

Using a crop water stress index based on a sap flow method to estimate water status in conilon coffee plants

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ABSTRACT

Measurements of water consumption by plants are not often used in irrigation management due to the high cost and difficulty of measurement, however new methods based on sap flow monitoring enable accurate estimation of plant transpiration. The objective of this work was to develop a sap flow sensor to reliably evaluate crop water stress index (CWSI_{sapflow}) to diagnose water status of coffee plants in response to 3 cycles of water suppression and recovery, thus proposing a critical water stress index for conilon coffee plants (*Coffea canephora*). Sixteen potted coffee plants were randomly monitored under two water status treatments. In eight plants, the soil was watered and maintained at field capacity moisture (irrigated treatment) and the other eight plants were subjected to water stress by water withholding (non-irrigated treatment). The calibration of the sap flow sensor allowed the conception of the proposed crop water stress index, which was compared to leaf water potential, stomatal conductance, transpiration and net photosynthetic CO₂ assimilation rate. A coefficient based on actual transpiration was calculated to validate the crop water stress index. The sap flow sensor can be used for determining sap flow in conilon coffee plants, as well as elaborating a crop water stress index and estimating crop water status quickly and at relatively low cost. A critical value (0.4) for the crop water stress index (CWSI_{sapflow}) in conilon coffee plants allows accurate scheduling the best time to initiate irrigation, avoiding physiological damage to the plant.

1. Introduction

Ongoing climate changes are expected to negatively affect coffee crops, which is one of the most heavily globally traded agricultural commodities (DaMatta et al., 2019). Indeed, several model-based studies have predicted serious impacts on coffee crops due to increases in temperatures and altered precipitation patterns, including substantial reductions in agro-climatic zoning and losses of areas adequate for coffee-production in many countries (Bunn et al., 2015; Magrach and Ghazoul, 2015; Moat et al., 2017). However, the negative effects of global warming on the coffee crops might be lower than previously assumed due to, among other things, the plants tolerance and

mitigation strategies (DaMatta et al., 2018; Ramalho et al., 2018)

Climate changes at the present time are causing shifts in the pattern of the world's rainfall, resulting in both extreme drought episodes and heavy precipitation (IPCC, 2019). Increased average global temperatures are likely to change precipitation and atmospheric moisture, including shifts towards more extreme precipitation during storms. In addition, warmer temperatures may lead to increased drying, accelerating the onset of drought episodes (IPCC, 2019).

Drought impairs coffee plants during all phases of their growth cycle, but impairments are much more pronounced during the bean-filling phase (DaMatta et al., 2018). A strong carbon imbalance has been observed during the phase between photoassimilate production,

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due to overall decreases in net CO₂ assimilation rate (A) and total leaf area, as well as in photoassimilate requirements, because fruits are the strongest and highest priority sinks for coffee assimilates (DaMatta and Ramalho, 2006).

At coffee leaf scale, stomatal conductance (g_s) is strongly correlated with A (Meinzer et al., 1992) and transpiration (E). The sensitivity of g_s to leaf water potential at predawn (Ψ_{PD}) and leaf-to-air vapor pressure difference ($VPD_{leaf-air}$) (Pinheiro et al., 2005) suggests that g_s is a key factor limiting both coffee transpiration and yield. As reductions in g_s occur, stomatal limitations to photosynthesis are thought to be intensified during drought progression, contributing to the limitation of coffee transpiration and, in turn, yield (DaMatta and Ramalho, 2006). In addition, under severe drought conditions, non-stomatal limitation of photosynthesis becomes important (DaMatta et al., 1997). Under rapidly imposed water deficit ($\Psi_w = -3.0$ MPa), initial Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase activity (RuBisCO) but not its concentration decreased by ca. 60 %, but when water deficit was imposed slowly the concentration was also reduced (Kanechi et al., 1991).

Cultivar-dependent responses to drought have been observed in coffee plants based on their physiological performance under water stress conditions (Tausend et al., 2000). From a study with excised branches of three arabica coffee cultivars at $\Psi_{PD} = -2.5$ MPa, hydraulic conductivity decreased between 29 and 50 %, depending on the cultivar. In another study, a threshold value of Ψ_{PD} at which net CO₂ assimilation rate begins to decline due to water limitation was suggested to be close to -1.0 MPa for potted arabica coffee plants growing in drying soil (Kumar and Tieszen, 1980). However, Almeida and Maestri (1997), from an experiment using four arabica coffee cultivars in pots, reported exponential decreases ($r^2 = 0.99$) in potential photosynthetic capacity (A_{max}) [saturating CO₂ (5 kPa)], which started to occur even at Ψ_{PD} as high as -0.5 MPa. DaMatta and Ramalho (2006) reported that these results are difficult to reconcile with field experiments in which A (expressed on a leaf area basis) measured throughout the morning were maintained at control levels with predawn Ψ_{PD} as low as -1.5 MPa.

In coffee plants, drought reduces (1) the number of fruiting nodes per tree, (2) the number of fruits per node, and/or (3) the beans size, depending on its timing and severity. The effect on final yield, however, will depend on whether the number of fruits is greater or less than the number that can be sustained by the tree, which is a function of leaf area. On the other hand, excessive fruiting will lead to reduction of the potential number of flowering nodes, with implications for yields in the following year (Carr, 2001). This study concluded that rainfall variability is one of the main factors contributing to this instability but the effects of drought are unable to be quantified with precision, indicating an uncertainty of where and when irrigation is worthwhile, and how should be performed effectively (Carr, 2001).

The use of irrigation has allowed the establishment of coffee cultivation in low altitude regions with high temperatures. It is accepted that irrigation is an increasingly important management option for efficient coffee production in the face of climate change (Ramirez-Villegas et al., 2012). One critical factor for the irrigation management of coffee crops is to accurately quantify the volume of water to be applied, since this directly affects the crop productivity.

Even though advances in water supply technologies are available for plants, irrigation management is still inadequate in most of the coffee producing regions. The lack of basic information on crop water demand is a major cause of the inefficient use of this resource (Marin et al., 2016). Carr (2001) reported that there is a need to interpret and apply scientific understanding of the water relations on the coffee growth and development, and this should be translated into practical instructions to assist farmers to plan and use the water efficiently, either by rainfall or using irrigation techniques to obtain reliable and high-quality yields.

Water consumption by irrigated crops is often assessed using evapotranspiration and crop coefficients (Pereira et al., 2015; Guimarães et al., 2019), universally adopted as a standard procedure for irrigation

management (Rosa et al., 2012; Taylor et al., 2015). However, there is room for improvement in this method in order to consider the different physiological responses of each culture to water deficit.

The determination of sap flow using thermoelectric methods allows estimation of whole-plant transpiration, as well as from leaves or branches. Estimation of whole-plant transpiration is ineffective using porometry, infrared gas analysis at leaf scale and pressure chamber. Sap flow measurements have shown satisfactory results enabling the evaluation of the direct response of the plant to irrigation (Angelocci et al., 2004; Cermak et al., 2004). In addition, sap flow measurement is a potential tool for irrigation management as it is a parameter indicative of plant water status, reflecting the interactions between the amount of water available in the soil and the atmospheric water demand (Ortuño et al., 2006), which is important for the characterization of the water stress. From the calculation of the evapotranspiration using consolidated methods (Pereira et al., 2015), and sap flow measurements by thermoelectric methods, it is possible to estimate transpiration rate and elaborate water stress indexes for different crops (Sakuratani, 1981; Cammalleri et al., 2013; Han et al., 2018).

