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## TECHNICAL PAPER

### **CALIBRATION OF THE THERMAL DISSIPATION PROBE FOR ATEMOYA (*Annona squamosa* x *A. cherimola*)**

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#### **KEYWORDS**

Annonaceous, sap flow, lysimeter, Granier method.

#### **ABSTRACT**

When determining plant transpiration (TR) using the thermal dissipation probe method, it is necessary to calibrate the sap flow (SF) estimation equation for each species under study, and thus obtain reliable information about the water demand of crops. This study aims to calibrate the equation used to determine SF using the thermal dissipation probe for atemoya cultivation. The experiment was conducted in the experimental field of the Universidade do Estado da Bahia, Juazeiro, Northeast Brazil. Atemoya plants in the initial phase of development were transplanted into 21.5-l pots arranged on weighing platforms that acted as lysimeters. The SF was determined by the thermal dissipation probe method using 1-cm-long probes. The angular coefficient of the Granier equation was adjusted by minimizing the absolute deviations between the accumulated daily SF and TR measured using the lysimeter. The results demonstrated that the application of the Granier method is satisfactory for estimating the TR of the atemoya and that it is necessary to adjust the angular coefficient of the equation. The mean absolute error between SF and TR was 3.1%.

#### **INTRODUCTION**

The hybrid atemoya (*Annona squamosa* x *Annona cherimola*) obtained from the crossing of sweetsop with cherimoya, is among the few commercial species of the Annonaceae family that has received special attention from Brazilian producers and consumers (Silva et al., 2009). This tropical fruit is one of the alternatives with high potential; it is appreciated by the internal and external markets for its pleasant taste as well as its nutritional and medicinal properties (Martin & Souza Neto, 2015). However, in the Northeast region, the expansion of its cultivation is still limited, primarily because of the lack of technical-scientific knowledge for its cultivation. Therefore, more studies on the management of this plant are required (Silva et al., 2009), which will provide reliable information that can assist producers in the production process, especially in relation to water use. This plant requires water in great abundance for its growth and functioning (Taiz et al., 2017). Therefore, irrigation is a decisive factor in maximizing production and product quality, especially in regions where low and

irregular rainfall are common, e.g., the semiarid region of Brazil. The estimates of plant evapotranspiration have usually been quantified from crop parameters and meteorological data (Bergamaschi & Bergonci, 2017). However, for atemoya, which presents discontinuous soil coverage and differentiated management with the occurrence of defoliation for production, the use of these methods is hindered.

Recently, agronomic research has improved in quantifying plant water consumption using simpler and more precise techniques. Among these, we highlight the thermal methods, which estimate the flow of sap based on the heat supply to the stem and the detection of convective heat transfer by the sap (Fuchs et al., 2017). Because they are easy to install and use and inexpensive, these methods have been used to quantify water use in various crops such as mango (Cotrim et al., 2019), banana (Hai-Jun et al., 2015), African mahogany (Sérvulo et al., 2017), and apple (Bhusal et al., 2019).

Granier's thermal dissipation probe method is an extensively used thermal method that can be applied in field conditions to provide continuous measurements over

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a long time period. The sap flow (SF) density is calculated by the temperature difference between the heated upper probe and the lower one, which is maintained at the stem's ambient temperature using an equation originally calibrated for several woody species proposed by Granier (1985). However, although the calibration coefficients are similar for several woody species, a standard equation should be applied with caution because it is dependent, among other factors, on the distribution of vessels, the thermal properties of the wood, and the radial distribution of SF density in the stem, which can lead to major errors in assessing the daily water use of the plant (Pasqualotto et al., 2019; Vergeynst et al., 2014). The application of the original Granier equation to all types of species and xylems can cause substantial errors when determining SF. Sérvulo et al. (2017), performing the calibration in African mahogany, observed that the original model underestimated transpiration (TR) by 44.4%. For atemoya, there were no studies that determine the SF using the Granier method. Thus, for this method to be applied to its cultivation, serving as a basis for ecophysiological research and irrigation management, it is necessary to calibrate the method. Thus, the aim of this study is to calibrate the equation for determining the SF using the Granier method for atemoya cultivation.

