Tabasco pepper transpiration by the heat dissipation probe method

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Abstract: Methods of heat supply in the trunk have been widely used to estimate transpiration of woody plants; however, there are few studies about its effectiveness in small plants. This study aimed to calibrate the Heat Dissipation Probe (HDP) method to estimate transpiration and study the influence of natural thermal differences, the estimation of the conductive area and the placement of sensors to estimate transpiration in Tabasco pepper plants. Heat dissipation probes were installed in twenty nine 8-month-old plants, planted in beds, in a greenhouse. The equation for sap flow estimation was calibrated with standard lysimetric measurements in one of the representative plants. The angular coefficient of the equation for estimating sap flow was adjusted by minimizing the absolute deviations between the sap flow and daily transpiration measured in the lysimeter. After ten days of sap flow measurements, all plants were cut above the installation location of the upper probe; images of certain areas were generated and the dimensions of the regions present in the stem were determined. The HDP method with original calibration adjustment, correction of natural thermal differences and with a small sampling to determine the conductive area of the xylem is accurate to estimate the transpiration of Tabasco pepper plants.

Key words: Capsicum frutescens, lysimeter, sap flow, xylem, pepper

Transpiração em pimenta tabasco pelo método da sonda de dissipação térmica

Resumo: Métodos de fornecimento de calor no tronco têm sido amplamente utilizados na estimativa de transpiração de plantas lenhosas. Entretanto, faltam estudos sobre a sua eficiência em plantas de pequeno porte. Esse trabalho teve como objetivo calibrar o método da sonda de dissipação térmica (SDT) e estudar os efeitos das diferenças térmicas naturais, da estimativa da área condutora do caule e do posicionamento do sensor na estimativa da transpiração de plantas de pimenta tabasco. Foram instaladas SDT em 29 plantas com 8 meses de idade, cultivadas em canteiros em ambiente protegido. A equação para estimativa do fluxo de seiva foi calibrada tendo como padrão medidas lisimétricas em uma das plantas representativa. O coeficiente angular da equação de estimativa do fluxo de seiva foi ajustado pela minimização dos desvios absolutos entre o fluxo de seiva e a transpiração diária medida pelo lisímetro. Após 10 dias de medição de fluxo de seiva todas as plantas foram recepadas acima do local de instalação da sonda superior. Em seguida, foram geradas imagens das superfícies e determinadas às dimensões das regiões presentes no caule. O método da SDT com ajuste da calibração original, com correção das diferenças térmicas naturais e com uma pequena amostragem para determinação da área condutora do xilema é preciso na estimativa da transpiração de plantas de pimenta tabasco.

Palavras-chave: Capsicum frutescens, lisimetria, fluxo de seiva, xilema

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Introduction

The increase in conflicts between sectors that use the water resources, the charging for water use and the commercial demand for certified products, derived from a sustainable agricultural activity, have increased the importance of efficient irrigation. Localized irrigation is a very efficient irrigation system, since the applied volume of water is small and restricted to a portion of the soil, reducing losses by evaporation and runoff. Since losses are reduced in localized irrigation systems, transpiration becomes the principal factor to be determined for water management.

Advances in the use and development of sap flow estimation techniques were observed throughout the years. These techniques can be used to quantify the transpiration and the water relations of various crops under different irrigation regimes, with salty water or even under biotic and abiotic stresses. Most of the studies about plant transpiration, especially in woody plants, are based on methods of heat supply in the trunk. Nowadays, there are three groups of systems to determine sap flow: heat pulse, heat balance and heat dissipation (Green et al., 2003; Davis et al., 2012; Renninger & Schäfer, 2012)

The heat dissipation probe (HDP) method developed by Granier (1985) presents a simple theoretical approach and, compared to the heat balance method, shows the advantages of facility to build and install the sensors, lower cost and the need for fewer channels in the data acquisition system, enabling the monitoring of a higher number of plants in the field. It has shown to be a promising method, being largely used worldwide (Sugiura et al., 2009; Ford et al., 2004; Gartiner et al., 2009; Gebauer et al., 2008). In Brazil, its use has been reported in studies of estimation of transpiration in crops such as "Valência" orange (Vellame et al., 2012); coffee tree (Pimentel et al., 2010); mango tree (Vellame et al., 2009); rubber tree, a latex-producing tree (Delgado-Rojas et al., 2006) and also in the determination of water regulation strategy of Rapanea guianensis and Roupala Montana, woody species from 'Cerrado', under water deficit conditions (Naves-Barbiero et al., 2000). These studies indicate the possibility of utilization of this method with positive results.