Studies using sap flow measurements have been carried out in many important crops, including coffee (Fu et al., 2016; López-López et al., 2018; Pereira et al., 2006; Zhao et al., 2015). Tausend et al. (2000) studied water use, hydraulic properties and xylem vulnerability to cavitation in three cultivars of *Coffea arabica* L., finding greater differences between cultivars mostly under water stress. They also found that all cultivars shared the same functional relationship between integrated daily sap flow and soil-leaf pathway apparent hydraulic conductance, even in different operating ranges. Sarmiento-Soler et al. (2019) monitored sap flux density using a Granier thermal dissipation method to access both the water consumption of coffee and water competition between coffee and shade tree species. These authors showed that daily water consumption of coffee plants was $1.2 \pm 0.64 \text{ l d}^{-1}$ and this did not differ between systems. However, further research using the sap flow method should be conducted to use sap flow in the management of irrigation in coffee plants.

The sap flow method has been used to assess the transpiration of several crops, however the use of this technology in the irrigation management depends on further studies, mainly for coffee cultivation. Thus, the objective of this work was to develop a sap flow sensor, based on heat pulse, to reliably evaluate crop water stress index ($CWSI_{sapflow}$) in coffee plant with the aim of diagnosing the coffee water status in response to 3 cycles of water suppression and recovery. These results were used to propose a critical water stress index for conilon coffee (*Coffea canephora*).

2. Material and methods

2.1. Experimental conditions

The experiment was conducted in a greenhouse at the State University of Northern Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro State, Brazil, located at $21^{\circ}44'47''$ S, $41^{\circ}18'24''$ W, 10 m above sea level, with climate Aw (wet tropical climate) with rainy summer, dry winter and the coldest monthly temperature above 18°C (Köppen, 1948).

Coffea canephora cv. conilon plants, denominated genotype 12 V, belonging to variety "Vitória Incaper 8142" were used in their early stages of development, six months after the seedlings. Implementation of the experimental treatments began six months after planting to allow better conditioning and plant development. The plants were placed in 20-liter plastic pots, filled with commercial substrate and earthworm humus, in the proportion 80 % and 20 %, respectively, of the pot volume, presenting moisture at field capacity equal to 24 %. The soil was maintained in chemical and physical conditions suitable for coffee cultivation. Three liters of gravel was placed in the bottom of the pots before adding the substrate, in order to avoid water accumulation.

A completely randomized arrangement was used, with 2 treatments and 8 replications, totaling 16 plants, which were watered to field capacity in the evening prior to starting all experiments. From the sixteen plants, eight were watered to soil field capacity (irrigated treatment - I) and the other eight plants were subjected to water withhold (non-irrigated treatment - NI). For the NI treatment, water supply was withheld abruptly, until they reached a leaf water potential (Ψ_w) between -2.0 and -3.0 MPa, which is considered as severe water stress for coffee plants (Silva et al., 2010). After that, the plants were irrigated for 7 days (recovery time of optimal plant water status).

The I and NI treatments were carried out in 2015 during three successive evaluation cycles, the first from June 9th to 23rd (14 days), the second from July 14th to 28th (14 days), and the third from August 18th to 28th (10 days). For the first cycle, the period of data acquisition was coincident with the water withhold duration, but for the second and third cycles, data acquisition continued after the irrigation was restarted for NI plants, for 3 days (up to July 31st) and for 4 days (up to September 1st), respectively.

Treatments were reversed after completion of each evaluation cycle, so the plants submitted to irrigation at field capacity (I) were those that had been stressed in the previous cycle (NI), while the non-irrigated plants (NI) were those that had been previously submitted to field capacity (I). As three evaluation cycles were carried out, the reversal of the treatments occurred twice. Irrigation was performed manually with the aid of a millimeter container every 2 days. Irrigation management was done via soil by weighing the pots and replacing the water difference between two irrigations.

2.2. Meteorological conditions

Data from the National Institute of Meteorology (Brazil) were used, collected from a meteorological station located 7 km from the experiment site. Average temperatures (T_{ave}) for first, second and third cycles ranged from 18.6 to 26.3 °C, from 21.0 to 27.4 °C, and from 19.8 to 25.1 °C, respectively (Fig. 1). Average maximum (T_{max}) and minimum (T_{min}) temperatures, for each cycle, were 31.2 and 15.3 °C, 31.3 and 18.8 °C, and 30.8 and 13.9 °C, respectively for the first, second and third cycles. For the same period, the relative humidity (RH) ranged from 72.5 to 92.2 % for the first cycle, from 70.0 to 81.3 % for the second cycle, and from 60.6 to 79.6 % for the third cycle (Fig. 1). The maximum photosynthetically active radiation inside the greenhouse was $700 \mu\text{mol m}^{-2} \text{s}^{-1}$.

According to Coste (1992); DaMatta and Ramalho (2006); Partelli et al. (2010); Pohlman and Janssens (2012) and Ngolo et al. (2018), the weather conditions regarding relative humidity and maximum, minimum and average temperature (Fig. 1) during cycle evaluation were suitable for *Coffea canephora*.

The reference evapotranspiration (ET_0) was estimated according to Penman-Monteith FAO-56 Bulletin (Allen et al., 1998), from Eq. (1),

using data for temperature, humidity, solar radiation and wind speed, with a wind speed equal to 0.2 m s^{-1} , recommended for protecting the environment (Guimarães et al., 2019).

$$ET_0 = \frac{0.408 s(R_N - G) + \gamma \frac{900}{t + 273} U_2 \frac{(e_s - e)}{10}}{s + \gamma (1 + 0.34 U_2)} \quad (1)$$

where s is the slope of the saturation pressure curve versus temperature ($\text{kPa } ^\circ\text{C}^{-1}$), R_N is the radiation balance ($\text{MJ m}^{-2} \text{day}^{-1}$), G is the soil heat flux ($\text{MJ m}^{-2} \text{day}^{-1}$), γ is the psychrometric constant ($\text{kPa } ^\circ\text{C}^{-1}$), t is the average air temperature ($^\circ\text{C}$), U_2 is the wind speed at a height of 2 m (m s^{-1}), e_s is the water vapor saturation pressure (hPa), and e is the current water vapor pressure (hPa).

2.3. Sap flow sensor – construction, installation and calibration

The sensors were built at the Agricultural Engineering Laboratory (LEAG), located at the Agricultural Sciences and Technologies Center (CCTA) of the State University of Northern Fluminense Darcy Ribeiro (UFENF), Campos dos Goytacazes, RJ, Brazil.

The sensors are based on the heat pulse method that relates the speed of the heat pulse with the speed of sap displacement in plant organs. The basic principle of this method is to promote sap heating at a given point of the plant organ followed by tracking the heat along the sap's path using temperature sensors (Marshall, 1958).

The proposed sensor (Fig. 2) consists of a set of thermocouple wires and a constantan heating wire, arranged parallel to one another and surrounded by an enameled copper wire, attached to one end of an acrylic plate (length 70 mm, width 12 mm, height 4 mm). The mounting of the wires on the acrylic plate meant that the thermocouple wire is 4.5 cm from the plate, and the heating wire is 7 cm from the acrylic plate. Thermocouple reference point was located at 10–12 cm from the other end, and then it was set on the acrylic plate.

The sensors were connected to dataloggers (model CR1000 Campbell Scientific, USA) for acquisition and storage of data. Data collector promoted the heating and cooling of the sensor by controlling the passage of electric current across the constantan wire during pre-defined time intervals, triggering and disabling a relay. The temperature variation was measured using the thermocouple wire and the values recorded continuously every 30 s, allowing the calculation of their variation between two times of interest, during both the heating and cooling cycles.

One sensor was installed per plant, in a plagiotropic branch from the lower third of the coffee plant, between the 5th and 6th pair of leaves. The sensors were fixed with the heating wire and the thermocouple wires wound twice around the chosen branch. To protect the sensor, glass wool coated felt on one side with aluminized kraft paper (length 2.5 cm and width 2.5 cm) was used in order to minimize the influence of ambient temperature on the sensor readings.