**MATERIAL AND METHODS**

The experiment was conducted in the experimental field of the Department of Technology and Social Sciences – DTCS of the Universidade do Estado da

Bahia (UNEB) – located in Juazeiro, BA (latitude 9° 24' 50" S; longitude 40° 30' 10" W; altitude 368 m), in the Submédio (Lower Middle) São Francisco region between March and April 2019. According to Koppen's classification, the climate is the hot semiarid type (BSwh) with an average annual rainfall of 540 mm (Teixeira, 2010). Note that Atemoya plants of the Gefner variety were used; they were aged 18 months, grown in 21.5 l pots, arranged on three weighing platforms that functioned as lysimeters. Moreover, the platforms had capacity of 120 kg (Alpha Instruments) and were connected to a datalogger (CR800, Campbell SCi), associated with a channel multiplexer (AM 16/32B Multiplexer, Campbell, SCi). The lysimeters were calibrated within the platform capacity range to a cumulative value of 65 kg, as per the procedure cited by Cabral (2013) and Schmidt et al. (2013). To calibrate the weighing platforms, masses of 500, 1000, and 5000 g of crushed stone were created using an analytical balance which, during the calibration process, was applied in three loading and unloading cycles. After each mass increase or decrease, the stability of the reading voltage detected in the datalogger was monitored for annotating the electrical signal. Information was generated every 30 s, and the average was calculated every hour. After data collection, the calibration equations were adjusted using linear regression, represented by the output values of the load cells, in millivoltage (mV) per volt of excitation and the mass equivalent of the set (kg), which demonstrated linear behavior between the variables (Table 1).

TABLE 1. Linear regression coefficients (Mass = signal\*A +b) for calibration of weighing lysimeters.

	Load			Unload		
	a	b	r <sup>2</sup>	a	b	r <sup>2</sup>
Lysimeter 1	93.274	-11.582	0.9999	93.305	-11.614	0.9999
Lysimeter 2	88.925	-13.077	0.9999	88.947	-13.100	0.9999
Lysimeter 3	91.470	-12.702	0.9999	91.494	-12.731	0.9999

The accuracy of the lysimeters was determined using the standard error (Figure 1). Throughout the calibration, the errors were small in magnitude with a dispersion of 0 and ranged from -0.020 to 0.027, from -0.022 to 0.020, and

from -0.025 to 0.026 kg for lysimeters 1, 2, and 3, respectively, with a mean absolute error value of 0.0089, 0.0072, and 0.0087 kg for the three lysimeters, respectively, thus indicating good accuracy of the system.

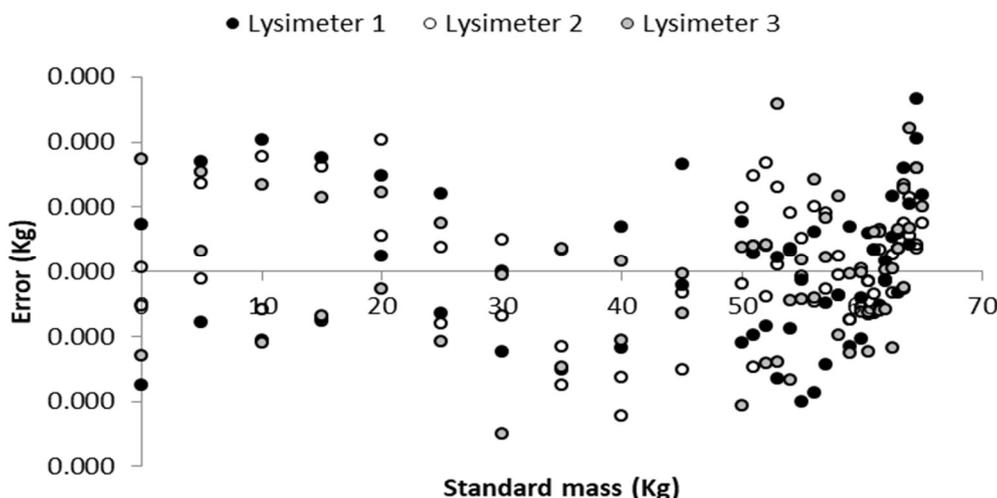


FIGURE 1. Mean error between the standard mass and that determined in the calibration of the lysimeters used for calibration.

The pots were filled with soil classified as Fluvic Neosol with a density of  $1.2 \text{ kg dm}^{-3}$ , which was collected in the experimental area in 0-20 cm deep layers. To facilitate drainage in the pot a 2.5-cm-thick layer of crushed stone, non-woven fabric (TNT), and irrigation logs were added. Irrigations were performed with the aid of a graduated beaker, and water replacement was defined based on the mass variation data of the lysimetric set to approximate the humidity values to that corresponding to the field capacity and favor the potential perspiration of plants. Field capacity was estimated in the pots with plants, containing the equivalent of 20 kg of soil, which was subjected to saturation and subsequent drainage, thus determining the water content retained after the mass of the set was stabilized.