However, the HDP method shows sources of error related to the natural thermal differences, the spatial distribution of the sap flow in the stem, the setting of the conductive area (the transversal area occupied by the xylem) and the installation position of the sensors on the stem. The area conducting sap varies according to the species, age and stem diameter of the plant. The setting of the conductive area is a limitation of the method because it demands the plant to be destroyed for the measurement. Thus, it is possible to correlate the sap conductive area with the external diameter; however, this information is not available in the literature for most crops and must be better studied.

There is also little research on the influence of the sensor position on the stem, its application for estimating transpiration and water relations in smaller-sized woody plants, like pepper plants. This crop has been highlighted as an important product of Brazilian agribusiness, generating employment and income for farmers in the rural areas (Miranda et al., 2006).

This work aimed at calibrating the heat dissipation probe method studying the effects of natural thermal differences, stem conductive area and sensor positioning for estimating transpiration of Tabasco pepper plants.

Material and Methods

The experiment was carried out in a greenhouse, in the Biosystem Engineering Department of "Luiz de Queiroz" College of Agriculture – ESALQ-USP, Piracicaba, São Paulo, 22°42'30" S and 47°38'00" W, at 580 m of altitude, from 20th July to 5th August, 2010.

In this study three arched, east-west oriented greenhouses were used (Ceiling height – 3.0 m; Width – 7.1 m; Length – 17.64 m; and Total Height – 4.7 m). The greenhouses had a 150 μ m transparent polyethylene plastic cover, and front and side walls made with anti-aphid net with a 0.3-m-high bottom part made of concrete. Sap flow was measured using the HDP method in 29 tabasco pepper plants at the age of 8 months (227 days after transplanting) after a production cycle and a period of irrigation.

Each sensor was comprised of a probe heated at constant power and a non-heated probe (reference probe), both having internally a thermocouple (Figure 1). The power applied to the heated probe followed the literature - 0.1 W cm⁻¹ on probe length (Delgado-Rojas, 2003). Adjustable current source were built in order to control the power dissipated in each sensor.

Granier (1985) correlated the sap flow density and the thermal difference between both probes, validating his equation (Eq. 1) for some species, in special forest plants: *Pinusnigra*, *Pseudotsuga*



Tc-heated probe; Tb-reference probe Adapted from: Delgado-Rojas, 2003

Figure 1. Schematic of heat dissipation probe method

menziensii and *Quercus pedunculata* (coniferous and ring-porous plants).

$$F_{d} = \alpha k^{\beta} = 118.99 \times 10^{-6} \left(\frac{\Delta T_{max} - \Delta T}{\Delta T} \right)^{1.231}$$
 (1)

in which,

 F_d - sap flow velocity, m.s⁻¹

 $\alpha e \beta$ - empirical coefficients

k - thermal variations

 $\Delta T\,$ - temperature difference between the two probes

 $\Delta T_{_{m \dot{a} x}}$ - maximum temperature difference between the two probes

Sap flow is calculated considering the effective section area of the xylem that conducts the raw sap, through Eq. (2):

$$F_{s} = F_{d}AS$$
 (2)

in which,

Fs - sap flow, $m^3 s^{-1}$

AS - raw sap conductive section area, m²

The 1-cm-long probes were built with copperconstantan thermocouples with 0.5 mm diameter, inserted in 1-mm-diameter needles and filled with resin for fixation.

In the probe installation two holes were made on the stem with diameters equal to the diameter of the capsule. The aluminum capsule is as long as the probe, with a slightly smaller inner diameter than it (1.5 mm). The probes were coated with thermal grease for a better heat conduction and inserted into the capsule. Silicone glue was externally applied to ensure a good fixation.

