Article

A Test of the Relationship between Sap Flow and Evapotranspiration, Normalized via Leaf Area, under Non-Limiting Soil Moisture

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Abstract: Sap flow is the movement of fluid within plants, whereas reference evapotranspiration (ET_o) occurs external to plants as the transfer of water vapor from a hypothetical grass crop. Yet, on daily time scales, and when soil moisture is non-limiting, sap flow has a positive linear relationship with ET_o. Furthermore, the $E_{2.88}$ model hypothesises that sap flow (Q) is equal to ET_o when parameters are normalized by leaf area (A_L) via the following relationship: $Q/A_L = ET_o/2.88$. The value of 2.88 is the supposed leaf area index of the hypothetical grass in the ET_o model. Therefore, the $E_{2.88}$ model potentially provides a null or expected value of sap flow based on independent ET_o parameters and leaf area. A test of the $E_{2.88}$ model was conducted via three sap flow methods (dual method approach [DMA], heat ratio [HRM], and Tmax method) on the measurement of three woody species: *Pyrus communis* L. (var. Beurre Bosc Pear), *Syzygium floribundum* F. Muell. (Weeping Lilly Pilly), and *Syzygium paniculatum* Gaertn. (Lilly Pilly). A data compilation of the literature expanded the sample size to include additional species. The measured trees and data compilation found a strong, positive correlation between sap flow and ET_o normalized by leaf area. However, the interpretation of the results was dependent on the sap flow method. The DMA had an average accuracy of 1.6%, whereas the HRM and Tmax significantly underestimated and overestimated sap flow, respectively. This study suggested that sap flow can be reliably estimated from accurate leaf area and ET_o measurements and when other variables, such as soil moisture, are non-limiting.

Keywords: dual method approach; heat pulse probe; hydrology; irrigation management; leaf area index; Penman–Monteith FAO-56; transpiration

1. Introduction

Total plant volumetric sap flow (Q) is the movement of fluid within xylem and is functionally correlated with transpiration, stem refilling and transport of solutes. In contrast, reference evapotranspiration (ET_o) is the transfer of water vapor from a land surface covered by a hypothetical grass with a uniform height of 0.12 m [1,2]. Despite the fundamental difference in definition between Q and ET_o, there is a significant positive correlation between the variables (e.g., [3–6]).

Under certain assumptions, it has been hypothesised that Q is not only positively correlated with ET_o, but it is equivalent. Pereira et al. [7] proposed a model, hereafter called the $E_{2.88}$ model, where Q, normalized per unit leaf area, is equivalent to ET_o, normalized per unit leaf area of a reference grass crop. The $E_{2.88}$ model has the form of $Q/A_L = ET_o/2.88$ where A_L is total plant leaf area (m^2). The 2.88 constant is derived from leaf area index (LAI) of the hypothetical reference grass crop of height 0.12 m [1,7]. The assumptions of the $E_{2.88}$ model include non-limiting soil moisture and transpiration is conditioned by leaf area [7]. The $E_{2.88}$ model has been explicitly tested in a limited number of studies with promising results [7–10]. Yet, if the generality of the $E_{2.88}$ model is demonstrated then it will prove valuable for plant–water relations, physiological comparisons, irrigation and

water resource management because sap flow may be estimated, under non-limiting soil moisture, without the need for site specific calibrations.

Aside from the $E_{2.88}$ model, Q is a commonly incorporated parameter in numerous models across many fields of application such as physiology, irrigation, hydrology, climatology, and others [11–13]. As Q occurs within xylem it is difficult to measure directly, and it is generally estimated indirectly via thermometric techniques. This approach involves the application of continuous heat or short duration heat pulses on, or within, the xylem and the conversion of temperature changes via empirical or theoretical equations [14]. Extensive research has been undertaken over many decades to improve the accuracy of thermometric methods so that the sap flow results produced by one method are significantly correlated with another method [15]. Consistency in results produced by different sap flow methods is critically important for the correct interpretation of results across studies, experiments and models, as well as the validity of large scale, global data sets [16]. Therefore, the inclusion of different sap flow methods in a model, such as the $E_{2.88}$ model, should yield a consistent interpretation of results.

The heat pulse family of methods are a popular thermometric approach to estimate Q [15]. Heat pulse methods are based on the physical theory of heat conduction and convection in porous media [17]. Examples of heat pulse methods include the heat ratio method (HRM), Tmax method, and dual method approach [17–20]. Following the application of a short duration (i.e., <3 s) heat pulse, the HRM measures the ratio of the temperature change downstream versus upstream of the heat source [17,19], whereas the Tmax method measures the time to the maximum temperature increase downstream of the heat source [18]. Forster [21] experimentally demonstrated that the HRM and Tmax are limited to slow and fast heat velocities, respectively. The dual method approach (DMA) theoretically combines slow and fast heat pulse methods to describe the entire observable measurement range of heat velocity in xylem [20]. As the HRM and Tmax methods are biased towards under- and overestimating sap flow, respectively, their inclusion in a model, without consideration of the alternative sap flow methods, may lead to varying interpretations of the results. Therefore, given the importance of consistency produced by various sap flow methods, this study explored the null hypothesis that there would be no difference in results generated via different sap flow methods in the $E_{2.88}$ model.