For this study, the soil surface of the lysimeters was covered with a plastic to avoid evaporation losses, thus leading to only the TR of the plants. The calculation of TR comprised the difference in mass of the set, from midnight of one day to midnight of the next day, thus discounting the irrigated volume. The days with occurrence of rainfall were then excluded (Equation 1).

$$TR_L = M_{i(0:00h)} - M_{i+1(0:00h)} \quad (1)$$

Where:

TR is the transpiration of the plants obtained by lysimeter ( $\text{L day}^{-1}$ );

$M_i$  is the mass recorded on the lysimeter at 0:00 h of the previous day, kg, and

$M_{i+1}$  is the mass recorded on the lysimeter at 0:00 am of the current day (kg).

Based on the mass data recorded in the lysimeters, the moisture in the soil was determined using the following equation (Equation 2):

$$\theta = \frac{M_L - (M_{ss} + ta)}{V} dw \quad (2)$$

Where:

$\theta$  is the volumetric humidity ( $\text{m}^3 \text{ m}^{-3}$ );

$M_L$  is the mass of the lysimetric assembly (kg);

$M_{ss}$  is the air-dried soil mass (kg);

V is the volume of soil ( $\text{m}^3$ );

tare is the mass of the pot-drainage system assembly (kg), and

dw is the specific mass of water ( $1000 \text{ kg m}^{-3}$ ).

Moreover, plant TR was determined using Granier's thermal method (1985). For this purpose, 1-cm-long thermal dissipation probes were constructed using copper-constantan thermocouples (diameter = 0.5 mm) inserted into hypodermic needles (internal diameter = 1.0 mm), which were filled with resin for fixation. A heating element, formed by a constantan wire that was wrapped around the outer part of the needle, was mounted on the upper probe, thus allowing heating with continuous dissipation via the Joule effect. To promote this heating, the voltage was regulated according to the electrical resistance of the heater element of each sensor with the

dissipated electrical power being  $\sim 0.2 \text{ W}$  as recommended by the literature for the method (Lu et al., 2004) using an adjustable voltage source developed on an Arduino platform. While installing these probes, two holes were drilled in the same axial line as the stem of the plants (spaced 7 cm apart). Next, the stem segment was coated with Al foil, and, at the sensor site, a skirt-shaped protector was developed to minimize the effect of natural thermal gradients by reflecting the radiant energy onto the trunk and minimizing the thermal load on the sensor, as proposed by Vellame et al. (2011). The sensors were connected to a data acquisition and storage system (datalogger CR800 Series, Campbell SCi.) associated with a channel multiplexer (AM 16/32B Multiplexer, Campbell SCi.). Information was generated every 30 s with averages recorded every hour. The SF was calculated using the equation proposed by Granier (1985), considering the area of the effective section of the xylem that conducts the crude sap, namely (Equation 3):

$$Fs = 118.99 \times 10^{-6} \left( \frac{\Delta T_m - \Delta T}{\Delta T} \right)^{1.231} \times AS \quad (3)$$

Where:

Fs is the SF ( $\text{m}^3 \text{ s}^{-1}$ );

$\Delta T_m$  is the temperature difference between the two probes in the zero-flow situation ( $^{\circ}\text{C}$ );

$\Delta T$  is the current temperature difference, and

AS is the area of the effective section of the xylem ( $\text{m}^2$ ).

To correct the effects of natural thermal differences (NTD) on the stem, the heating of the probes was switched off for 24 h, according to the methodology used by Vellame et al. (2009). Thus, the thermal differences obtained with the unheated sensor were measured in a period distinct from the measurement of SF and, from there, estimation models were generated by linear regression as a function of air temperature (AT) for each probe (NTDe) individually. Subsequently, the data obtained were corrected based on these models using the following equation (Equation 4):

$$\Delta T = \Delta T_m - NTDe \quad (4)$$

Where:

T is the corrected current thermal difference ( $^{\circ}\text{C}$ );

$T_m$  is the uncorrected thermal difference measured by the probe ( $^{\circ}\text{C}$ ), and

NTDe is the estimated natural thermal difference for each probe ( $^{\circ}\text{C}$ ).