After probe installation, the stem segment was covered above and below with aluminum foil, and a skirt-shaped protection of aluminum foil was placed on the sensor location. This procedure minimizes the effect of natural thermal differences (Vellame et al., 2011).

The thermal difference measurements were performed every 30 seconds and the mean values stored every 15 minutes with a system composed of a data logger CR10X (Campbell Sci.) and multiplexer (AM 1632 Relay Multiplexer, Campbell Scientific Inc.).

The natural thermal differences (NTD) were measured by the non-heated probes on 20^{th} , 21^{st} and 22^{nd} July, 2010. The NTD from 28 plants were correlated with the NTD from two plants, one in each greenhouse, taken as "NTD_{ref}" to generate, through linear regression, estimation models for each monitored stem segment, according to Eq. (3).

$$NTD_{E} = a \times DTD_{ref} + b$$
 (3)

in which,

 NTD_{E} - natural thermal difference estimated for each probe, °C

 $\mathrm{NTD}_{\mathrm{ref}}$ - natural thermal difference for the reference probe, ${}^{\mathrm{o}}\mathrm{C}$

a and b - empirical coefficients

The thermal difference with the heated sensor was corrected through Eq. (4).

$$\Delta T = \Delta T_{\rm m} - \rm NTD_{\rm E}$$
⁽⁴⁾

in which,

 ΔT - corrected thermal difference, ^oC

 ΔTm - uncorrected thermal difference measured by the probe, $^{\rm o}C$

 NTD_{E} - natural thermal difference estimated for each probe, °C

The application of the heat dissipation probe method for estimating sap flow requires the knowledge of the conductive section area of the stem. In most studies with this method, due to the destruction of the plant, the determination of the area is correlated with the outer diameter or perimeter of the stem, usually calibrated with plants other than those used for sap flow measurements.

After the period of sap flow measurement, all plants were cut right above the insertion location of the upper heat dissipation probe. Images of the cut surfaces were generated and, using a CAD (Computer-aided design) software, the dimensions of the regions in the stem were determined.

In the Laboratory of Plant Anatomy of the "Luiz de Queiroz" College of Agriculture – USP, the

stem structure was identified through transversal cuttings using sliding microtome Leica SM 2000R. After that, they were stained with a 0.25% aqueous safranin solution and a 1% aqueous Astra blue solution, and then photographed (Figure 2). The distinction between the phloem, xylem and medulla zones was visually made (Figure 3).



Figure 2. Transverse section of the stem of 'Tabasco' pepper



Figure 3. Transverse section of the stem of 'Tabasco' pepper

The relation between measured and estimated area, and also the mean error of this relation (E), were calculated for all plants for different numbers of samples (n), 1 to 29, through Eq. (5).

$$E = \frac{t\alpha}{\sqrt{n}}$$
(5)

in which,

 α - standard deviation; and

t - Student coefficient for the 29 measurements at 95% confidence limit

Through Eq. (6), correction coefficients were determined from the measurements of flow density for each plant as a function of the percentage of probe length inserted in non-sap conductive regions.

$$K = \frac{1}{1 - \%A_{NC}}$$
(6)

in which,

K - correlation coefficient of the sap flow density measurements; and

 ${\rm A}_{_{\rm NC}}$ - percentage of the probe length inserted in the non-sap conductive region

A weighing platform with a load cell (Alfa GL 50) was built, centralized between two carbon steel plates and connected to the datalogger. A polyethylene box (250 L) was placed on the platform, working as a weighing lysimeter. The lysimeter calibration was performed applying known soil masses and recording the respective electrical signals (mV). With the mass variations and voltage data, an equation was adjusted by linear regression for converting electrical signals into mass.

In order to calibrate the equation for estimating sap flow, lysimetric measurements in one of the plants were performed. The soil surface in the lysimeter was covered with a plastic to prevent evaporation. The angular coefficient of the Eq. (1) was modified to minimize the absolute deviations between the sap flow accumulated in 24h and the daily transpiration measured by the lysimeter.

In the greenhouses, global solar radiation values were obtained using a pyranometer LI200X, connected to a CR10X (Licor Inc.) datalogger, with measurements at every 1 second and the mean stored at every 15 minutes.