The primary aim of this study was to test the hypothesis that Q is equivalent to ET_o when both parameters are normalized by leaf area (i.e., the $E_{2.88}$ model). This study also explored the hypothesis that the interpretation of results was consistent across three heat pulse sap flow methods. To broaden the scope of the study, a compilation of data from the broader literature was included that incorporated a larger sample size of measured plant species and sap flow methods.

2. Material and Methods

2.1. Study Species and Site Descriptions

Three species were measured in this study: *Pyrus communis* L. (var. Beurre Bosc Pear), *Syzygium floribundum* F.Muell. (Weeping Lilly Pilly), and *Syzygium paniculatum* Gaertn. (Lilly Pilly). Table 1 summarises tree characteristics including average height, leaf area, diameter, and sapwood radius.

Table 1. Tree characteristics of the three woody species that were measured in this study. Values are means ($\pm$ S.D.); n is sample size of the number of trees instrumented with a sap flow sensor; LAI is leaf area index.

Species	Variety	n	Height (m)	Crown Area (m^2)	LAI	Leaf Area (m^2)	Trunk Diameter (m)	Bark Radius (m)	Sapwood Radius (m)
Pyrus communis L.	Beurre Bosc Pear	5	5.040 ± 0.336	13.480 ± 1.698	2.586 ± 0.248	35.011 ± 6.670	0.190 ± 0.014	0.007 ± 0.001	0.161 ± 0.025
Syzygium floribundum F.Muell.	Weeping Lilly Pilly	3	1.083 ± 0.162	0.599 ± 0.018	1.675 ± 0.092	1.003 ± 0.057	0.023 ± 0.003	0.002 ± 0.000	0.007 ± 0.001
Syzygium paniculatum Gaertn.	Lilly Pilly	3	1.480 ± 0.121	0.711 ± 0.059	1.985 ± 0.062	1.410 ± 0.108	0.028 ± 0.003	0.002 ± 0.000	0.010 ± 0.001

P. communis were mature ~5 m tall trees, 15 years of age, growing in an established orchard in the Gruyere, Yarra Valley, Victoria, Australia (37.73° S, 145.45° E). The site elevation was ~90 m a.s.l., consisting of low rises and swales, with mean maximum/minimum annual temperature of 20.5/7.4 °C and mean annual rainfall 732 mm. The soils comprised of a silty clay or clay loam to a depth of approximately 300 to 500 mm below which there was a gradation to a semi-impermeable sub-soil layer. Soil moisture was maintained at a high level (>−100 kPa) with frequent irrigation and was monitored with TEROS 21 soil water potential sensors (METER Group, Pullman, WA, USA), installed at 0.2 m and 0.4 m depth. Across the growing season and measurement campaign, irrigation occurred every two to three days via a drip irrigation system, dripper spacing of 2.5 m, delivery rate of 8 L h^{-1} over a 6-h period. Two sites were randomly chosen across the orchard and three and two trees, within a radius of 10 m, were randomly selected at site 1 and site 2, respectively, for sap flow instrumentation. Sap flow sensors (see details below) were installed in early November, 2019 and removed in mid-June, 2020. For this study, a 35-day period between mid-December 2019 and mid-January 2020 was selected for analysis because tree growth and canopy development had reached a seasonal maximum and there was no change in leaf area.

The *S. floribundum* and *S. paniculatum* were potted saplings, purchased locally from a commercial supplier, grown in an open common garden in Melbourne, Victoria (37.99° S, 145.03° E). The site elevation was approximately 10 m a.s.l., with mean air temperature, during the measurement campaign, of 18.4 °C and a maximum/minimum of 43.5/7.6 °C. The soil volume of the pots was 15 L and the soil was a standard potting mixture of 2:1:1 pine bark, peat, and sand. Soil moisture was maintained at a high level through the application of 5 L of irrigation, per pot, every two days. Sap flow sensors were installed on *S. paniculatum* in mid-January, 2020, for a 28-day measurement period, and *S. floribundum* in mid-March, 2020, for a 34-day measurement period. The length of the measurement period was chosen because there was minimal trunk and canopy development and no change in leaf area.