Table 2 lists the outer diameter, conductive section area and leaf area of the plants used during the experiment. To estimate the conductive area of the xylem, branches of atemoya plants were selected prior to the experimental period, comprising representative diameters of the plants used in the study, and then cross-sections were obtained. The sections were imaged, and the main anatomical structures of the stem were delimited. Using linear regressions, it was possible to determine the area of the

conductive section, considering the outer diameter of the stem of the plants and disregarding the region occupied by the area of the medulla. For estimating the leaf area, the total number of leaves was counted, and then the lengths and widths of all the leaves were measured. Next, the leaf area was calculated by [eq. (5)] and determined for the culture in the phase prior to the experiment.

$$LA = 0.7039 \times LW \quad (R^2 = 0.999) \quad (5)$$

Where:

LA is the leaf area (cm<sup>2</sup>), and

LW is the product of the length and width of the leaves (cm).

TABLE 2. Values of outer diameter, conductive section area and leaf area of atemoya plants in the initial stage of development.

	Outer diameter (cm)	Conductive Section Area (cm <sup>2</sup> )	Leaf Area (m <sup>2</sup> )
Plant 1	1.45	1.63	0.187
Plant 2	1.46	1.65	0.231
Plant 3	1.59	1.96	0.222

To adjust the coefficient of the equation proposed by Granier (1985) for the atemoya plant, the SF and lysimetric TR data, integrated in the 24-h period, were correlated by linear regression, with forced intercept at 0. Subsequently, the angular coefficient of the Granier equation was adjusted by minimizing the absolute deviations between the accumulated daily SF and the daily TR measured using the lysimeter. The micrometeorological data were obtained from an automatic meteorological station, located at the Universidade do Estado da Bahia, at a distance of ~90 m from the experimental area. The following rainfall data were used: AT, correction of natural thermal gradients, and determination of saturated air vapor pressure; dew point temperature to determine the current vapor pressure of the air and subsequent calculation of the water vapor pressure deficit (VPD); global solar radiation; and reference evapotranspiration using the Penman–Monteith method.

## RESULTS AND DISCUSSION

The estimated area of the crude sap conduction section in the stem of the atemoya plant, as a function of the outer diameter, is shown in Equation 5. For this ratio, the sapwood area (conductive section) was represented by discounting only the area of the medulla. The 2nd degree polynomial model expressed good data adjustment ( $R^2 = 0.999$ ). On an average, the area of the sapwood with the bark represented 99.8% and the medulla represented 0.2% of the total area of the straight section of the stem.

$$AcS = 0.7844ED^2 - 0.0046ED - 0.0117 \quad (R^2 = 0.999) \quad (6)$$

Where:

AcS is the area of the conductive section, and

ED is the external diameter.

Figure 2 shows the course of NTDs of each atemoya plant and of the AT, observed for 24 h. The thermal differences were more significant in the afternoon, starting at 3 PM, along with variations in AT. Pamponet et al. (2012) in a study with adult cocoa plants grown in full sun confirmed a good correlation of AT with NTD. The mean NTD among the three atemoya plants was 0.43°C with a maximum of 3.9°C. Vellame et al. (2011), working with different types of insulation in branches and trunks of cocoa trees, in the Recôncavo Baiano region, reported a mean thermal difference of 0.14°C and a maximum of 0.83°C when the insulation was performed with open laminated paper. The amplitude of NTDs among the plants presented a maximum value of ~6°C. Cotrim (2009), in a study with mango orchards in the semi-arid region of Bahia, reported a value of ~3°C. Cotrim reported that regions with high thermal amplitude, such as semi-arid regions, can contribute to the occurrence of high natural thermal differences recorded by unheated sensors. In Juazeiro, the thermal amplitude in the period was registered at 10.3°C.

