dissipation probe data were recorded. The maximum temperature difference between each sensor pair was recorded under zero flow conditions, following the experimental procedure. In order to determine the zero flow value, stems remained in the same position associated with flow measurements, where they were left overnight with the upstream (lower) end of each stem still submerged in a beaker of water. The

time required to obtain a stable zero flow value took anywhere from less than an hour to several hours depending on the measurement segment.

Sapwood area determination

In order to determine sapwood area, stems were submerged in a beaker of Safranin O solution (0.1%) following a series of flow rate measurements, and dye was pulled by vacuum through each stem until it was visible in the downstream reservoir (Erlenmeyer vacuum flask). In most cases, dye was pulled through the stems for an additional 5–10 min after it was visible in the downstream reservoir. Directly following the dye perfusions, the stems were again submerged in KCl solution and flushed for the same time period in order to inhibit the spread of dye to inactive xylem. Measurement segments were then sectioned using a band saw, and images of cross-sections located near the heated sensors were obtained using a scanner. Scanned images were used to calculate the total conducting sapwood area with Image J software (Image J, NIH, USA, http://rsbweb.nih.gov/ij/).

Calculations and statistical analysis

A correction to calculate the temperature difference and sap flux density associated with active sapwood was applied to diffuse-porous calibration data, where the active sapwood depth was less than the sensor length according to Clearwater et al. (1999):

$$\Delta T_{sw} = \frac{\Delta T - d\Delta T_m}{c} \qquad (3)$$

where ΔT_{sw} is the temperature difference between the portion of the heated and reference sensors associated with active sapwood, ΔT_m the temperature difference between the portion of the heated and reference sensors associated with inactive xylem and c and d the fraction of the sensor length associated with active and inactive xylem, respectively.

Statistical analyses were performed using GraphPad Prism software (www.graphpad.com). Species calibration curves were obtained by fitting a least-squares power function to pooled data of all measurement segments for each species. Comparison of calibration data across species was made by log transforming the data, followed by performing standard least-squares regression, where differences in slopes (exponent b) and intercepts (ln(coefficient a)) were assessed.

Results

Ring-porous species

Calibration results were different from the Granier calibration for all ring-porous species considered in this study. In contrast to coefficient a of the Granier equation (0.0119, Eq. (1)), the coefficient a obtained from all measurements, combined by

species, varied from 0.93 to 5.81, exceeding the Granier coefficient by two to almost three orders of magnitude. Values obtained for exponent b were more similar to the reported Granier value and ranged from 1.24 to 1.88 (Figure 1). We also observed a fairly high degree of variation across stems obtained from different trees of the same species. However, in general, there was less variability in measured sap flux density values at the low end of the applied pressure range with correspondingly smaller k values (Figure 1).

Our results from dye perfusions generally showed that only the current year xylem was active in the ring-porous species (Figure 2). The only exception to this pattern was *E. angustifolia*, where some active xylem was present in previous growth rings (Figure 2a). However, the active xylem in previous years growth comprised only a small fraction of the total stained area. The average conducting sapwood area ranged from 0.35 ± 0.06 to 1.70 ± 0.18 cm^2, whereas the average sapwood depth ranged from 0.88 ± 0.04 to 1.63 ± 0.23 mm (Table 2 and Figure 2).

Calibration differences between ring-porous species varied depending on the range of applied pressure gradients used in the laboratory to induce different flow conditions. Species-specific differences in the scaling exponent b and coefficient a were assessed by comparing the slopes and intercepts of log-transformed regression

models for each species. Differences in the scaling exponent b were observed when data across the entire pressure gradient range were considered (0.001–0.14 MPa m^{-1}, Table 3, $P < 0.05$). However, no statistical differences in b were observed between species for data within the expected range of in situ pressure gradients (<0.02 MPa m^{-1}, Table 3, $P > 0.05$). This was also true for all data collected within the pressure gradient ranges of <0.03 and <0.04 MPa m^{-1} (Table 3, $P > 0.05$). The point at which statistical differences in b between species were observed occurred when data associated with a pressure gradient range of up to 0.05 MPa m^{-1} and greater were included in the analysis (Table 3, $P < 0.05$). Species-specific differences in coefficient a were observed across all pressure gradient ranges considered (Table 3, $P < 0.05$). However, when *E. angustifolia* was removed from the regression comparison, no differences in intercepts were observed between *G. triacanthos*, *Q. gambelii* and *S. japonica* at the lower end of the pressure gradient ranges considered (<0.02 and 0.03 MPa m^{-1}, $P > 0.05$).