2.2. Reference Grass Crop Evapotranspiration Measurements

An assessment of the accuracy and performance of ET_o methods was beyond the scope of this study and it has been conducted in numerous reviews (e.g., [2,22,23]). Instead, the FAO-56 Penman–Monteith method was used because it is considered a standard method to estimate ET_o [1,2,23] and was the most widely used model from the data compilation. The following Penman–Monteith model was applied in this study [1,7]:

$$ET_o = \frac{0.408\Delta R_n + \frac{900\gamma D_a U_2}{T_{mean}+273}}{\Delta + \gamma(1 + 0.34U_2)} \tag{1}$$

where Δ is the slope of the saturation vapour pressure curve at ambient air temperature (kPa °C^{-1}), R_n is daily net radiation (MJ m^{-2} day^{-1}), γ is the psychometric constant (kPa °C^{-1}), D_a (kPa) is daily average vapour pressure deficit at 2 m height, U_2 (m s^{-1}) is daily average wind speed at 2 m height, and T_{mean} (°C) is daily average air temperature at 2 m height. Equation (1) is the reference grass crop evapotranspiration equation because of the 0.34 U_2 component. This component was derived by dividing the reference grass crop boundary layer conductance ($U_2/208$ m s^{-1}) by stomatal conductance (1/70 m s^{-1}) which equals 0.34 U_2.

For the *P. communis* field site, daily (midnight to midnight) estimates of ET_o were collated from an Australian Government Bureau of Meteorology (BOM) official weather station approximately 3 km distance from the field site (Site number: 086383, Coldstream, Victoria, Australia). For the *S. floribundum* and *S. paniculatum* measurement campaigns, ET_o was calculated from a local weather station installed proximate to the instrumented trees. The weather station model was ATMOS 41 (METER Group, Pullman, WA, USA) that measured solar radiation (±5%), air temperature (±0.2 °C), relative humidity (±1.5%), and wind speed (±0.3 m s^{-1} or ±3% of measurement). The weather station was installed at

2 m height and data were logged at 10-min intervals and hourly or daily averages used for data analysis.

2.3. Sap Flow Measurements

Sap flow sensors were heat pulse variety (Implexx Sap Flow Sensor, Implexx Sense, Melbourne, Australia) that consisted of three stainless steel needles of 0.03 m length, 0.0013 m diameter, and spaced at 0.006 m equidistance in the axial direction. There were two negative temperature coefficient (NTC) thermistors, with an accuracy of ± 0.015 °C, located in the downstream and upstream needles, respectively, spaced at 0.01 m and 0.02 m distance from the needle tip. The central needle was a heater element of nichrome wire with a typical resistance of 38 Ω and total energy applied per heat pulse of ~400 J m^{-1}. Thermistors and heater element were controlled by a serial to digital interface (SDI-12, Implexx Sense, Melbourne, Australia) that measured temperature at a resolution of 0.2 s and precisely controlled a 3 s heat pulse. Data were logged at 10 min intervals, and then averaged hourly, by a commercially available data logging system (ES-SYS, Edaphic Scientific, Melbourne, Australia).

Heat velocity was measured via three heat pulse methods: heat ratio method (HRM), Tmax method, and dual method approach (DMA) following the methods outlined by Forster [20,21]. Heat velocity estimates were corrected for wounding and probe misalignment following the methods of Forster [20]. Corrected heat velocity was converted to sap flux density (J, m^3 m^{-2} s^{-1}) via the following equation [24]:

$$J = \frac{V_c \rho_d (c_d + m_c c_w)}{\rho_w c_w} \tag{2}$$

where V_c is corrected heat velocity (m s^{-1}), ρ_d is sapwood density (kg kg^{-1}), c_d is dry wood specific heat capacity (1200 J kg^{-1} K^{-1}; [25]), m_c is sapwood gravimetric moisture content (kg kg^{-1}), c_w is sap solution specific heat capacity (4182 J kg^{-1} K^{-1}; [25]), and ρ_w is the density of sap (1000 kg m^{-3}; [25]). Destructive samples of sapwood were taken from the trees, at the end of the measurement campaign, and proximate to the sap flow sensor installation site, for the measurement of ρ_d and m_c following methods outlined by Forster [21]. Finally, total tree sap flow (Q, kg or L) was calculated as J multiplied by sapwood area (A_S, m^2; [26]). Sapwood area was calculated based on a visual assessment as there was a clear colour demarcation between sapwood and heartwood within the xylem.

2.4. Total Leaf Area Measurements

Total leaf area for *P. communis* was measured at start, mid-point and end of the 35-day measurement campaign by multiplying LAI by canopy projection area. LAI was estimated via two, independent light interception techniques: ceptometer (AccuPAR, model LP-80, METER Group, Pullman, WA, USA) where an average of 20 below canopy measurements were taken at random positions beneath each tree approximately 0.5 m distance from the trunk; and digital imagery where images at four locations (north, south, east, west) beneath the canopy approximately 0.5 m distance from the trunk were converted to LAI via a MATLAB based software algorithm developed by Fuentes et al. [27]. The two techniques yielded similar values and averages were taken as tree LAI. Canopy projection area was assumed to be circular following averaging of eight radii measurements, between the trunk and outer canopy, around the circumference of the tree.