Results and Discussion

Global solar radiation, sap flow and transpiration data throughout 4 days, calculated using hourly means for the plant used in the calibration, are shown in Figure 4.



Figure 4. Course of solar radiation (Rs), transpiration (lysimeters) and sap flow in Tabasco pepper, calculated as hourly mean (Piracicaba, São Paulo, August 1-4, 2010)

The sap flow, as well as the transpiration, increases with the increase in solar radiation, from the morning to part of the afternoon, with a sharp drop between 11:00h and 13:00h. When solar radiation reaches its maximum, high values of sap flow and transpiration are observed, decreasing again until the night time. It is possible to notice the existence of sap flow and transpiration during nighttime, possibly in order to recover from water deficit accumulated throughout the day. Peaks of Fs values were reported in some studies (Pimentel et al., 2010; Naves-Barbiero et al., 2000; Shackel et al., 1992).

There is also a good relation between solar radiation and sap flow by HDP calibrated with the lysimeter and the AS values with the correction of the probe location (Figure 5), showing linear trend with a good adjustment (R^2 = 0.94). González-Altozano et al. (2008) verified the highest sap flow value at the time of highest demand for the pear tree in the North of Spain, with a regression coefficient of R^2 = 0.32 between the solar radiation and the sap flow by the Granier equation without calibration.



Figure 5. Relationship between sap flow by the equation of Granier calibrated to Tabasco pepper and solar radiation as an hourly mean (Piracicaba, São Paulo, July 25 to August 4, 2010)

One can also notice that both transpiration and sap flow do not follow solar radiation precisely over time, which occurs because of the hydraulic resistance found in the plantissues, according to Angelocci (2002). So, the ability of pepper plants to avoid excessive loss of water occurs probably by stomatal regulation.

It is noteworthy that the lag between sap flow and transpiration, so commonly observed in studies with large plants was not so expressive in this study, as found by Delgado-Rojas et al. (2007) who verified a little lag between transpiration and sap flow in young "Tahiti" acid lime plants using Granier method.

Sap flow and transpiration followed solar radiation trend, with abrupt variations and lag of the transpiration obtained by lysimetry compared to the daily progress of solar radiation, having as a probable cause the low resolution of the lysimeter, which impedes hourly measurements. Similar results were found by Coelho et al. (2012) studying transpiration in "Valência" oranges, in Piracicaba-SP.

Since lysimetric measurements were not sufficiently accurate on an hourly basis and there is a lag between sap flow and transpiration, the linear coefficient of the general Granier equation was modified, approaching the values corresponding to daily basis according to the Eq. (7). The β coefficient (1.231) of the Eq. (1) was kept as originally suggested by Granier (1985).

$$F = 0.000353k^{1.231}AS$$
 (7)

The relation between the transpiration measurements obtained from lysimeters and the sap flow calculated using Eq. (7) and the general Granier equation (Eq. 1) can be observed in Figure 6. Sap flow estimated by the original Granier equation, underestimated plant transpiration obtained by the lysimeters.



Figure 6. Relationship between transpiration measurements made with lysimeter and sap flow accumulated daily by the heat dissipation probe

In Table 1 the analysis of variance is shown for the regression between daily accumulated sap flow and transpiration, and Table 2 shows the statistical parameters of regression. Results indicate a good relation between variables (adjusted R^2 > 0.94). The mean deviation between sap flow and transpiration on a daily basis was 6.1%. Coelho et al. (2012) observed mean deviation of 10% between daily values of sap flow and transpiration in "Valência" orange.

Table 1. Variance analysis for the regression between sapflow and transpiration of the Tabasco pepper

	DF	SS	MS	F	Р
Regression	1	1.04670	1.0467	143.73223	2.15912E-06
Error	8	0.05826	0.0073		
Total	9	1.10496			

Table 2. Statistical indices for the regression between

 sap flow and transpiration Tabasco pepper

Indices	Values
Intercept	-0.150
Slope	1.111
\mathbb{R}^2	0.947
R ² Adjusted	0.941
Standard error of estimate	0.085
Number of observations	10

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Figure 7. Relationship between the average daily sap flow period (FS) for each plant with and without compensation of the natural temperature differences in the stem - NTD (A) and relationship between the lysimeter measured and cumulative daily sap flow with and without NTD compensation (B)

The mean values of sap flow estimated by Eq. (7), with and without compensation for NTD, are correlated in Figure 7A. There is a strong trend of underestimation in the method when NTD are not compensated (average of 14.9%). Vellame et al. (2011) found underestimation trends of 13.15% for sap flow in adult mango trees, using the heat dissipation probe method.