The calibration equation coefficients and scaling exponents obtained depended on the range of applied pressure gradients considered (Figure 3 and Table 3). In general, increasing the range of data included according to pressure gradient conditions was associated with increasing a and b values (Figure 3 and Table 3).
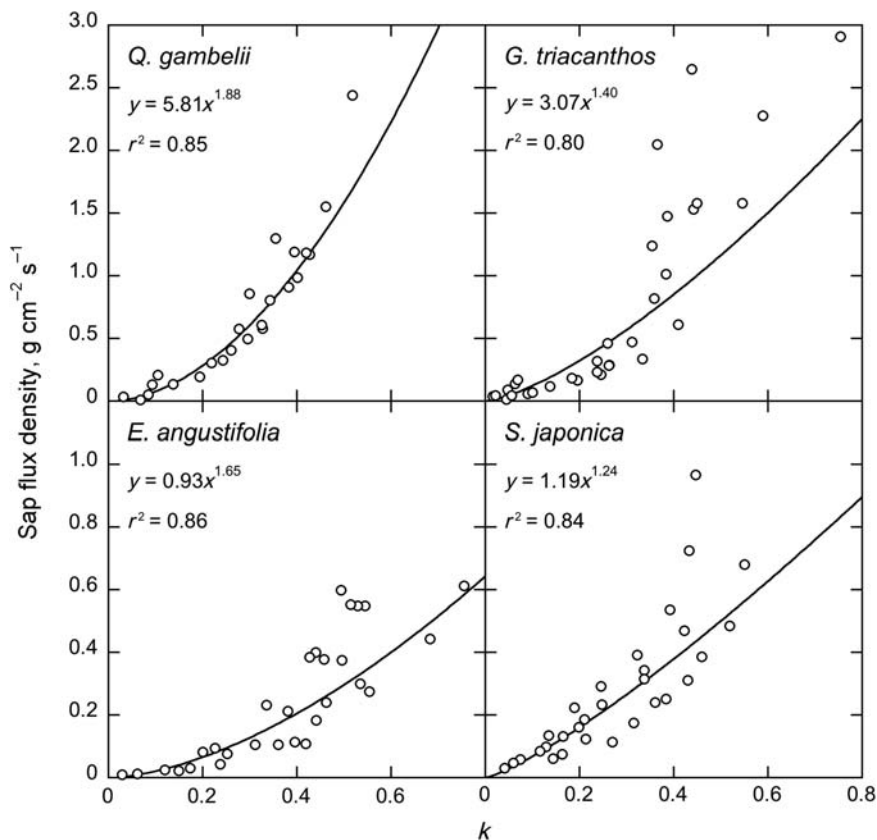