Total leaf area for *S. floribundum* and *S. paniculatum* was measured at the start and end of the measurement campaign by multiplying average leaf area by total number of fully expanded leaves on each tree (approximately 700 to 1000 leaves per individual). Average leaf area was measured by harvesting 100 random leaves at the end of the campaign and measuring via a flatbed scanner and Easy Leaf Area v2 software [28].

2.5. Data Compilation

A comprehensive search of the literature was undertaken to collate data to test the hypothesis that the slope of the linear relationship between transpiration (i.e., $T = Q/A_L$) and ET_o was 0.347 (i.e., the reciprocal of a LAI of 2.88). Collated data conformed with the following criteria: experimental or measured trees were well-watered (this criteria regularly excluded forest or natural experiments and limited studies to intensively managed orchards), Q was estimated on a per stem (or individual) basis, Q was estimated with a thermometric sap flow method (other estimates of transpiration, such as lysimeters, porometers, chambers, Eddy covariance, or other instrumentation, were excluded), Q was divided by A_L, ET_o was calculated via the Penman–Monteith method, all other methods of ET_o calculation were excluded, and crop evapotranspiration (ET_c) data were excluded. Literature was searched on the Web of Science and Google Scholar databases up until April, 2020, with the following search terms: sap flow (or sapflow), leaf area, transpiration, evapotranspiration, ET_o, Penman–Monteith. References and citations were also searched for additional data. Only peer-reviewed, published data were included in the data compilation.

2.6. Data Analysis

Linear regression analysis was used to test the hypothesis that T (Q/A_L) can be estimated from $ET_o/2.88$. All data were untransformed because the analysis tested whether T was linearly related to $ET_o/2.88$ and a log or other transformation would bias this assumption. Slopes of the linear regression curve were fitted through the origin because it was assumed the relationship was equal. A slope and r^2 of 1 indicate a perfect relationship. A slope less than 1 indicates $ET_o/2.88$ underestimates T and a slope greater than 1 indicates $ET_o/2.88$ overestimates T. The accuracy of the DMA, HRM and Tmax sap flow methods were further assessed via a root mean square error (RMSE) test where smaller values indicate a better model.

3. Results

The three sap flow methods used in this study showed differences in the diel pattern of sap flow. Figure 1 displays an example 7-day period of sap flow data around the period of maximum sap flow during the respective species measurement campaigns. The Tmax had high night-time flows whereas the HRM had low peak daytime flows. This observation was evident for the three species measured in this study. The DMA successfully merged the optimal sap flow data from the HRM and Tmax.

