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GAS EXCHANGE AND FLUORESCENCE IN ‘SUTIL’ LIME (*Citrus aurantifolia* Swingle) UNDER DIFFERENT SOIL MOISTURE LEVELS

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ABSTRACT

The Portoviejo valley in the Manabi province, Ecuador, is an important ‘Sutil’ lime (*Citrus aurantifolia* Swingle) producer, but since the water resources in the region are of vital importance, a good management of the irrigation is needed. The objective of this research was to know the physiological response of the plant expressed in terms of gas exchange and fluorescence, under different levels of irrigation. The study zone is located at Maconta site, 40 masl, in an orchard with 15 year old plants grafted on rootstock ‘Cleopatra’, planted at 6 x 6 m. The following irrigation treatments were tested, according to the atmospheric demand: 0.3ET$_0$, 0.5ET$_0$, 0.7ET$_0$, 0.9ET$_0$ and a control (farmer's irrigation). A randomized complete block design with five treatments and six replications was established. Significant differences (P≤0,05) were found among treatments measured in the dry season, where the photosynthesis rate (A) was higher in the control treatment, decreasing in those treatments with water restrictions (0,3ET$_0$) or excess (0,9ET$_0$) in the soil. Similar behavior was observed in stomatal conductance (g_s) and transpiration (T); the increase or decrease of A, would be associated with T and g_s. The water use efficiency (WUE) was higher in the treatment of 0,5ET$_0$ than in the rest of treatments, suggesting that the increase in this variable would be due to a good equilibrium of the C_i/C_a, ratio, avoiding water loss and still maintaining active physiological processes. Low values of the F_v/F_m ratio existed in conditions of excess moisture. The performance of g_s in both periods leads to deduce the rapid adaptation of the crop to different levels of soil moisture.

Additional keywords: Photosynthesis, quantum efficiency, stomatic conductance, transpiration

RESUMEN

Intercambio gaseoso y fluorescencia en lima ‘Sutil’ (*Citrus aurantifolia*, Swingle) en diferentes niveles de humedad del suelo
El valle de Portoviejo en la provincia de Manabí, Ecuador, es un importante productor de lima ‘Sutil’ (*Citrus aurantifolia* Swingle), pero dado que el agua es un recurso vital en la región, se requiere de un buen manejo del riego. El objetivo de esta investigación fue conocer la respuesta fisiológica de la planta expresada en términos de intercambio gaseoso y fluorescencia, bajo diferentes niveles de riego. El sitio del estudio está ubicado en Maconta, 40 msnm, en un huerto con plantas de 15 años de edad, injertadas sobre ‘Cleopatra’ y plantadas a 6x6 m. Se compararon cinco tratamientos de riego, calculados según la demanda atmosférica, de la siguiente manera: 0,3ET$_0$, 0,5 ET$_0$, 0,7 ET$_0$, 0,9 ET$_0$ y un testigo (riego del agricultor), y se usó un diseño experimental de bloques al azar con seis repeticiones. Se encontraron diferencias significativas (P≤0,05) entre tratamientos medidos en época seca, donde la tasa de fotosíntesis (A) fue mayor en el tratamiento testigo, disminuyendo en aquellos tratamientos con restricción hídrica (0,3ET$_0$) o exceso de agua (0,9ET$_0$). Se observó un comportamiento similar en la conductancia estomática (g_s) y transpiración (T), lo cual sugiere que el aumento o disminución de A estaría asociado a T y g_s. La eficiencia en el uso del agua (EUA) fue mayor en el tratamiento de 0,5ET$_0$ respecto a los demás tratamientos, sugiriendo que el aumento de esta variable se debe a un buen equilibrio en la relación Ci/Ca, lo cual evitaría pérdidas de agua a la vez que mantiene activos los procesos fisiológicos. La relación Fv/Fm fue baja en condiciones de exceso de humedad. El comportamiento de g_s en ambos periodos sugiere una rápida adaptación del cultivo ante diferentes niveles de humedad en el suelo.