Figure 1. Relationship between sap flux density and k obtained for excised branch segments of ring-porous species. Solid lines represent curve fits of least-squares power functions.
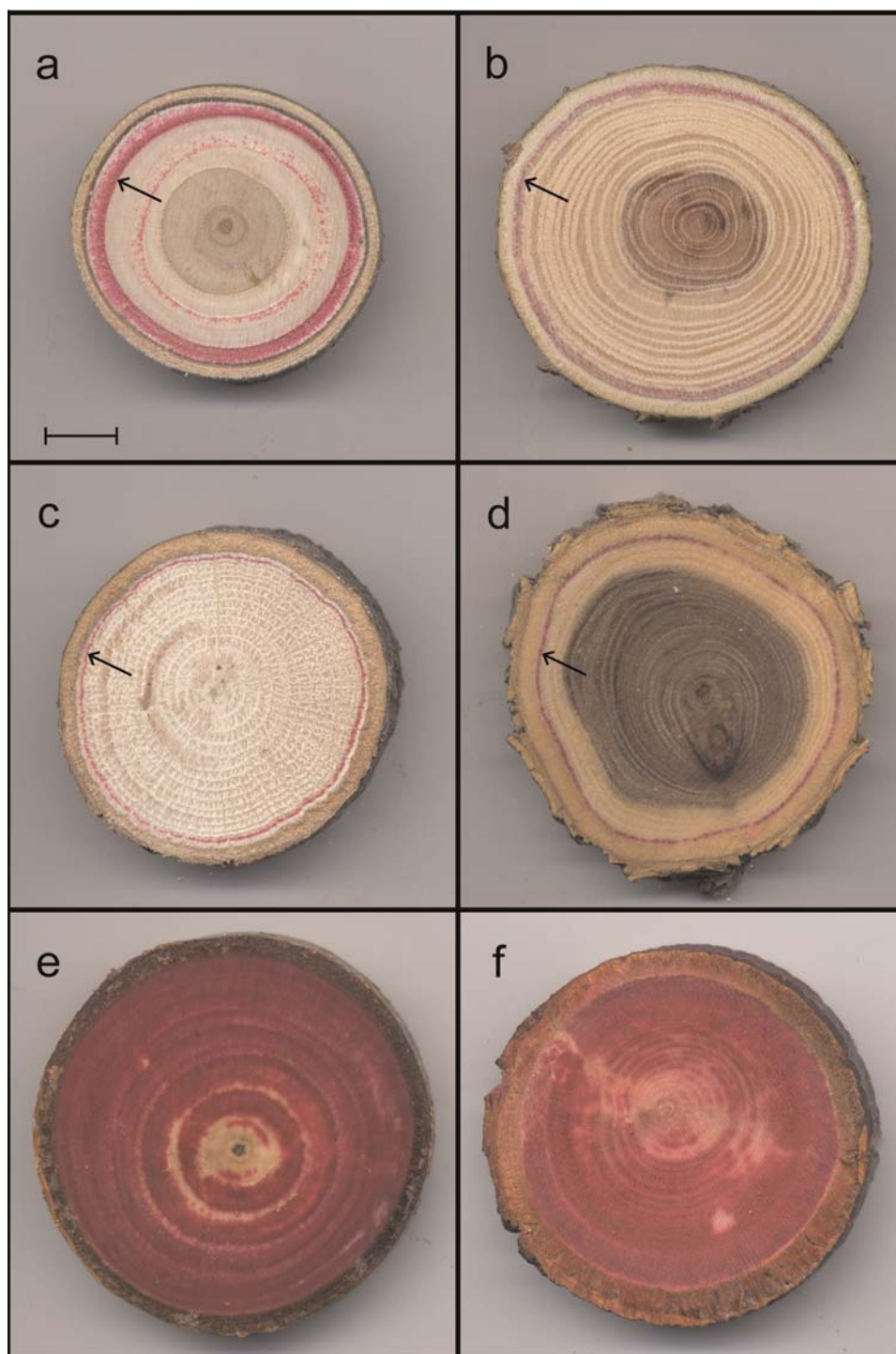
Figure 2. Images of representative branch cross-sections obtained from the location between the heated and reference thermal dissipation probes for both ring-porous and diffuse-porous species. (a–d) Ring-porous species *E. angustifolia*, *G. triacanthos*, *Q. gambelii* and *S. japonica,* respectively. (e and f) Diffuse-porous species *P. fremontii* and *T.cordata*, respectively. Active sapwood area is shown as the stained area indicated with arrows. The scale bar represents a length of 1 cm.

Diffuse-porous species

Calibration results for diffuse-porous species were generally in good agreement with Granier's calibration (Figure 4). In addition, comparison of coefficient *a* and exponent *b* values yielded no differences across the entire range of pressure gradient conditions considered ($P > 0.05$). In most cases, the active sapwood depth was less than the sensor length (20 mm) for both *P. fremontii* and *T. cordata*. Because of

Table 2. Mean sapwood area and depth ± standard error for all branch segments associated with calibration measurements.