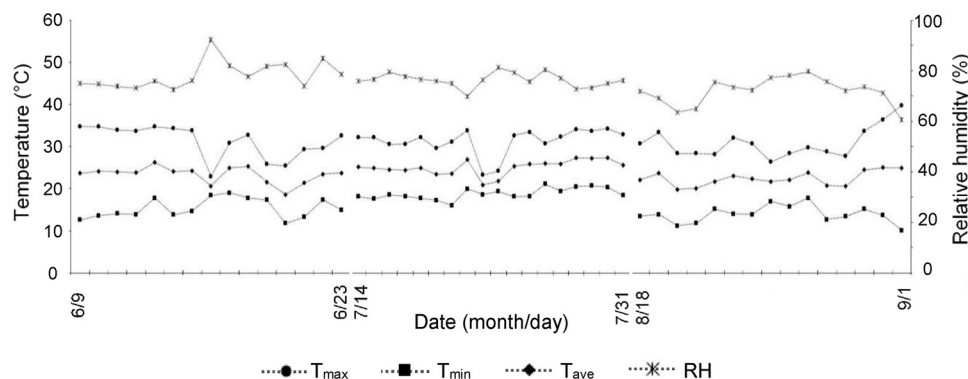


Fig. 1. Maximum (T_{max}), minimum (T_{min}) and average (T_{ave}) daily temperature ($^\circ\text{C}$), and average daily relative humidity (RH), for first cycle (9/6 - 23/6), second cycle (14/7 - 31/7) and third cycle (18/8 - 1/9). Campos dos Goytacazes, RJ, Brazil.

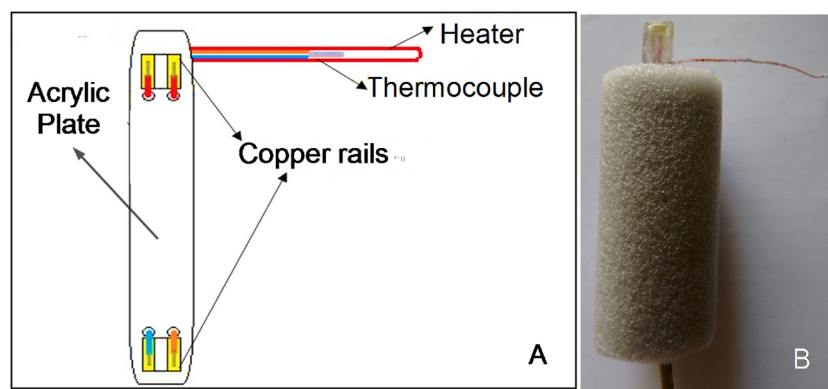


Fig. 2. (A) Illustration of the sap flow sensor and its constituent parts; and (B) sap flow sensor, showing the insulation material protecting the heater wires and thermocouple assembly (which was encased by enameled copper wire).

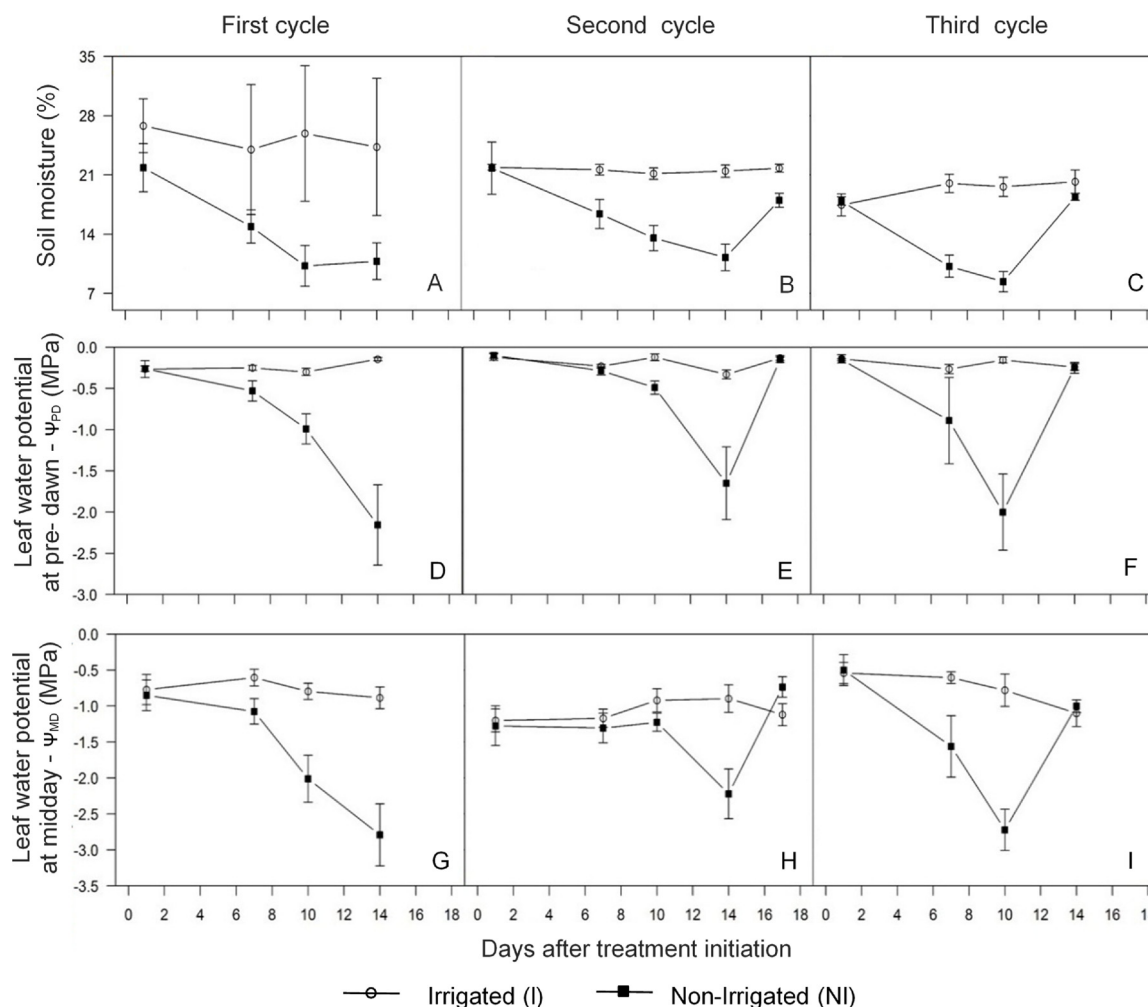


Fig. 3. Soil and plant water status for conilon coffee plant (clone 12 V) comparing irrigated (I) and non-irrigated (NI) treatments: soil moisture (%); and leaf water potential at pre-dawn (Ψ_{PD} , MPa) and midday (Ψ_{MD} , MPa) for the three evaluated cycles. Confidence interval of 95 %. Vertical bars represent \pm SDM. of measurements for each treatment. The return of irrigation in the NI treatment occurred on the 14th and 10th day of cycle 2 and 3, respectively.

To calibrate the sap flow sensors, data was assessed at daytime using the daily reference evapotranspiration rate (ETo mm h^{-1}) during the period before the evaluation period.

2.4. Evaluated variables

All analyzed variables were collected during the 2 treatments (irrigated and non-irrigated) and with 8 repetitions, totaling 16 plants

sampled for each of the 3 cycles.

Soil moisture was monitored daily in all treated plants at a depth of 10 cm from the surface and 5 cm distance from the stem using a portable meter (Soil Moisture Meter-Extech Instruments, A Flir Company, MO 750). Three readings were carried out per pot and after the reading, the average humidity values were calculated for all the treatments.