Palabras clave adicionales: Conductancia estomática, eficiencia cuántica, fotosíntesis, transpiración

INTRODUCTION

Citrus are perennial species that are often exposed to constant climatic variations, such as water deficiency or water excess in field conditions, facing an alteration in the reduction of

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the photosynthesis rate, product of stomatal closure, causing growth inhibition and losses in crop productivity (Miranda et al., 2021). Apart from the species factor, stomatal conductance is sensitive to changes in the environment (Cohen and Cohen, 1983). Abiotic stress caused by soil water deficit greatly threatens agriculture by limiting the water supply to the aerial part of the plant. Water deficit reduces growth and metabolism, and it also reduces stomatal conductance (g_s), leaf transpiration (*T*) and net CO_2 assimilation, thereby decreasing the photosynthetic rate (Pérez et al., 2009; Whellyson et al., 2019), causing a decrease in efficiency and alterations in the fruit quality (Colmenero et al., 2020).

As with water deficit, excessive water in soil stresses crops, generating a reduction in the photosynthesis rate (*A*), transpiration (*T*) and stomatal conductance (g_s). Waterlogging reduces the transmission and absorption of nutrients in the root zone, and if this continues, it could result in weakening the trees, making them more vulnerable to the entry of diverse pathogens (Syvertsen et al., 1983). Stress directly affects the photosynthetic rate by decreasing CO_2 availability, causing stomatal closure and changes in the photosynthetic metabolism (Osakabe et al., 2014). In general, plants make a balance between the need to conserve water and assimilate CO2, therefore transpiration and biomass accumulation depend largely on gas exchange through stomata (Mejía, et al., 2014).

Stomatal conductance is a physiological parameter that represents the control of water loss done by plants. When there are adverse exogenous factors, g_s decreases, limiting gas exchange (Dos Santos et al., 2013). It is essential to mention that crop yield is closely related to the amount of water available, as well as to the efficiency of water use in plants (Riccardo, 2019). As already mentioned, photosynthetic activity is directly affected by stress (Abbas et al., 2019). One way to estimate photosynthetic activity is through chlorophyll fluorescence. Under stress circumstances, maximum fluorescence (Fm) is significantly affected, thus affecting the maximum quantum yield of PSII (Pereira et al., 2000; Šajbidorová et al., 2015).

Due to increasing and extended dry periods, producers face a harder competition for available water sources (Kusakabe et al., 2016). Under water shortage conditions, proper water management comprises any strategy that achieves adequate yield (García et al., 2007). The water management condition for an adequate physiological response in ‘Sutil’ lime in Ecuador has not been studied. Furthermore, its response is unknown, despite being a relevant crop for the Manabí region, where water resources are of vital importance.

Equator has 2.256 ha under production of Sutil´´ lime, out of which 47 % corresponds to the province of Manabi, with Portoviejo valley being the most outstanding (INEC, 2018). Lime yield in Ecuador is 24,144 $Mg\cdot ha^{-1}$, an average that is lower than that reported in other countries such as Brazil and Mexico, as their yields are 27,883 and 14,4907 $Mg\cdot ha^{-1}$, respectively (FAO, 2019). Manabí has a bimodal climatic regime with six dry months (July-December period), where the average amount of rainfall is 10 mm. Hydric stress becomes a limiting factor for lime growth and production in this region. Rainfall in the January-June period becomes a problem for lime growers. It is therefore essential to understand the physiological responses that affect lime yield. The calculation of effective water depth, irrigation scheduling, and crop response would be the basic tools to contribute to the improvement of production in this region of Ecuador. Therefore, the objective of the research was to know the physiological response of the ‘Sutil’ lime expressed in terms of gas exchange and fluorescence, under different levels of soil moisture.

MATERIALS AND METHODS

Location of trial site: The commercial farm San Nicolás is located at the Maconta site (at 40 masl), in the parish of Colón-Los Ángeles in the canton of Portoviejo, province of Manabí, Ecuador.