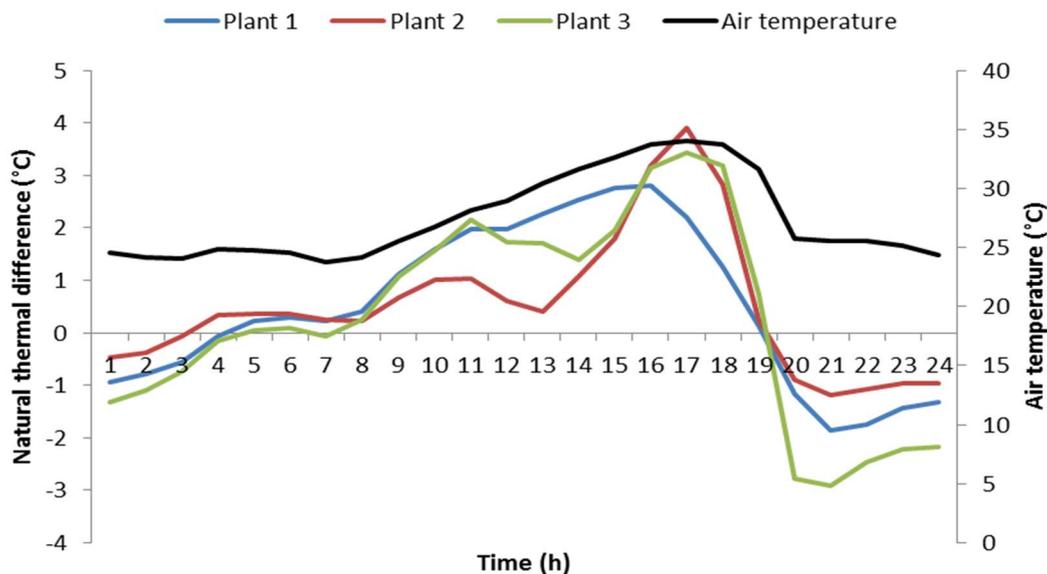


FIGURE 2. Air temperature and natural thermal differences measured over a day in atemoya plants.

Figure 3 shows the course of the SF with solar radiation, the water VPD, and the average AT over three sequential days of evaluation. It is observed that over a sequence of days, the SF patterns showed similar behavior, beginning at 7 AM and proceeding with increasing and positive values, before reaching a maximum at between 3 PM and 4 PM, and then decreasing until the end of the day. The VPD and AT followed a similar pattern; however, the start of the process, at 6 AM, occurred concomitantly with solar radiation. Despite the dynamism of the SF with solar radiation ( $R_g$ ), the processes do not simultaneously occur, and a time lag between them is observed throughout the day. The maximum values of SF occurred 3–4 h after the maximum peak of  $R_g$ , coinciding with the VPD and AT. At the end of the day, although there is no more energy supply,

the SF continues to occur to replace the water present in the tissues and lost throughout the day (Coelho Filho et al., 2011), conducting the movement of water, which can be detected by the SF sensors.

Although there is strong interaction between environmental variables that affect SF, these can often reveal opposite effects on plant physiology. This makes it difficult to understand the responses of these variables on the SF; therefore, it is the simultaneous relationship between them that will provide the answers (Liu et al., 2011; O'brien et al., 2004). In studies with African Pride atemoya, George & Nissen (2002) reported that the greatest variation in TR was attributed to changes in relative air humidity while AT had more influence in the variation of leaf water potential.