Species	Wood type	Sapwood area (cm^2)	Sapwood depth (mm)	N
E. angustifolia	Ring-porous	1.70 ± 0.18	1.63 ± 0.23	7
G. triacanthos	Ring-porous	0.73 ± 0.09	0.98 ± 0.09	6
Q. gambelii	Ring-porous	0.35 ± 0.06	0.88 ± 0.04	6
S. japonica	Ring-porous	0.51 ± 0.12	1.08 ± 0.14	6
P. fremontii	Diffuse-porous	16.02 ± 1.01	12.80 ± 1.46	6
T. cordata	Diffuse-porous	13.08 ± 0.75	15.20 ± 0.80	5

Table 3. Slope and intercept values ± standard error of log-transformed calibration data obtained by standard least-squares linear regression.

Pressure gradient range ($MPa\ m^{-1}$)	Species	Slope	Intercept	r^2
<0.02[a]	*E. angustifolia*	1.09 ± 0.16	−1.42 ± 0.34	0.91
	G. triacanthos	0.87 ± 0.25	−0.51 ± 0.69	0.59
	Q. gambelii	1.55 ± 0.61	0.84 ± 1.43	0.57
	S. japonica	0.94 ± 0.14	−0.56 ± 0.28	0.82
<0.03[a]	*E. angustifolia*	1.15 ± 0.15	−1.20 ± 0.30	0.86
	G. triacanthos	0.91 ± 0.21	−0.23 ± 0.52	0.60
	Q. gambelii	1.65 ± 0.47	1.10 ± 1.05	0.68
	S. japonica	1.08 ± 0.13	−0.21 ± 0.23	0.82
<0.04[a]	*E. angustifolia*	1.16 ± 0.14	−1.18 ± 0.26	0.88
	G. triacanthos	1.00 ± 0.19	0.03 ± 0.45	0.66
	Q. gambelii	1.70 ± 0.29	1.33 ± 0.58	0.76
	S. japonica	1.16 ± 0.11	0.00 ± 0.18	0.83
<0.05[a,b]	*E. angustifolia*	1.29 ± 0.14	−0.88 ± 0.25	0.87
	G. triacanthos	1.07 ± 0.17	0.24 ± 0.40	0.70
	Q. gambelii	1.72 ± 0.27	1.39 ± 0.53	0.77
	S. japonica	1.24 ± 0.10	0.17 ± 0.16	0.84
<0.15 (all data)[a,b]	*E. angustifolia*	1.65 ± 0.13	−0.07 ± 0.17	0.86
	G. triacanthos	1.40 ± 0.13	1.12 ± 0.24	0.80
	Q. gambelii	1.88 ± 0.17	1.76 ± 0.27	0.85
	S. japonica	1.24 ± 0.10	0.17 ± 0.16	0.84

The slope data correspond to the scaling exponent *b*, and the antilog of the intercept corresponds to coefficient *a*. Different ranges in applied pressure gradient conditions associated with data collection are shown.
[a]Species-specific differences in intercept values were observed over all pressure gradient ranges analyzed.
[b]Species-specific statistical differences between slope values (exponent *b*) were not observed until the pressure gradient range included all data collected <0.05 $MPa\ m^{-1}$.

this, the use of the Granier equation initially underestimated sap flux density for both species. However, the active sapwood depth was large enough that it was possible to apply the correction according to Clearwater et al. (1999, Eq. (3)), which brought the calibration results for both species within the range consistent with the original Granier calibration.

Dye perfusion results for both *P. fremontii* and *T. cordata* showed that xylem representing multiple years of growth was active (Figure 2). This was the case for all the measurement segments of both species considered. In addition,

xylem associated with previous years' growth was often stained uniformly throughout. The average active sapwood area was 16.02 ± 1.01 and 13.08 ± 0.75 cm^2 for *P. fremontii* and *T. cordata*, respectively.

Discussion

Calibration data for all four ring-porous species gave equations that were substantially different from the original Granier equation, particularly with respect to coefficient *a*, where differences of two to three orders of magnitude were observed (Figure 1 and Table 3). These results are not consistent with the originally reported results of Granier or subsequent work (Granier 1985, Cabibel and Do 1991, Catovsky et al. 2002). However, Taneda and Sperry (2008) also obtained calibration results that differed substantially from Granier's equation for *Q. gambelii*, where reported coefficient *a* values ranged from 2.38×10^{-3} to 1.81×10^{-2} m s^{-1} (corresponding to 0.238–1.81 in g $cm^{-2}\ s^{-1}$ units reported here) and exponent *b* values ranged from 1.05 to 1.50 for different stems. The exact values for coefficient *a* and exponent *b* reported by Taneda and Sperry were smaller relative to those reported here, which may have been associated with differences in applied pressure gradients for measurement stems. However, both Taneda and Sperry (2008) and the current study showed that the largest differences compared with the original Granier equation were associated with the coefficient *a* value, where in both cases, differences that exceeded two orders of magnitude were observed, and the use of the Granier equation would have resulted in substantial underestimation of sap flux density.