The physiological status of the plants was assessed at specific dates during the monitoring cycles after the implementation of the

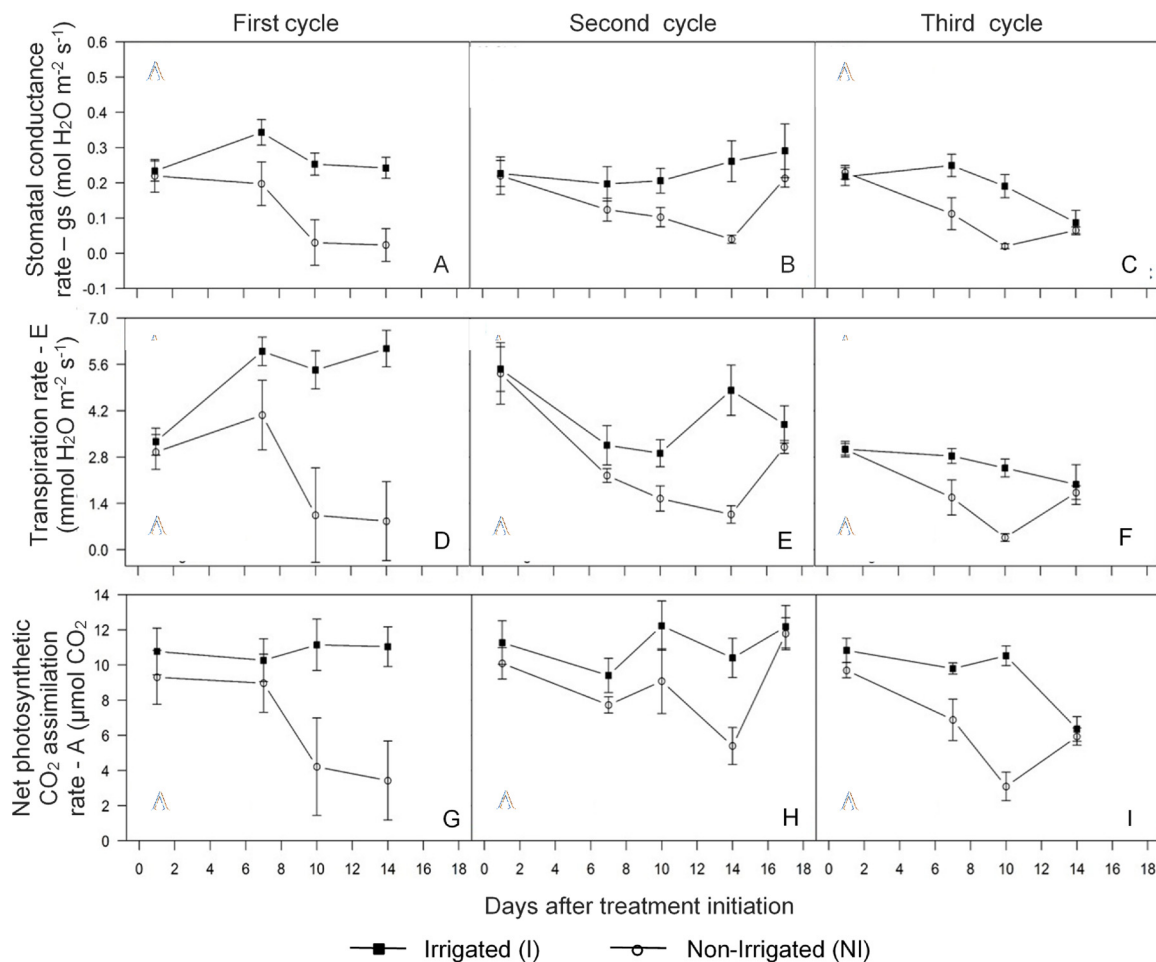


Fig. 4. Physiological traits for conilon coffee plant (clone 12 V) determined for irrigated (I) and non-irrigated (NI) treatments: stomatal conductance (g_s), transpiration (E) and net photosynthetic CO_2 assimilation rate (A) in three evaluated cycles. Confidence interval of 95 %. Vertical bars represent \pm SDM. of measurements for each treatment. The return of irrigation in the NI treatment occurred on the 14th and 10th day for cycle 2 and 3, respectively.

treatments, on the 1st, 7th, 10th and 14th days for first and third cycles; and the 1st, 7th, 10th, 14th and 17th days for the second cycle.

Leaf water potential was obtained using a Scholander-type pressure chamber (Soil Moisture - Plant Water Status Console, model 3115). Measurements were performed at predawn - Ψ_{PD} (between 4:30 and 5:30 a.m.) and midday - Ψ_{MD} (between 12:00 and 13:00 p.m.), according to Scholander et al. (1965).

Net photosynthetic CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration (E) were measured using a portable infrared gas analyzer (IRGA, LI-6400 model, LI-COR, USA), carried out on a fully expanded leaf from the same plagiotrophic branch used to measure the sap flow. The measurements were performed from 08:00 to 10:00 a.m. and from 12:00 a.m. to 2:00 p.m. with an artificial light source (LED) and photosynthetic photon flux adjusted to $1500 \mu mol m^{-2} s^{-1}$. During the evaluations, the initial concentration of CO_2 in the chamber was kept at approximately $380 \pm 30 \mu mol mol^{-1}$. Air temperature, relative humidity inside the chamber and the leaf area used in the equipment were 30.1 ± 0.15 °C, $61 \pm 0.13\%$, and $6 cm^2$, respectively.

Daily average values for the daytime period (12-h interval, from 6:00 a.m. to 6:00 p.m.) for variables A, g_s or E (Zd_x) were obtained from the weighted average between the values of the measurements made from 08:00 to 10:00 a.m. and from 12:00 a.m. to 2:00 p.m., on the same day (Eq. (2)).

$$Zd_x = \frac{3Z_{\text{morning}} + 2Z_{\text{afternoon}}}{6} \quad (2)$$

Where: Z_{morning} = A or g_s or E values, measured from 8:00 to 10:00

a.m.; $Z_{\text{afternoon}}$ = A or g_s or E values measured from 12:00 a.m. to 2:00 p.m.; "x" varied from 1 to 8, as replications of each treatment; and the numbers "3", "2" and "6" are weighting factors that resulted from the following assumptions: (i) the value of variables A, g_s or E increase linearly both from zero to Z_{morning} values, between 6:00 a.m. and 8:00 a.m., and from Z_{morning} to $Z_{\text{afternoon}}$ values, between 8:00 a.m. and 12:00 p.m.; and (ii) the value of variables A, g_s or E between 12:00 a.m. to 4:00 p.m. and between 4:00 and 6:00 p.m. decrease linearly in a symmetrical form with the increment assumed in the morning period (that is, with the same slopes but with opposite signs).

2.5. Statistical analysis

Regression analysis to compare the actual output of the sensor and the reference evapotranspiration was carried out to calibrate the sap flow sensors.

Data processing and statistical analysis were carried out using the program "R Core Team", which calculated mean, standard deviation and confidence interval ($\alpha = 5\%$) for each variable analyzed.