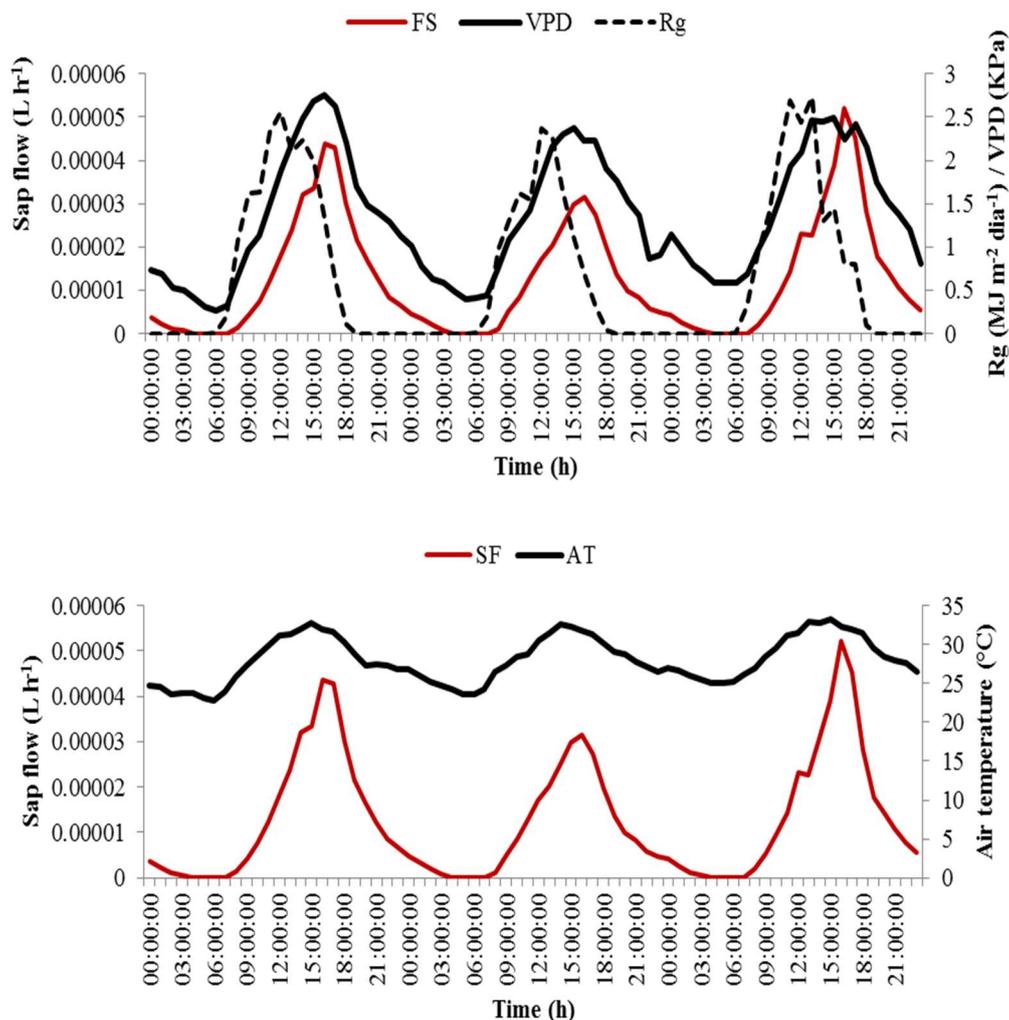


FIGURE 3. Course of sap flow, solar radiation, water vapor pressure deficit and average air temperature during three sequential days of evaluation.

Table 3 lists multiple regression coefficients for estimating the mean TR of atemoya plants as a function of ETo and soil humidity ( $\theta$ ). The coefficients relating ETo to SF and TR were significant ( $p < 0.01$ ); however, for the soil moisture factor, only TR presented a significant coefficient ( $p < 0.01$ ).

TABLE 3. Multiple regression parameters with reference evapotranspiration (ETo) and soil humidity ( $\theta$ ), for estimation of sap flow (SFe) and plant transpiration (TRe) of atemoya in the early development phase.

Sap Flow		
	Regression	PR>Fc
	$SFe = \beta_0 + \beta_1 * ETo + \beta_2 *$	0.0482
	<b>Coefficients</b>	<b>P-value</b>
$\beta_0$ (Intercept)	-0.2505	0.3893
$\beta_1$ (ETo)	0.0833	0.0165
$\beta_2$ ( $\theta$ )	0.2398	0.6990
R <sup>2</sup>	0.49	
Transpiration		
	Regression	PR>Fc
	$TRe = \beta_0 + \beta_1 * ETo + \beta_2 *$	0.0003
	<b>Coefficients</b>	<b>P-value</b>
$\beta_0$ (Intercept)	-0.4582	0.0028
$\beta_1$ (ETo)	0.0729	0.0001
$\beta_2$ ( $\theta$ )	0.8361	0.0076
R <sup>2</sup>	0.84	

Figure 4 shows a graphic representation of the variation of soil humidity and the SF/ET<sub>o</sub> ratio and ET/ET<sub>o</sub> during the study period. The volumetric content of water in the soil presented variation of up to 12% in relation to that corresponding to the humidity at field capacity (H<sub>fc</sub>). Between April 5 and 7, 2019, the data that was gathered was discarded because of rainfall. Moreover, a dynamic relationship was observed in the SF transfer process and in the TR of atemoya plants with decrease or increase in the volumetric content of water in the soil;

however, this relationship was not always linear. The processes of water loss by plants are driven by the availability of water in the soil; however, the response is determined by factors such as the available energy and evaporative demand of the atmosphere, as well as the plant's own resistance. Rodrigues et al. (2011), in a study on the variability of stomatal conductance in an Amazonian mangrove ecosystem, reported that stomatal conductance proportionally varied to the VPD in the absence of water restriction in the soil.