Sap flux density values associated with ring-porous calibration measurements in this study were quite high, although much of the data were collected under pressure gradient conditions that likely exceed those experienced in situ. However, measured *k* values associated with previous field installations (Bush et al. 2008), and the same populations of ring-porous species considered here, were generally < 0.5. Field measured *k* values combined with species-specific calibration curves indicated that in almost all cases that, even when all of the calibration data were considered (up to applied pressure gradients of 0.14 $MPa\ m^{-1}$), flux rates did not exceed maximum midday velocities previously reported for trees with wide vessels (up to 13 mm s^{-1} or 1.3 in g $cm^{-2}\ s^{-1}$ units reported here) (Tyree and Zimmermann 2002).

The large calibration differences observed are likely due to the small conducting sapwood area and small sapwood depth relative to the total sensor length obtained in all cases for the ring-porous species (Figure 2 and Table 2). The stained sapwood area ranged from 0.35 ± 0.06 to 1.70 ± 0.18 cm^2 for the entire cross-sectional area of the measurement segments and was generally limited to current year xylem. The stained area was substantially smaller than the apparent sapwood based on visual distinction between
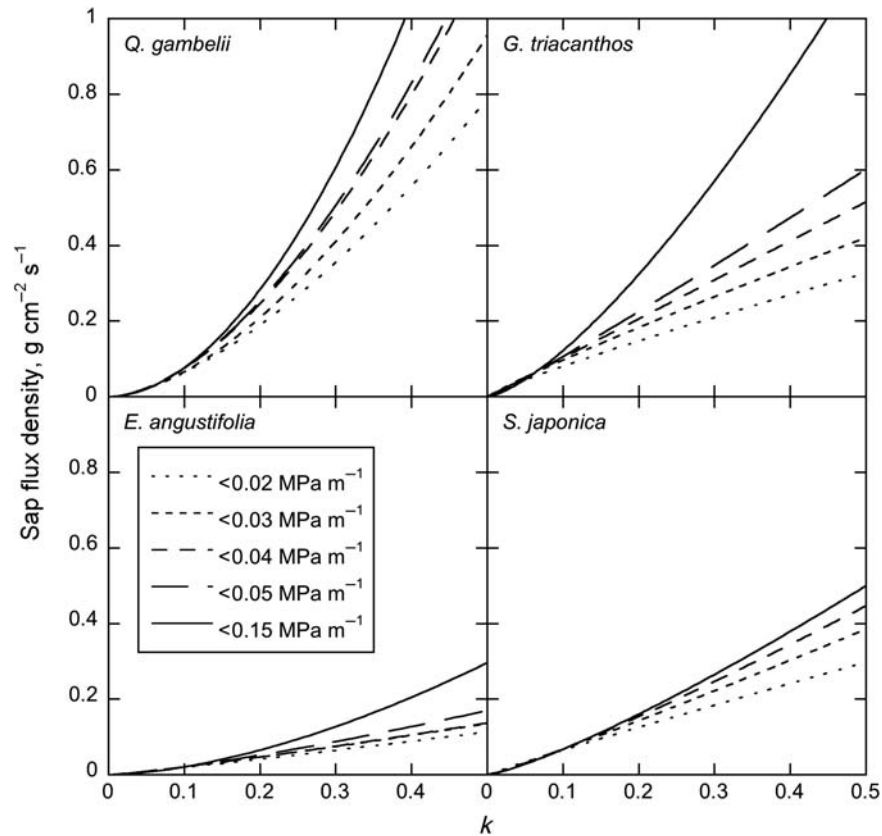
Figure 3. Influence of the range of applied pressure gradient conditions associated with calibration data collection on calibration results. Lines show least-squares power functions fitted to different pressure gradient ranges for all ring-porous species considered. The coefficients and r^2 values are given in Table 3 (slope and antilog of intercept values correspond to exponent b and coefficient a, respectively). No slope differences (exponent b) were observed for data collected below a pressure gradient of 0.04 MPa m^{-1}. However, differences in intercepts were observed over all analyzed pressure gradient ranges.
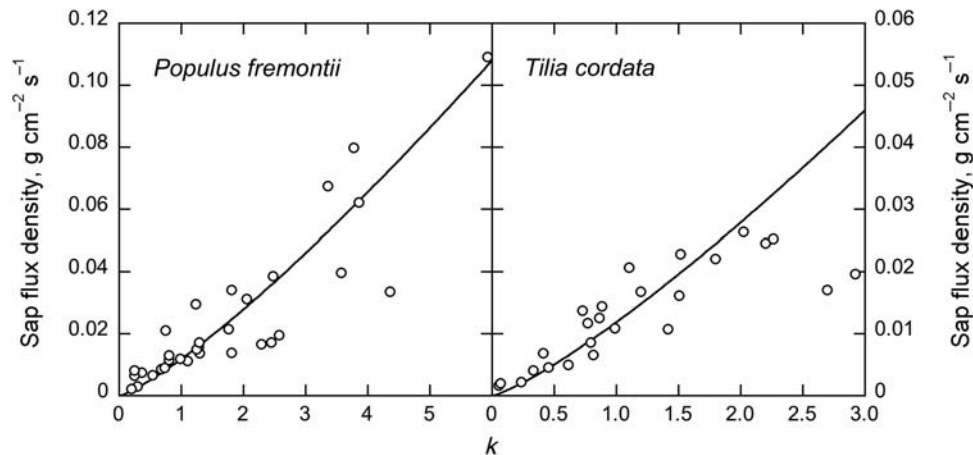