There was a significant linear correlation between ET_o and the sap flow methods for all three species (Figure 2). For *P. communis*, there was a significant positive linear correlation between daily ET_o (mm d^{-1}) and Q (L d^{-1}) when sap flow was estimated via the DMA ($r^2 = 0.824$, $p < 0.001$, $n = 35$), HRM ($r^2 = 0.811$, $p < 0.001$, $n = 35$) and Tmax ($r^2 = 0.177$, $p = 0.012$, $n = 35$). For *S. floridbundum*, there as a significant positive linear relationship between ET_o (mm d^{-1}) and Q (L d^{-1}) when sap flow was estimated via the DMA ($r^2 = 0.816$, $p < 0.001$, $n = 34$), HRM ($r^2 = 0.691$, $p < 0.001$, $n = 34$) and Tmax ($r^2 = 0.733$, $p < 0.001$, $n = 34$). There was also a significant positive linear relationship between ET_o (mm d^{-1}) and Q (L d^{-1}) for *S. paniculatum* via the DMA ($r^2 = 0.806$, $p < 0.001$, $n = 28$), HRM ($r^2 = 0.742$, $p < 0.001$, $n = 28$) and Tmax ($r^2 = 0.344$, $p = 0.001$, $n = 28$).
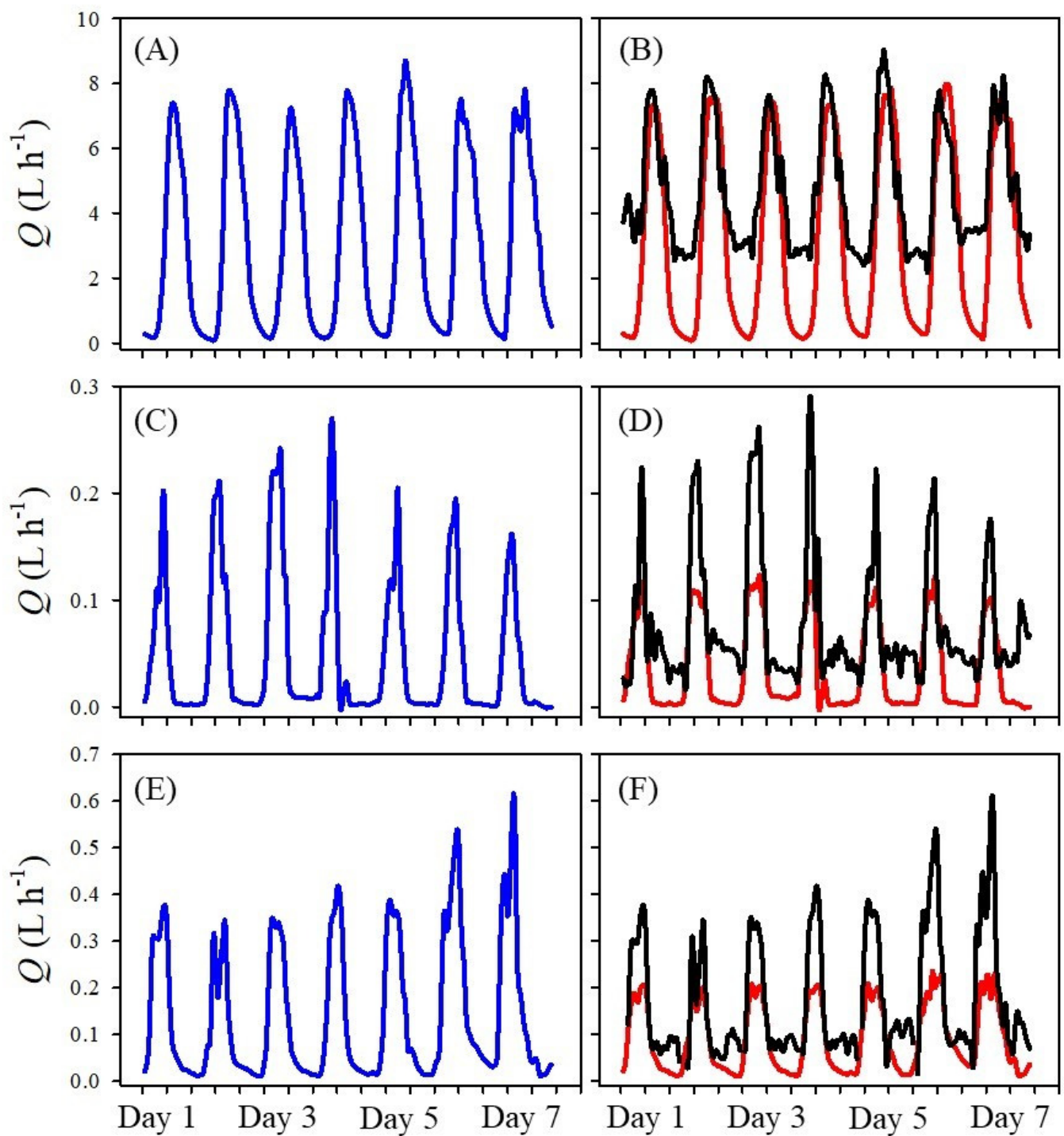
Figure 1. An example 7-day subset of total tree sap flow (Q, L h^{-1}) data for (**A,B**) *P. communis*, (**C,D**) *S. floribundum*, and (**E,F**) *S. paniculatum* highlighting the measurement range differences for the three sap flow methods. (**A,C,E**) Dual method approach (DMA, blue line), (**E,D,F**) heat ratio method (HRM, red line) and Tmax method (black line).
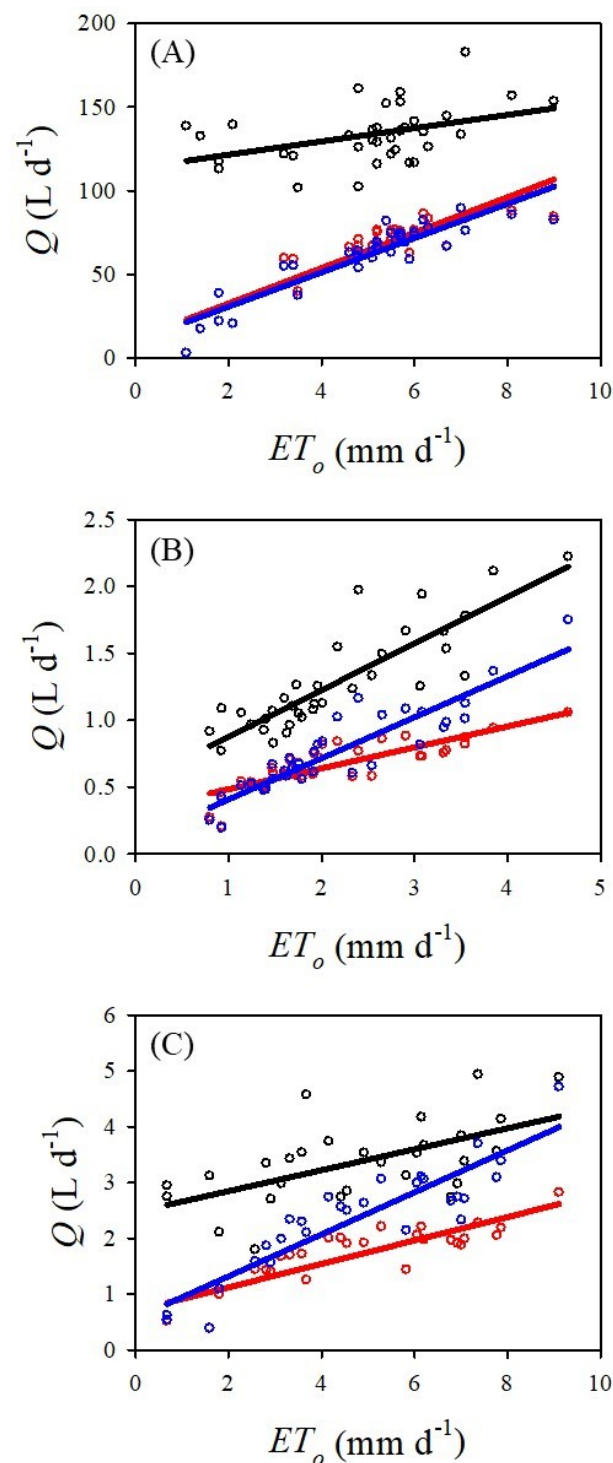
Figure 2. The linear relationship between total tree sap flow (Q, L d^{-1}) and evapotranspiration (ET_o, mm d^{-1}) for (**A**) *P. communis*, (**B**) *S. floribundum*, and (**C**) *S. paniculatum*, and the three sap flow methods: dual method approach (DMA, blue), heat ratio method (HRM, red), and Tmax method (Tmax, black).