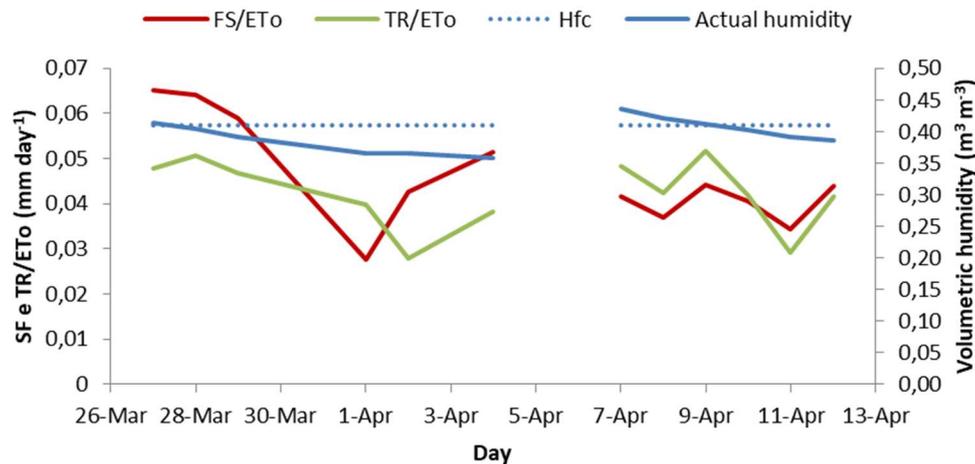


FIGURE 4. Soil humidity at field capacity (H<sub>fc</sub>) and the course of soil humidity, the reference sap flow/evapotranspiration ratio (SF/ET<sub>o</sub>) and the reference transpiration/evapotranspiration ratio (TR/ET<sub>o</sub>) during the evaluation period.

Observing the fluctuations between the SF and TR measurements of the atemoya over the 12-day evaluation period, a maximum of 0.290 L day<sup>-1</sup>, minimum TR of 0.025 L day<sup>-1</sup>, and average TR of 0.179 L day<sup>-1</sup> were confirmed. The SF reached a maximum of 0.350 L day<sup>-1</sup>, a minimum of 0.054 L day<sup>-1</sup>, and an average of 0.194 L day<sup>-1</sup>. Figure 5 shows the relationship between the TR measured by the lysimeter and the SF, estimated by the Granier general equation from the data of the thermal dissipation probe. The SF values were slightly higher than the TR values directly obtained from the lysimeter, thus presenting an overestimate of ~7.9%. The differences between TR processes and SF change the dynamics and storage of water in the plant (Maltese et al., 2018). One of these differences is attributed to the occurrence of nocturnal SF, responsible for the redistribution of water in

the xylem and conducts the movement of water that can be detected by the SF sensors, even when TR ceases. The relationships between SF and TR reported in the literature vary considerably between species. Cotrim (2009), evaluating the thermal dissipation method in mango orchards in the semi-arid region of Bahia, confirmed an overestimate of SF of ~6.44% using the Granier method. Niu et al. (2015), working with oil palm in Indonesia, confirmed an overestimate of 17.3%. In mango cultivation in the Recôncavo Baiano, Vellame et al. (2009) reported an underestimate of SF of ~31% in relation to the TR measured directly on the lysimeter. Marinho et al. (2013), working with tabasco peppers in a protected environment in Piracicaba - SP, confirmed the underestimation of SF values in relation to lysimeter TR with a mean deviation of 6.1%.

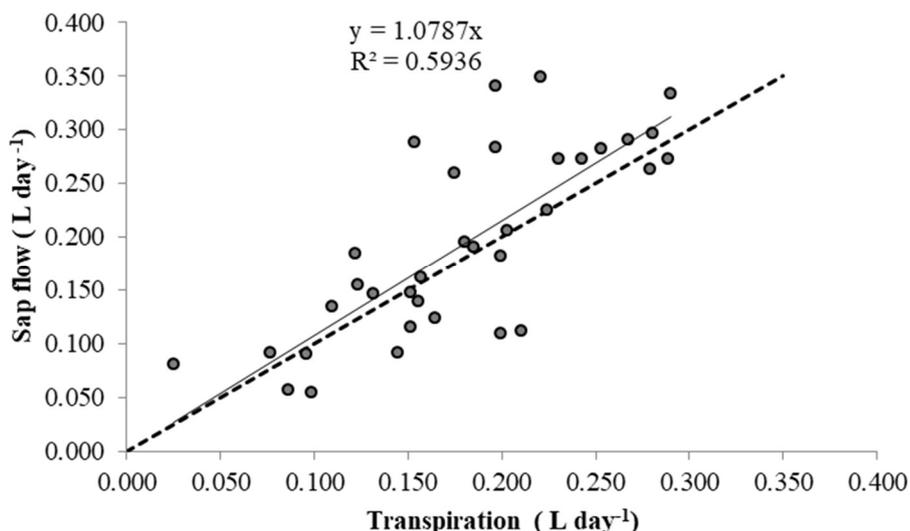


FIGURE 5. Relationship between the average sap flow estimated by the thermal dissipation probe and the transpiration measured through the lysimeter in atemoya seedlings.