Figure 4. Relationship between sap flux density and k obtained for excised branch segments of diffuse-porous species. The solid line represents the original Granier calibration. Data shown were corrected according to Clearwater et al. (1999) for all stems where sensor length exceeded active sapwood depth.

darker heartwood (where present) and lighter sapwood over the cross-sectional area of the stem (Figure 2 and Table 2). In addition, the average sapwood depth was substantially smaller than the sensor length for both the 10 and 20 mm

probes used (Table 2). Correcting for this problem according to Clearwater et al. (1999) was not possible because the active sapwood depth was so small that the corrected temperature difference between the sensor probes, representing

the area in contact with active sapwood, becomes a negative value. This also has implications for scaling sap flux data to the whole-tree and stand levels. Our data suggest that sap flux data should be scaled using total sapwood area associated with current year xylem, unless there is evidence to suggest that xylem from previous years' growth is substantially contributing to whole-tree sap flow.

Our results also showed species-specific differences between ring-porous species, which varied depending on the range of applied pressure gradients considered. Exponent b values were only different between species when data associated with a pressure gradient range of up to $0.05\,\mathrm{MPa\,m^{-1}}$ and above (likely in excess of native gradients) were included. Below a $0.04\,\mathrm{MPa\,m^{-1}}$ cut-off, all species were statistically the same. Coefficient a values differed between species across all pressure gradient ranges considered. However, the removal of *E. angustifolia* from the regression comparison showed no differences in coefficient a between the remaining three ring-porous species at pressure gradient ranges of <0.02 and <0.03 $\mathrm{MPa\,m^{-1}}$. In addition, obtained a and b coefficients were sensitive to the pressure gradient range considered, where increasing the range of pressure gradients associated with data collection was associated with increasing values of both a and b (Figure 3 and Table 3). Because of these results, we suggest that thermal dissipation probes used to measure absolute rates of water use in ring-porous species should be independently calibrated where possible and that calibration data used to derive equation coefficients be constrained to include only applied pressure gradient conditions likely associated with in situ pressure gradients. Our results differed considerably from the widely used Granier equation, particularly with respect to the coefficient a values, and suggest that previous calculations of absolute rates of water uptake in ring-porous species using the Granier coefficients may be substantially underestimated.