The $E_{2.88}$ model was mostly confirmed for the three species measured in this study. However, there were differences in the precision and accuracy of the three sap flow methods. Figure 3 displays the linear relationship between $A_L ET_o / 2.88$ (L d^{-1}) and Q (L d^{-1}) for the three species and sap flow methods. Table 2 presents the results from the linear regression analyses and the performance of each model via RMSE. For all three species, the DMA

was the most accurate sap flow method followed by the HRM and Tmax. The DMA had an error range between 0.5 to 3.3%, an average precision of 0.972 and the smallest RMSE values for all three species. For the HRM, there was a bias towards an underestimation (slope less than 1) in two of the species, whereas the Tmax showed a bias towards an overestimation (slope greater than 1) for all three species.
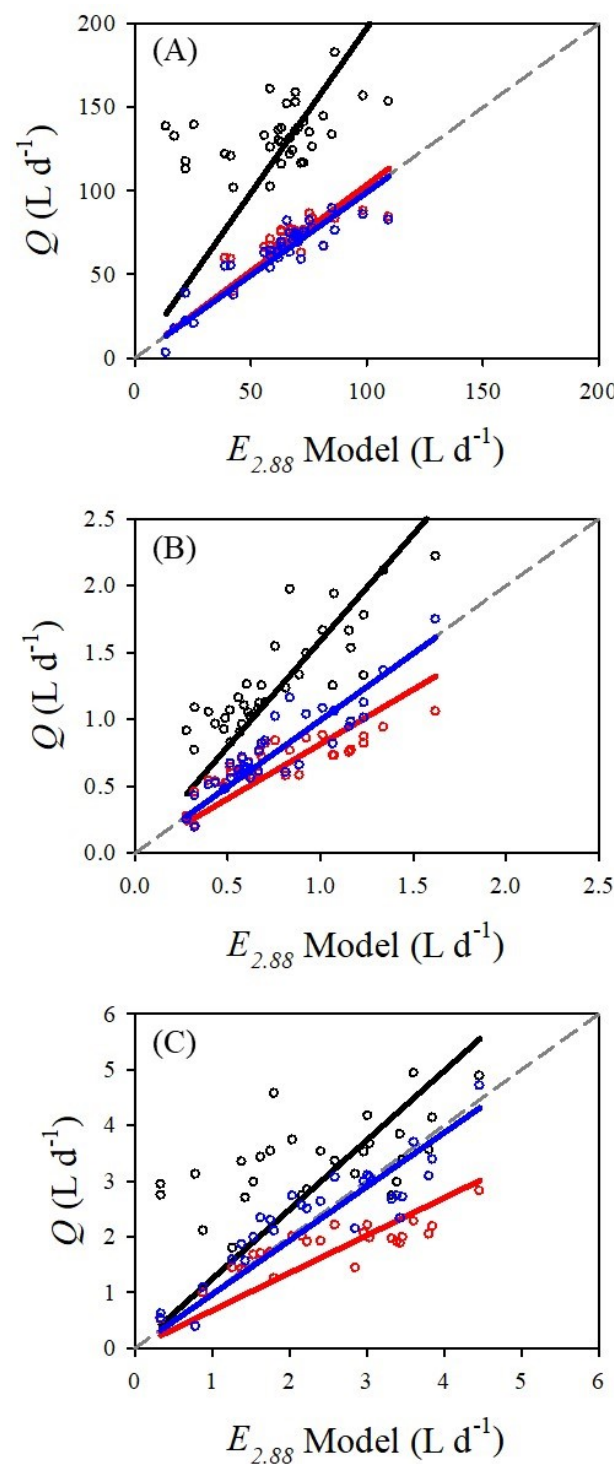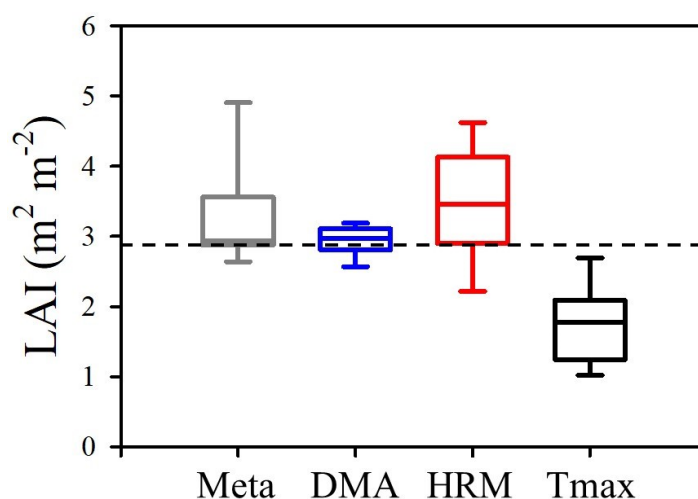