2.6. Crop water stress index using the sap flow sensor ($CWSI_{\text{sapflow}}$) and the IRGA equipment ($CWSI_i$)

The temperature variation produced by intermittent cycles of heating and cooling due to application of heat pulses, in alternate intervals of 90 s of heating and 90 s of cooling was defined as measured variable of the sensor. Intermittent heating and cooling cycles occurred

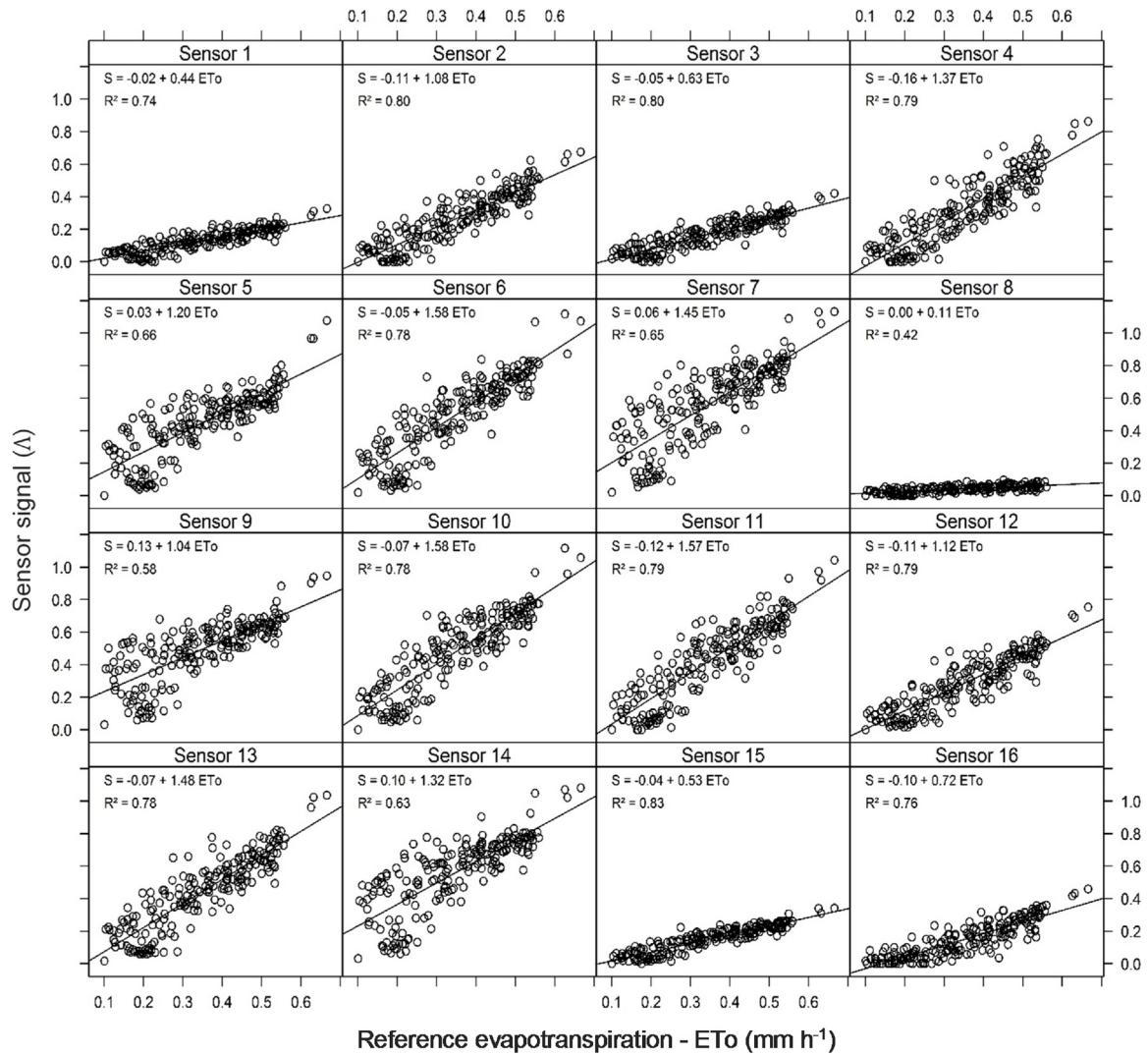


Fig. 5. Relationship between actual sensor signal (Λ) and daily reference evapotranspiration (ET_0) at daytime in mm h^{-1} for each sensor (sensor 1 to 16) for each conilon coffee plant. 1st cycle: sensors with irrigated treatment (1, 2, 4, 8, 9, 12, 13 and 16); sensors with non-irrigated treatment (3, 5, 6, 7, 10, 11, 14 and 15). 2nd cycle: sensors with irrigated treatment (3, 5, 6, 7, 10, 11, 14 and 15); sensors with non-irrigated treatment (1, 2, 4, 8, 9, 12, 13 and 16). 3rd cycle: sensors with irrigated treatment (1, 2, 4, 8, 9, 12, 13 and 16); sensors with non-irrigated treatment (3, 5, 6, 7, 10, 11, 14 and 15).

at regular intervals of three minutes. Eq. (3) was used to determine the temperature variation (ΔT_h), the temperature values measured at 10, 90, 100 and 180 s, after the start of heating supply:

$$\Delta T_h = \frac{(T_{90} - T_{10}) + (T_{100} - T_{180})}{2} \quad (3)$$

Where: ΔT_h = Temperature changes in the plant during the pulse; T_{10} = Temperature measured by the sensor 10 s after the start of the pulse; T_{90} = Temperature measured by the sensor 90 seconds after the start of the pulse; T_{100} = Temperature measured by the sensor 100 s after the start of the pulse; and, T_{180} = Temperature measured by the sensor 180 s after the start of the pulse.

With the change in temperature, the actual output of the sensor was defined (Λ) (Eq. (4)).

$$\Lambda = 1 - \frac{\Delta T_h}{\Delta T_h^0} \quad (4)$$

Where: Λ = Sensor signal (dimensionless); ΔT_h^0 = Temperature range measured by the sensor installed on the plant at zero flow condition at night; and ΔT_h = Temperature range measured by the sensor installed on the plant at one point in time during the day.

Assuming a linear relationship between the signal measured by the

sensor (Λ) and sap flow, the amount of sap flow can be estimated using Eq. (5).

$$J = k \cdot \Lambda \quad (5)$$

Where: J = Sap flow density ($\text{m}^3 \text{s}^{-1} \text{m}^{-2}$); and k = Coefficient depending on the wood and sap thermal properties (diffusivity and thermal capacity), and the sensor geometry.

The current transpiration (water loss by a plant through its leaves) was estimated by Eq. (6).

$$E_l = J \frac{S_a}{LA} \text{ or } E_l = k \cdot \Lambda \frac{S_a}{LA} \quad (6)$$

Where: E_l = Transpiration per unit of leaf area ($\text{m}^3 \text{s}^{-1} \text{m}^{-2}$); S_a = Cross section of the branch where the sensor was installed (m^2); and, LA = Leaf area above of the branch where the sensor was installed (m^2).

Relating the hourly average of the sensor signal (Λ) with the hourly reference evapotranspiration (ET_0), under conditions that show no water stress to the plant, that is, a situation in which the water deficit is equal to zero ($Def = 0$); and assuming a linear relationship between Λ and ET_0 , Eq. (7) is obtained:

$$\Lambda_{Def=0} = A \cdot ET_0 \quad (7)$$

Where: 'A' is the slope coefficient adjusted by linear regression between

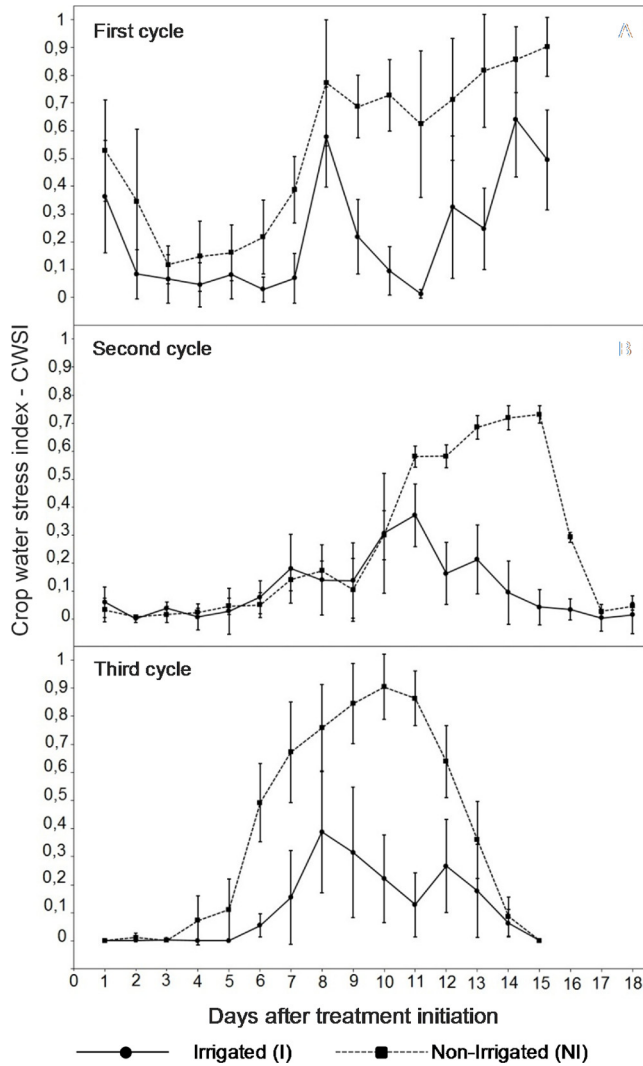


Fig. 6. Crop water stress index ($CWSI_{sapflow}$) for conilon coffee plants for the irrigated (I) and non-irrigated (NI) treatments during three evaluated cycles. Confidence Interval was 95 %. Vertical bars represent \pm SDM of measurements for each treatment. The return of irrigation in the NI treatment occurred on the 14th and 10th day for cycle 2 and 3, respectively.

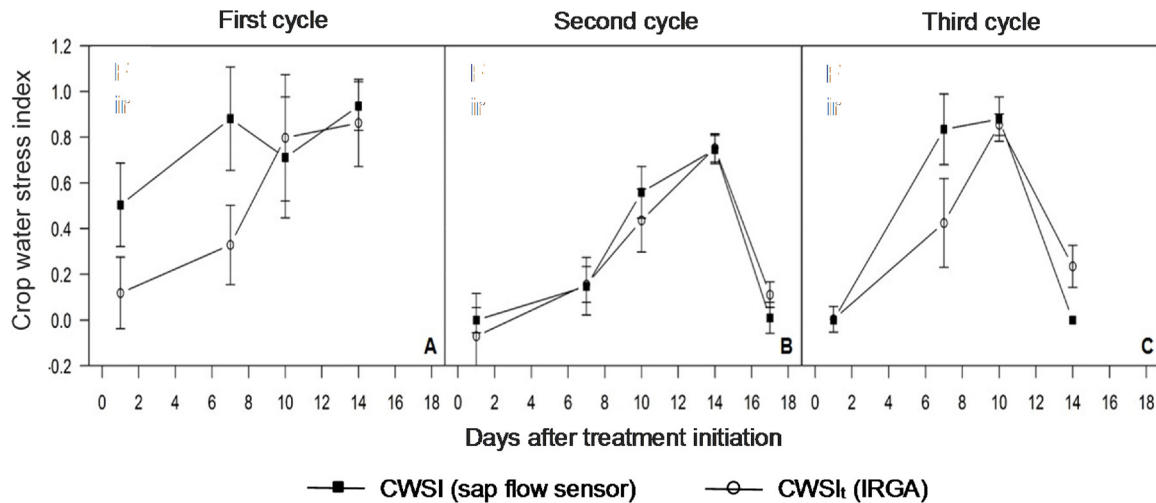


Fig. 7. Water stress coefficients based on transpiration rates measured by IRGA ($CWSI_t$) and compared to the results from the sap flow sensor ($CWSI_{sapflow}$), for conilon coffee plants during the non-irrigated treatment (NI) over three evaluated cycles. Confidence Interval was 95 %. Vertical bars represent \pm SDM. of measurements for each treatment. The return of irrigation in the NI treatment occurred on the 14th and 10th day for cycle 2 and 3, respectively.

the sensor signal and hourly reference evapotranspiration (ETo).

Leaf potential transpiration (\hat{E}_l) for plants without water stress was estimated by Eq. (8).

$$\hat{E}_l|_{Def=0} = kA(ETo) \frac{S_a}{LA} \Big|_{Def=0} \quad (8)$$

Therefore, crop water stress index ($CWSI_{sapflow}$) was estimated by the ratio between the current transpiration and leaf potential transpiration by applying the previous equations (Eq. (9)).

$$CWSI_{sapflow} = 1 - \frac{\sum E_l}{\sum \hat{E}_l|_{Def=0}} = 1 - \frac{\sum \Lambda}{A \sum (ETo)} \quad (9)$$

Where: $\sum E_l$ = the sum of daily transpiration of plant subject to water restrictions (NI); and, $\sum \hat{E}_l|_{Def=0}$ = the sum of daily transpiration of the plant without water restriction (I).

Therefore, the crop water stress index can be estimated only with the sensor signal and its relationship to reference evapotranspiration, when considering that the relationship between the cross-section of the branch and the leaf area remains constant during the evaluation period.

For $CWSI_{sapflow}$ validation, a crop water stress index ($CWSI_t$) based on the daily actual plant transpiration values (E), obtained by the IRGA equipment, was calculated using Eq. (10).

$$CWSI_x^{ni} = 1 - \frac{Ed_x^{ni}}{EM} = 1 - \frac{Ed_x^{ni}}{\left(\sum_{j=1}^8 Ed_x^{ir}\right)/8}$$

$$\therefore CWSI_t = \frac{\sum_{j=1}^8 CWSI_x^{ni}}{8} \quad (10)$$

Where: "x" varies from 1 to 8, according to the identification of each repetition (each plant) for each treatment; $CWSI_x^{ni}$ is the IRGA proposed stress index value for each plant (each replication) of the non-irrigated treatment, using the IRGA measurements; Ed_x^{ni} and Ed_x^{ir} are the average daily transpiration values of each plant obtained by Eq. (2) from the IRGA measurements in non-irrigated and irrigated treatments, respectively; and EM is the daily transpiration mean value representing the irrigated treatment.

3. Results and discussion

3.1. Evaluated variables

For both the coffee water (soil and plant) status (Fig. 3) and the physiological traits (Fig. 4), a significant statistical difference is