It is not clear why our results were markedly different from the previous calibrations for *Quercus* species and *C. sativa* (Granier 1985; Cabibel and Do 1991; Catovsky et al. 2002). It is possible that if a large number of early season vessels were open at both ends of the measurement segments used in the current study, observed sap flux density could have been considerably higher due to decreased resistance in the flow path, with correspondingly little or no flow occurring in the much higher resistance pathways associated with late wood. However, to minimize this potential effect, our final measurement segments were relatively long, ranging on average from 57.5 ± 10.0 to $79.6 \pm 8.3\,\mathrm{cm}$ (Table 1). Furthermore, if a large fraction of early season vessels were open at both ends of the measurement segments, we would expect that the stained xylem would include open-ended, early season vessels from previous years' growth as well, provided that vessel length distributions were unchanged across growth rings and tyloses formation was not a large factor. However, this was not observed, as stained xylem was generally limited to current

year growth. We cannot rule out the possibility that tyloses were not a factor, as the measurement segments in this study were not examined for their presence. In addition, because we did not generate vessel length distributions for each species and for this diameter size class, we cannot completely eliminate the possibility of open-ended vessels in our final measurement segments. Still, if our measured sap flux density values were in fact higher than what would be observed in situ, we would expect that the value obtained for k would increase accordingly and would not necessarily expect increased sap velocity conditions to have a large impact on the calibration results, unless the relationship between sap flux density and k changes considerably at high sap velocities.

In contrast to our results associated with ring-porous species, we found that the calibration results for the two diffuse-porous species agreed well with the original Granier calibration (Figure 4). Our results also provide further support for the use of the Clearwater equation (Eq. (3)) to correct for errors associated with installations, where the length of thermal dissipation probes exceeds the active sapwood depth. Our results reported here for diffuse-porous species are also consistent with many earlier reports that provided subsequent validation of the Granier calibration for a number of diffuse-porous species (Cabibel and Do 1991, Lu and Chacko 1998, Braun and Schmid 1999, Clearwater et al. 1999, McCulloh et al. 2007). Recently, however, Steppe et al. (2010) found that the original calibration underestimated sap flux density in *F. grandifolia* on average by 60% when compared with paired gravimetric measurements. In addition, calibration data collected on excised stems of *T. ramosissima × chinensis* (Hultine et al. 2010b) also showed that the Granier equation underestimated sap flux density by 50%. The average value obtained for coefficient a in the latter case was $240\,\mathrm{g\,m^{-2}\,s^{-1}}$ (compared with 119 for the Granier equation in the same units) and 1.16 for the scaling exponent b. While the results for diffuse-porous species reported here were consistent with the Granier equation and many other previous reports, the results of Steppe et al. (2010) and Hultine et al. (2010b) indicate that the Granier equation is not universally applicable to diffuse-porous species. However, the differences reported in Steppe et al. (2010) and Hultine et al. (2010b) (60 and 50%, respectively), while significant, are much smaller relative to differences reported for ring-porous species in Taneda and Sperry (2008) and this study (in excess of two orders of magnitude).

It is important to note that the calibration data reported here are associated with excised branch segments, where substantial circumferential variability in sap flow may occur due to the presence of compression or tension wood, which was not accounted for in this study. The degree to which calibration results may vary depending on sampling location in a given tree (branches vs. boles, for example) is still largely unknown. To our knowledge, we are the first to

report calibration data for five of the six species in this study. The number of species, genera and xylem types examined, particularly with respect to the ring-porous species, for which data were relatively scarce and potentially higher error relative to other xylem types may be expected based on anatomical features of the xylem and theoretical considerations (Clearwater et al. 1999), represents a step forward in advancing our overall understanding of the widely used Granier method and its potential limitations.

Thermal dissipation probes are widely used for calculating sap flow of trees and forest stands. However, our results show that it should not be assumed that the original calibration is appropriate in all cases. We found that the original calibration did not accurately calculate sap flow for all four ring-porous species considered in this study. In addition, we found species-specific differences in calibration results between ring-porous species, particularly with respect to the equation coefficient *a*. We also show that the Granier calibration was consistent with calibration data collected for two diffuse-porous species in this study. We suggest that independent calibration of the Granier method should be conducted where possible, as the data reported here and elsewhere suggest that the use of the original Granier equation is not universally applicable across species and xylem types.

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