Figure 3. The linear relationship between sap flow estimated via the $E_{2.88}$ model and via a thermometric sensor using a dual method approach (DMA, blue), heat ratio method (HRM, red) or Tmax method (Tmax, black) sap flow method for (**A**) *P communis*, (**B**) *S. floribundum*, and (**C**) *S. paniculatum*. The dashed grey line represents the 1:1 relationship.

Table 2. The accuracy of a sap flow methods in the relationship between Q/A_L and $ET_o/2.88$. Slope is derived from the linear regression of Q/A_L, where Q was estimated from the various sap flow methods, against $ET_o/2.88$ with the intercept forced through the origin. Error is the percent deviation of the slope from one. RMSE is the root mean square error of the method with lower values indicating a more accurate result. DMA: dual method approach; HRM: heat ratio method; Tmax: Tmax method.

Species	Method	Slope	Error (%)	r^2	RMSE	n
P. communis	DMA	0.991	0.9	0.980	9.132	35
	HRM	1.037	3.7	0.979	10.169	35
	Tmax	1.974	97.4	0.908	75.351	35
S. floribundum	DMA	0.995	0.5	0.971	0.140	34
	HRM	0.816	18.4	0.946	0.221	34
	Tmax	1.590	59.0	0.955	0.562	34
S. paniculatum	DMA	0.967	3.3	0.964	0.468	28
	HRM	0.674	32.6	0.948	1.061	28
	Tmax	1.245	24.5	0.878	0.978	28
Average	DMA	0.984	1.6	0.972	3.247	3
	HRM	0.842	18.2	0.958	3.817	3
	Tmax	1.603	60.3	0.914	25.630	3

The data compilation was conducted on 15 samples across 9 species and 14 varieties with the complete results presented in Supplementary Table S1. Box plots of the reciprocal of the slope of the linear regression between ET_o and T (Q/A_L) for the samples from the data compilation, are presented in Figure 4. The mean slope of the data compilation was 3.16 which was not significantly different from the hypothesised value of 2.88 from the $E_{2.88}$ model (Student's $t = 2.107$, two-tailed $p = 0.054$, $n = 15$). The mean slope of transpiration measured via the DMA in this study was 2.94 which was not statistically different from 2.88 (Student's $t = 1.058$, two-tailed $p = 0.315$, $n = 11$). The mean slope for the HRM was 3.45 and Tmax was 1.72 which was significantly greater (Student's $t = 2.417$, two-tailed $p = 0.036$, $n = 11$) and lesser (Student's $t = -7.060$, two-tailed $p < 0.001$, $n = 11$) than 2.88, respectively.

Figure 4. Box plots showing the median, 25th and 75th percentiles, and whisker caps representing the 5% and 95% confidence intervals, for the slope of the linear relationship between Q and $A_L ET_o/2.88$. The null hypothesis value of 2.88 (black dash) is derived from the $E_{2.88}$ model and represents the leaf area index (LAI) of the reference grass crop in the Penman–Monteith ET_o model. The box plots summarise results from the data compilation (Meta, grey box, see Supplementary Table S1 for details), and the three species from this study measured via the dual method approach (DMA, blue box), heat ratio method (HRM, red box), and Tmax method (Tmax, black box).