Vegetative material*:* ‘Sutil’ lime trees (*Citrus aurantiifolia* Swingle) were selected in productive condition, aged 15 years, grafted on Cleopatra rootstock, with a planting spacing of 6 x 6 and in open field conditions.

Climatic characteristics: The experiment was carried out in two periods: dry and rainy. In dry season conditions the mean temperature and ET_0 was 26.23 °C and 4.40 $mm\cdot day^{-1}$, while precipitation in that period reached 69.3 mm; on the other hand, in rainy season conditions the temperature reached 27 °C (Figure 1B), with 3.57 $mm \cdot day^{-1}$ ET_0; rainy periods were registered that reached 571.1 mm, with February and March being the months with the highest precipitation (Figure 1A).

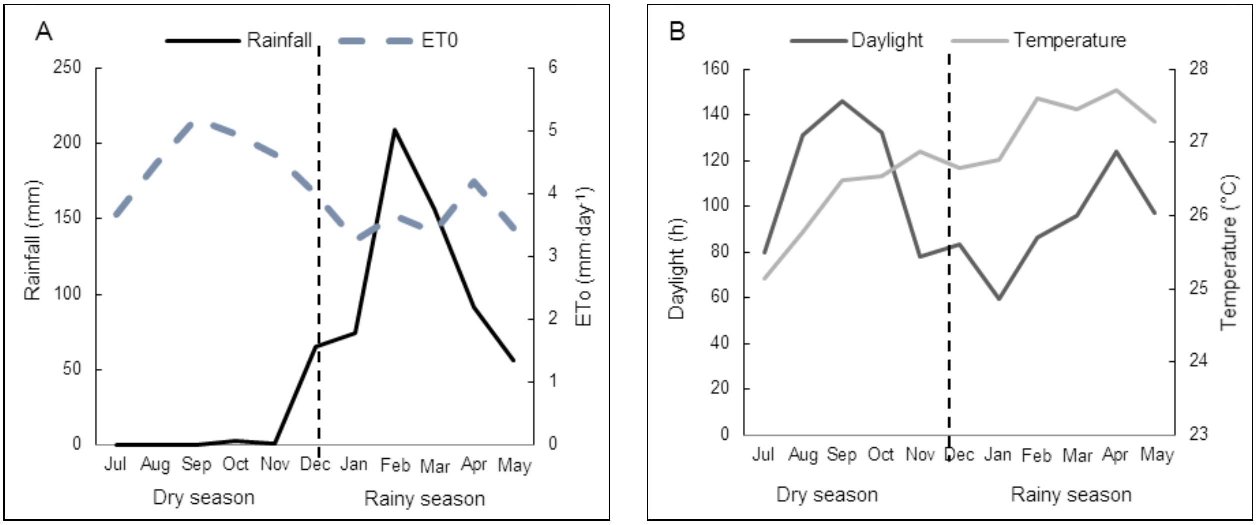

Figure 1. (A) Precipitation and reference evapotranspiration (ET_0) and (B) Light hours and temperature, during the evaluation period. Data collected from the meteorological station "La Teodomira"

Selection of treatments and experimental design*:* The treatments were defined according to the reference evapotranspiration (ET_0). The variation of each treatment is generated with the crop coefficient (Kc), giving values under and over the values reported for *Citrus*, 0.85 in rainy months and 0.65 for dry months (FAO, 2015). As treatments, differential irrigation volumes were applied as a function of 30%, 50%, 70% and 90% of the reference evapotranspiration (ET_0) and a control (similar as the one of the producers). The experiment was set up under a randomized full blocks experimental design, with 6 replications, the experimental unit is represented by a tree; for a total of 30 trees in the experiment.

Irrigation management: The experiment was carried out from July 2018 to May 2019, considering dry periods (irrigation application) and during the rainy season, the moisture continued to be monitored until the levels of the treatments were reached.