When thermal differences are corrected by subtracting the estimated natural thermal differences, the trend of underestimating the flow by the method decreases and the accuracy in estimating the sap flow increases, as one can see by the increase of the determination coefficient (R^2) shown in Figure 7B.

Delgado-Rojas (2003) and Delgado-Rojas et al. (2004) found, in coffee and lemon plants, respectively, that the mentioned gradient shows high interference on the estimations.

Girardi et al. (2010) noted that errors inherent to the NTD and injuries in the stem tissues affected the accuracy of the sap flow measurements, with the heat dissipation probe method of "Valência" orange tree (*Citrus sinensis* (L.) Osbeck) grafted on "Cravo" lemon (*Citrus limonia* Osbeck). However, Delgado-Rojas et al. (2006), studying rubber trees, verified no interference of NTD on the sap flow estimations (Fs) due to the closure of plant canopy, resulting in nearly 100% shading. According to these authors, it seems that when plants have a low basal area (young plants) or low population density, there may be a higher incidence of thermal load from the environment on them, suffering more easily with the interference of natural thermal gradient (NTD). Also, the smaller the plants the stronger the effects, especially because of the reduced stem diameter, thus more easily affected, both for the energy transferred by advection and for the energy transformed on that place.

The xylem and supporting structures (conductive area) occupied an area of 70.9%, medulla, 9.5% and epidermis, cortex, fibers and phloem, 19.6% of the total stem area of Tabasco pepper plant.

The relation between the conductive section area and the outer perimeter of the stem had a good adjustment following a quadratic polynomial, Eq. (8).

$$AS = 0.112P^2 - 0.819P + 2.765 \quad R^2 = 0.991$$
 (8)

in which,

AS - area of the conductive section, cm² P - outer perimeter of the stem, cm

In this study, using all the plants as destructive samples, it was possible to establish the number of sampled stems that allows a reliable estimation of the active xylem area.

With a small sampling (around 10% or 3 stem segments) the errors are smaller than 5%, which allows studies of this nature with a reduced number of destroyed plants (Figure 8).



Figure 8. Percentage error in the relationship between measured and estimated conductive area (ASest/ASmed) versus percentage of stems sampled

The coefficients of variation (CV) for the daily measurements of sap flow from the 29 plants with and without correction to the sensor positioning by Eq. (6) may be seen in Figure 9. It is noted that part of the variability of the daily measurements of sap flow between plants can be explained by the positioning of the sensor in areas not conducting raw sap. For the entire period, the difference between CV values of the



Figure 9. Coefficient of variation of daily measurements of sap flow in Tabasco pepper with and without correction of the effect of positioning the probe

measurements with and without correction was of 5.3%, which explains only 12.5% of the variability

Conclusions

1. Granier equation calibrated to Tabasco pepper (F=0.000353 k^{1.231} AS) with correction of the natural thermal differences in the stem was efficient to estimate transpiration (mean absolute deviation smaller than 7% and R² coefficient of 0.92), indicating great accuracy of the method and possibility of its utilization in studies of water relations in conditions similar to those in this study.

2. The estimation of the conductive section area as a function of the outer perimeter of the stem and the adopted sensor probe positioning was satisfactory for the calibration of the heat dissipation probe method for Tabasco pepper.

3. The positioning of the heat dissipation sensor probe in areas not conducting raw sap was responsible for part of the variability in the estimations of sap flow in Tabasco pepper plants, which highlights the influence of its location as a source of error in the HDP method.

4. Sampling 10% of the Tabasco pepper stems for measuring the actual sap conductive area was enough to establish a reliable estimation of the active xylem area, with errors smaller than 5%, allowing studies of this nature with a reduced number of destroyed plants.

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