4. Discussion

The $E_{2.88}$ model was generally supported by the sap flow measurements of the three woody species in this study. The results of this study were further supported by a data compilation of an additional 15 samples across 9 species and 14 varieties from previous studies [7–10]. The $E_{2.88}$ model was only validated under non-limiting soil moisture. Therefore, the results of this study suggest a positive correlation between Q, A_L, and ET_o when soil moisture, and most likely other stress factors, are non-limiting.

A general confirmation of the accuracy of the $E_{2.88}$ model will improve the reliability of plant water use measurements. Many models have been proposed to correlate Q with ET_o that have also shown good accuracy and predictive power (e.g., [9,29]). However, incorporating plant factors directly into evapotranspiration models is complex, site specific, requires extensive measurement and instrumentation, such as satellites or eddy covariance towers, to determine parameters such as stomatal and canopy conductance, and qualitative decisions such as partitioning the canopy into sun and shade leaves (e.g., [30,31]). The $E_{2.88}$ model may remove the requirement to estimate a crop coefficient to convert ET_o to ET_c [7]. The crop coefficient (K_c), or basal crop coefficient (K_{cb}) in the dual crop coefficient approach, converts ET_o to ET_c which is evapotranspiration for a specific leaf surface [2]. The $E_{2.88}$ model is analogous to K_c or K_{cb} because it also estimates a plant specific evapotranspiration based on its leaf surface, in this case total leaf canopy area (A_L). Yet, the $E_{2.88}$ model has an advantage over other approaches to estimate K_c or K_{cb} because it is comparatively simple to implement [7,9,10]. However, extensive research, explicitly comparing the $E_{2.88}$ model against traditional K_c, K_{cb} and ET_c models, which was beyond the scope of this study, is required.

The $E_{2.88}$ model estimates maximum potential plant water use that is driven by atmospheric evaporative demand based on the assumption that all other conditions are non-limiting [7,10]. The non-limiting assumption may prove useful because it provides a null model against which measured Q can be compared. For example, if Q is not equal to the $E_{2.88}$ model then, by inference, Q is not equal to atmospheric evaporative demand and some other biophysical or physiological component is the limiting factor. Experimental designs or comparative studies can explore potentially limiting factors to Q. For instance, Pereira and Villa Nova [10] used the $E_{2.88}$ model to experimentally test phenological and biophysical effects of water use in Tahiti acid lime; and Fernández et al. [9] used the $E_{2.88}$ model in a study on regulated deficit irrigation.

In this study, only the DMA sap flow method was consistent with the $E_{2.88}$ model whereas the HRM and Tmax under- and over-estimated transpiration. This outcome supports previous research demonstrating that the DMA sap flow method can accurately estimate a wide range of tree sap flow and that the HRM and Tmax methods are limited at fast and slow flows, respectively [20,21]. The HRM underestimates sap flow because it estimates sap flow when conduction is the dominant thermal process in sapwood [20]. In this study, the Tmax method overestimated sap flow in all three species which contrasts with previous studies that found Tmax generally underestimates sap flow [15]. The overestimation from the Tmax method observed in this study was due to the high nocturnal sap flow which consequently contributed to a higher cumulative daily total. This study highlights that the measurement range limitation of certain sap flow methods may lead to varying interpretations of the $E_{2.88}$ model. A method that is known to measure the entire observable range of sap flow in plants, such as the DMA, should be prioritized in assessing evapotranspiration-based models.

The accurate measurement, or estimation, of A_L is also critical for the $E_{2.88}$ model. In this study, A_L was measured by multiplying leaf area index (LAI) by canopy projection area. Consequently, A_L is dependent on the accurate measurement of LAI and canopy projection area. Testing the accuracy of LAI and canopy area methods was beyond the scope of this study. Many studies have estimated LAI, via light interception, digital imaging, ceptometers, remote sensing, drones, or satellite, with varying degrees of accuracy (e.g., [27,32,33]). Similarly, various methods are available to measure canopy projection area which can be dif-

ficult given geometrical irregularity and dealing with clumping or sparseness (e.g., [34,35]). Inevitably, LAI, canopy area and A_L will rarely be measured precisely, and these parameters will need to be treated with caution when used with the $E_{2.88}$ model.

5. Conclusions

The $E_{2.88}$ model, or the hypothesis that the sap flow is equivalent to evapotranspiration, normalized via leaf area, was verified in this study when sap flow was measured via the DMA. There was a discrepancy between observed and modelled values when the HRM and Tmax sap flow methods were used highlighting the importance of methodology. The $E_{2.88}$ model was additionally supported by the data compilation of the literature which included additional species.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12070875/s1, Table S1. Data compilation results of the linear regression of transpiration (sap flow divided by total leaf area, mm) versus reference crop evapotranspiration (ETo, mm) for (A) a data compilation of the literature and (B) the results found in this study.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Supporting data can be found via this weblink: https://implexx.io/supplementary-materials/, accessed on 14 February 2021.

Conflicts of Interest: The author is an owner of a company that manufactures sap flow sensors that were used as a part of this study.

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