After confirming the overestimation between SF and TR, a modification was made to the linear coefficient of the general Granier equation, bringing the values closer on the daily scale, as shown by [eq. (7)].

$$SF = 0.000103 K^{1.231} AS \quad (7)$$

The mean absolute deviation between the SF and the lysimeter TR in the period studied was ~7.9%, indicating an overestimation of the SF values determined by the Granier method in relation to the lysimetric measurements. Using the modified equation (Eq.6), the mean absolute error was 3.3%, thus representing 0.061 kg of water. Considering the

errors inherent in the measurement system (0.027 kg in the lysimeter weighing system), this value is considered acceptable. Moreover, differences between the processes of SF and TR do not evenly occur in all plants. Individually, there are differences in the thermal properties of the stem, and variation in the rate of water transport, (Figure 6) which increases the dispersion in the relationship between SF and TR even on a daily scale. Therefore, to adjust the equation to estimate SF based on lysimetric measurements, the requirement to use more than one plant and varying soil moisture conditions and evaporative demand of the atmosphere is justified.

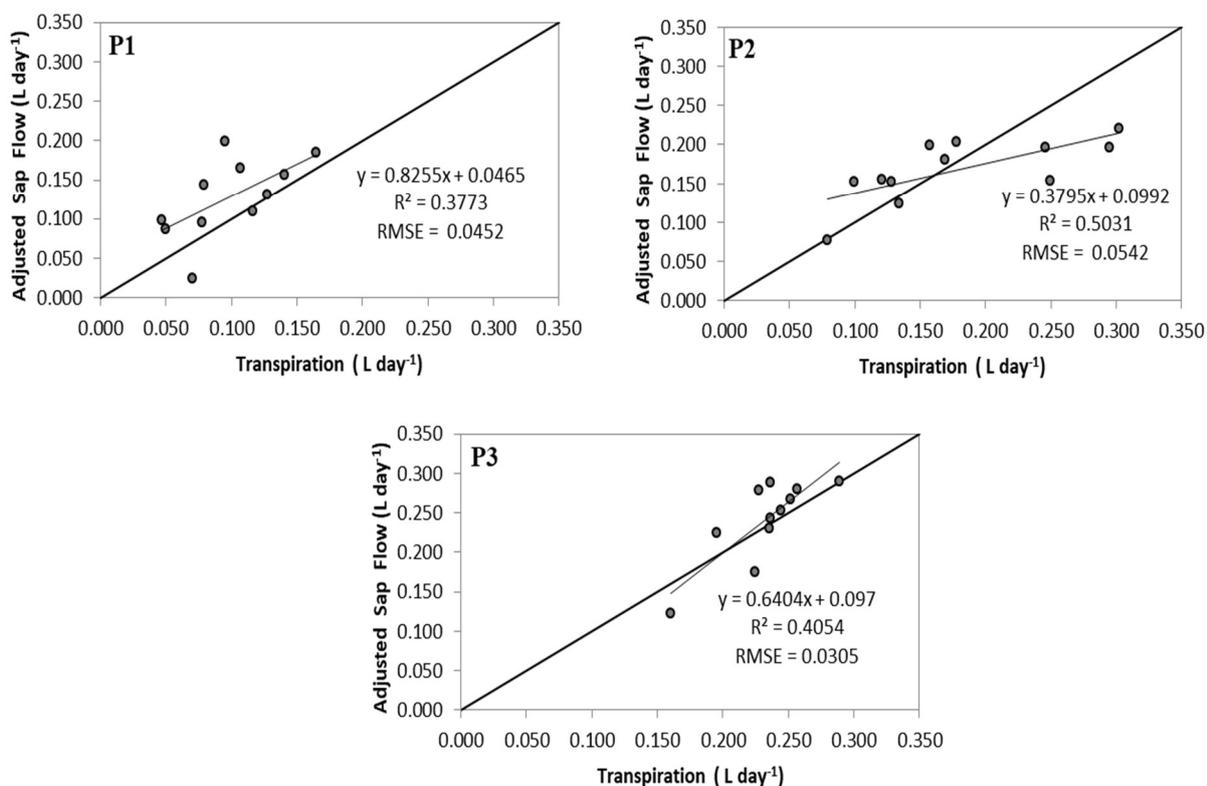


FIGURE 6. Relationship between adjusted sap flow and transpiration measured through lysimeter for the three atemoya plants (P1, P2 and P3).

## CONCLUSIONS

The application of the Granier method, with correction of natural thermal differences and the modification of the angular coefficient of the equation, was satisfactory to estimate the TR of atemoya with an absolute mean error of 3.1%.

The overestimation of the SF in relation to the lysimetric measurements may be related to the redistribution of water in the plant in the occurrence of nocturnal SF.

Moreover, temporal differences between SF processes and TR, accentuated by measurement system errors, explain the dispersion of the calibration data.

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