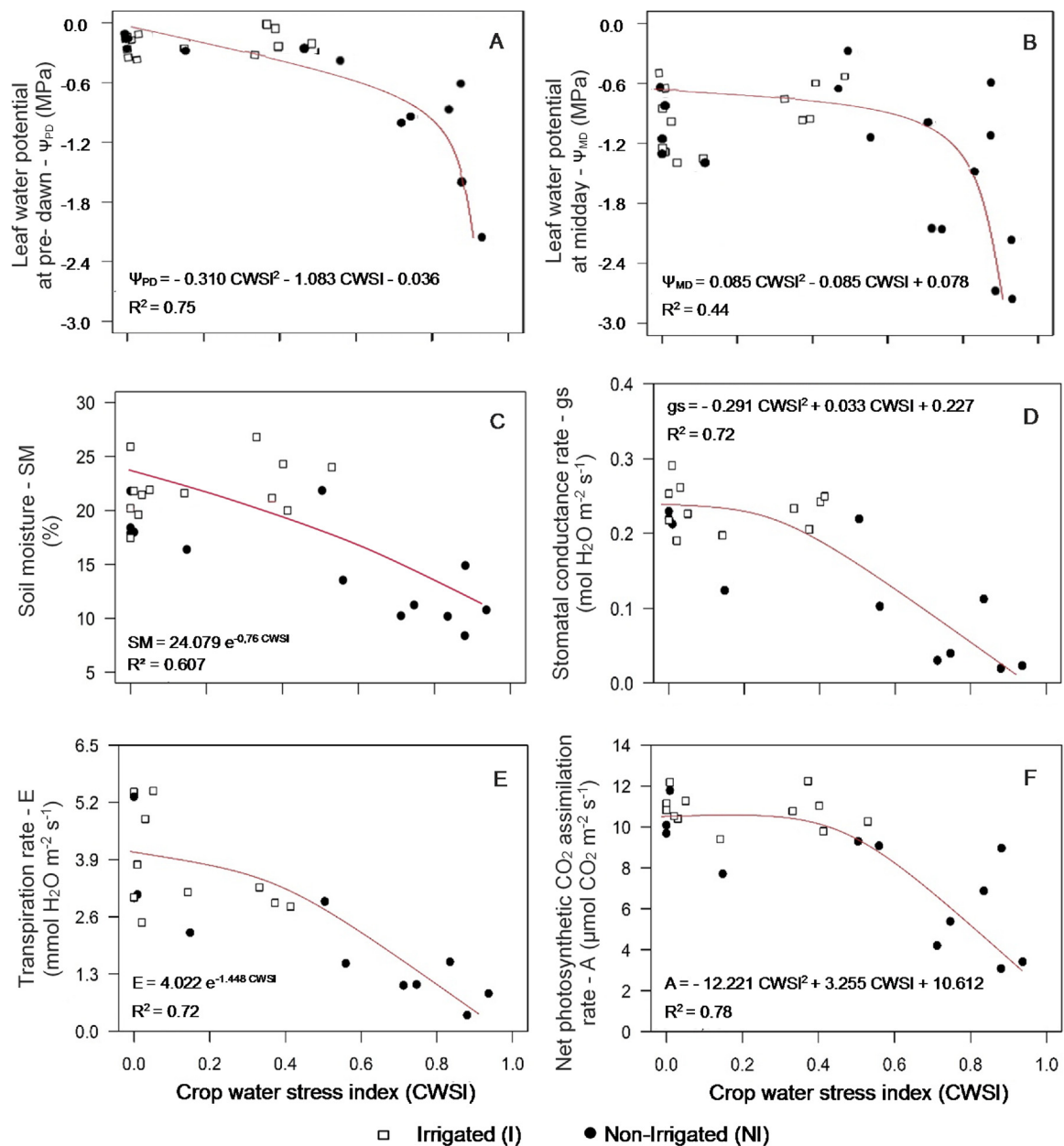


Fig. 8. Relationship between crop water stress index ($\text{CWSI}_{\text{sapflow}}$) and evaluated variables, for irrigated (I) and non-irrigated (NI) conilon coffee plants from June 9 to September 01, 2015.

observed when the error bars that represent the standard deviations of the mean (SDM) for I and NI no longer overlap on the same date (day after initiating treatment).

Significant differences between values of soil moisture, leaf water potential at pre-dawn (Ψ_{PD}) and at midday (Ψ_{MD}), were detected for all evaluated cycles, in which NI presented lower averages in comparison with I (Fig. 3).

Periods of significant difference (Figs. 3 and 4), indicate that the reduction of soil water content by evapotranspiration and water redistribution inside the soil, especially for NI, were enough to cause the distinction between the treatments. With reestablishment of irrigation at the end of the 14th day of second cycle and 10th day of the third cycle, soil moisture, Ψ_{PD} and Ψ_{MD} showed no significant difference between treatments for the next date of evaluation (Fig. 3). That is, irrigation replenished the soil water lost by evapotranspiration, eliminating the stress in the evaluated plant.

Ψ_{PD} and Ψ_{MD} values (Fig. 3, from D to I), reflected the decrease in

soil moisture (Fig. 3A–C) for NI, reaching significantly negative values, and ensuring the implementation of severe water stress for the treatment in question. According to Silva et al. (2010), for a particular clone of conilon coffee, Ψ_{PD} of between -1.5 and -3.0 MPa, causes moderate to severe water stress, respectively.

By the time NI plants reached the maximum negative Ψ_{PD} and Ψ_{MD} , no symptoms of leaf wilting were observed in any of the cycles. Conilon plants maintain high relative water content even at significantly negative leaf water potential. Visible symptoms of leaf wilting are rare because turgor loss point, usually around 90 %, is related to high rigidity of cell walls, presenting leaf water potential ranging from -1.7 to -2.2 MPa for irrigated clones and reaching more negative values for non-irrigated clones (DaMatta et al., 1993, 2002; Pinheiro et al., 2005).

The greater dispersion of soil moisture for cycle 1 (Fig. 3A) influenced stomatal conductance, transpiration and net photosynthetic CO_2 assimilation rate during this cycle (Fig. 4J, M and P), since they are directly dependent processes of soil water availability (Bergonci and

Pereira, 2002; Larcher, 2004; Andrade et al., 2015).

The significant difference between I and NI treatments (Figs. 3 and 4) confirmed the water stress condition of the plant, proposed by the present research, but it did not quantify the level of stress, being indicated to elaborate the $CWSI_{sapflow}$ for this purpose, based on the aforementioned stress condition, for convenience and ease in automation.

3.2. Calibration of sap flow sensors

The main objective achieved from the calibration of the sap flow sensor (Fig. 5) was to determine the adjustment coefficient "A" of Eq. (6) for the continuity of the $CWSI_{sapflow}$ elaboration process based on the subsequent equations.

The proposed sensor model showed good correlation with ETo based on the coefficient of determination, with satisfactory linear correlation (Fig. 5). The results corroborate with the findings of Conejero et al. (2006), that after studying sap flow by a compensation Heat Pulse Method, in peach trees, under water deficit and recovery from drought stress, found linear correlation between sensor signal and ETo with valued of R^2 equal to 0.77.

The statistically significant linear relationship (Fig. 5) obtained between the actual sensor signal (Δ) and the reference evapotranspiration (ETo) for the irrigated condition (I) confirmed the linearity condition of Eq. (6), allowing us to obtain the adjustment coefficient "A" of the same equation. Thus, Δ was only in function of ETo. As ETo is directly related to plant transpiration (Eq. (7)), the $CWSI_{sapflow}$ could be elaborated, relating the current transpiration with maximum potential plant transpiration (Eq. (8)).

For the $CWSI_{sapflow}$ developed here, the value 0 (zero) indicates absence of water stress and the value one (1) indicates maximum water stress. In the present study, for NI, the $CWSI_{sapflow}$ varied within the estimated range, so that it is possible to identify water status of the plants, with a statistically significant difference in relation to I treatment (Fig. 6).

It is important to emphasize that, since the $CWSI_{sapflow}$ is not an absolute value but a relation between the current transpiration (measured indirectly by the sensor signal) and the potential transpiration of the plant in question (previously determined), the plant water status can be identified only by the actual sensor signal (Δ) received, without the need to determine transpiration itself (a more laborious and relatively expensive process).

This sap flow sensor estimated plant transpiration faster and cheaper than IRGA method (infrared gas analyzer).

3.3. Proposed crop water stress index ($CWSI_{sapflow}$)

Variation of the $CWSI_{sapflow}$, between zero (no drought) and 1.0 (severe water stress) in response to water stress (NI), and full-irrigated conditions (I) is showed in Fig. 6.

The statistical differentiation of $CWSI_{sapflow}$ starts on the 6th day after initiation of the treatments for the first and third cycles, and on the 11th day for second cycle (Fig. 6). For NI, after the application of the irrigation on the 15th and 10th days, for the second and third cycles, respectively (days with $CWSI_{sapflow}$ maximum values), there was no significant difference between treatments, agreeing with Figs. 3 and 4.

The higher $CWSI_{sapflow}$ variation of I treatment in the first cycle (Fig. 6) is justified by the greater soil moisture variation presented for the same trait and the same cycle (Fig. 3A), represented by a higher standard deviation of the mean, compared to soil moisture in the other two cycles.

The increase in $CWSI_{sapflow}$ for I treatment on the 8th, 12th and 14th day for the first cycle, from the 10th and the 14th day for the second cycle and on the 8th and 12th day for the third cycle (Fig. 6) suggests low sap flow related to low plant transpiration on the days in question. What was to be expected, then, for the same period, lower values of

T_{max} and T_{med} (Fig. 1) were observed. Low temperatures negatively affect stomatal conductance and transpiration (Praxedes et al., 2006; Partelli et al., 2009, 2010, 2011a, 2011b; Fernandes et al., 2012). According to Pimentel et al. (2010), the sap flow decreases influenced by low temperatures and high relative humidity, with correlation between variables with R^2 value of 0.6 and 0.7, respectively.

3.4. $CWSI_{sapflow}$ validation

The $CWSI_{sapflow}$ was compared to a $CWSI_t$ (calculated based on the measurement of the actual plant transpiration using IRGA equipment) for NI treatment (Fig. 7).

For first and third cycles, the $CWSI_t$ presented significantly lower values for some dates, in relation to the $CWSI_{sapflow}$ (Fig. 7). However, for the second cycle, throughout all the evaluation period, there was no significant difference between $CWSI_{sapflow}$ and $CWSI_t$.

Low difference between the two coefficients of water stress (Fig. 7) was observed. Both $CWSI_{sapflow}$ and $CWSI_t$ reflected the plant water status, under conditions of low water availability (values close up to 1.0) or in easily available water conditions (values close to zero). Thus, the sap flow sensor can be used to reliably determine the level of water stress of conilon coffee plants.

3.5. Relationship between evaluated variables and crop water stress index ($CWSI_{sapflow}$)

Fig. 8 demonstrates the relationship between the evaluated variables and $CWSI_{sapflow}$, besides indicating critical values of $CWSI_{sapflow}$, from which the water stress condition started.

Results obtained from the sap flow sensor (Fig. 8) allow one to infer plant water condition, considering the good correlation ($R^2 = 0.75$) between $CWSI_{sapflow}$ and Ψ_{PD} , widely used as a crop water status indicator (Silva et al., 2003; Larcher, 2004).

Fig. 8(A and B) presents data assessed during the conduction of cycles for I and at the beginning of each cycle for NI, when the plants were not under influence of water stress. It shows the wide variation in the values for $CWSI_{sapflow}$ (0.0 and 0.5), low variation of Ψ_{PD} (0.0 and -0.5 MPa) and Ψ_{MD} (-0.5 and -1.3 MPa). However, for NI, with the intense increase of water stress, there was a decrease in the variation of $CWSI_{sapflow}$ values (from 0.6 to 0.9) and an increase in the variation of Ψ_{PD} (-0.6 to -2.3 MPa) and Ψ_{MD} (0.6 to -2.5 MPa).

The reduction of soil moisture (Fig. 3), caused a decrease in the amount of water available for the plant (Fig. 4) and a reduction in transpiration (Maestri and Vieira, 1958; Tenhunen et al., 1987; Larcher, 2004). This resulted in increase of $CWSI_{sapflow}$ and decreased stomatal conductance (g_s) values for NI plants (Fig. 8D), corroborating with DaMatta et al. (2002) and Silva et al. (2010).

$CWSI_{sapflow}$ had a polynomial relationship with net photosynthetic CO_2 assimilation rate (A), presenting relatively low rates of net photosynthetic CO_2 assimilation rate for conilon coffee plants (Fig. 8) compared to other woody species (Pinheiro et al., 2004, 2005; Ronchi et al., 2005; Praxedes et al., 2006), recording maximums values around $11 \mu mol CO_2 m^{-2} s^{-1}$, similar to Andrade et al. (2015). However, for conilon coffee plants (clone 12 V) there was a variation in the net photosynthetic CO_2 assimilation rate, without water stress, of between 7.73 and $12.24 \mu mol CO_2 m^{-2} s^{-1}$, which was higher than the values found in the literature.

Water deficit in coffee causes a reduction in the net photosynthetic CO_2 assimilation rate, due to a reduction in stomatal conductance. Under water stress conditions, the influx of CO_2 in the substomatal chamber is reduced, due to stomatal closure or the direct effects of dehydration on the photochemical or biochemical reactions of photosynthesis (Andrade et al., 2015; Costa and Marengo, 2007). Results observed for soil moisture, Ψ_{PD} , Ψ_{MD} (Fig. 3), g_s , E and A (Fig. 8D–F), during the evaluated cycles, corroborate with above-mentioned authors.

NI plants presented lower values of A in comparison to I plants, in the same period in which g_s was significantly lower as well. According to Cornic (2000), under moderate water stress conditions, stomatal closure severely restricts net photosynthetic CO_2 assimilation, so that stomatal control has been considered as the main limiting factor of net photosynthetic CO_2 assimilation. Araujo et al. (2008) states that low photosynthetic rates in some woody species as *Coffea arabica* can be attributed mainly to lower values of stomatal conductance.

Silva et al. (2010) verified a sharp reduction in stomatal conductance of conilon coffee plants under water deficit, compared to a small reduction in the net photosynthetic CO_2 assimilation rate. It was also verified that stomatal closure caused proportionately greater decreases in transpiration than net photosynthetic CO_2 assimilation, since instant water use efficiency (A/E) increased under water stress conditions. These data are indicative that the reduction of photosynthesis due to water stress was associated mainly to CO_2 diffusion limitations, and not to biochemical limitations of the photochemical or biochemical reactions of photosynthesis (DaMatta and Ramalho, 2006).

During the beginning of each cycle, the coffee plants had statistically equal values for soil moisture, Ψ_{PD} and Ψ_{MD} , g_s , E and A , among the treatments (Fig. 8). However, with the worsening in water stress for the NI treatment, decreases in the values of the variables in question was observed, concomitant with increasing $CWSI_{sapflow}$.

The reduction on values of the abovementioned variables began with values of $CWSI_{sapflow}$ between 0.4 and 0.6, which are suggested, by the present study, as critical values for $CWSI_{sapflow}$ for conilon coffee plants (clone 12 V) in early stages of development, subjected to water stress, from June to September in the region studied here.

Finally, studies of irrigation management based on $CWSI_{sapflow}$ are encouraged by the authors, since, as seen in the present study, $CWSI_{sapflow}$ values above 0.4 provide a significant drop in the processes of stomatal conductance, transpiration and net photosynthetic CO_2 assimilation (Fig. 8), initiating a water stress condition in the plant. Thus, with the constant monitoring of the $CWSI_{sapflow}$ variation using the sap flow sensor for a particular plant, irrigation will be necessary whenever the $CWSI_{sapflow}$ reaches the suggested critical value (0.4) for the crop in question.

There is also the possibility of automated irrigation based on the current sensor response, to start water application automatically every time the sap sensor reading reaches a value corresponding to the critical value of $CWSI_{sapflow}$.

4. Conclusions

The proposed sap flow sensor can be used for determining sap flow in young conilon coffee plants by measuring crop water stress index ($CWSI_{sapflow}$) and estimating crop water status more quickly and at lower cost than previous methods.

The $CWSI_{sapflow}$ showed good correlation with the percentage of soil moisture, leaf water potential, stomatal conductance, transpiration, and net photosynthetic CO_2 assimilation of the conilon coffee plant clone studied here.

The present study suggests a critical value for $CWSI_{sapflow}$ of 0.4, from which point conilon coffee plants suffer from water stress during the early stages of their development.

A critical value of crop water stress index ($CWSI_{sapflow}$) for conilon coffee plants allows the estimation, with good precision, of the critical time to start irrigation, avoiding physiological damage to the plant.

The present work proposes a new methodology for estimating the $CWSI$ with satisfactory applicability in automated irrigation management for coffee cultivation.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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