Drip irrigation was applied, installing 15 drippers of 8 $L \cdot h^{-1}$ in each tree, distributed in two rings around the trunk, for a total discharge per tree of 120 $L \cdot h^{-1}$. The irrigation schedule was reviewed and adjusted with daily resolution, by calculating crop evapotranspiration (ET_c), estimating ET_0 using the CROPWAT 8.0 software. The information was taken from the La Teodomira-Inahami meteorological station. With the results of ET_0 and given the Kc, ET_c was estimated, using equation $ET_C = K_C \cdot ET_0$.

With the obtained result ETc ($mm \cdot día^{-1}$) was substituted in equation V = ETc x Ah to obtain the volume to irrigate, where, V is the volume of water to irrigate and Ah is the wetted area. V was substituted in equation $t = V/Q$ to obtain the irrigation application time (t). Q refers to the total flow rate of the drippers per tree.

In order to achieve greater accuracy in the interpretation of the treatments' application, which was not possible with the spatial method alone, the gravimetric method was used to determine the moisture condition reached by the soil with the application of irrigation (each treatment), thus a monitoring information was made over time. These data were also used to verify the proximity of moisture to the limits, volumetric field capacity (FC) and permanent wilting point (PWP), which were 36 and 20 %, respectively of the clay-loam soil in the farm

The differential application of irrigation (depending on each treatment) allowed defining the Kc value to which the 'Sutil' lime crop responds best in physiological terms. The gravimetric method was used to determine the average moisture status reached by the soil with the application of each treatment. The irrigation frequency responded to the depletion of 20 % of each treatment.

Physiological conditions: Photosynthesis rate (*A*), stomatal conductance (g_s), transpiration (*T*), and the ratio of intra and extracellular CO_2 (C_i/C_a) were measured with the portable LI-6400 XT Photosynthesis System under dry season conditions. Chlorophyll fluorescence was measured with a fluorometer (Opti-Sciences OS5) under actinic light of 1200 $\mu mol \cdot m^{-2} s^{-1}$ intensity. Prior to this measurement the leaves were subjected to 30 min of darkness (Liu et al., 2019) by covering them with reflective paper. Stomatic conductance (g_s) was measured, using a porometer (Decagon Devices). For this measurement, 10 exposed leaves were selected, without mechanical damage, free of pests and diseases; the measurement was allowed to stabilize for 5 minutes, with the clamp covering the middle zone of the leaf, to ensure the accuracy of the reading. Data collection was biweekly.

The variables were measured in both dry and rainy seasons, although it is important to clarify that measurements of photosynthesis were taken only during the dry season; however, the measurement of g_s were done in both seasons and allowed interpreting the behavior of gas exchange.

In order to know the crop photosynthetic activity in both dry and rainy seasons, the quantum efficiency expressed as F_v/F_m, which represents the ratio of basal fluorescence and maximum fluorescence, was determined

Statistical analysis: To define the differences between treatments, an ANOVA was performed and to define their significance, the Duncan test for comparison of means ($P \leq 0.05$) was performed with the program Duncan ($P \leq 0.05$), with the program Statistical Analysis System, SAS (version 9.4. Cary, NC).

RESULTS

Soil hydric conditions. Moisture measurements taken 24 hours after the soil had drained showed the average soil moisture associated with each irrigation treatment (Table 1). Thus, the average is the result of the sum of the moisture values, divided by the number of irrigation events. During the dry season, the application with a treatment of 0,3ET_0 accumulated a volumetric moisture level (θ) of 29.17 %, which was different ($P \leq 0.05$) from the rest of the treatments, providing a more restrictive water sheet. The analysis indicated that treatments 0.5ET_0 and control were statistically similar ($P > 0.05$), as well as treatments 0.7 and 0.9ET_0. While during the rainy season, only the 0.9ET_0 treatment was statistically different ($P \leq 0.05$) from the other treatments. It is observed (Table 1) that during the dry period 0.5ET_0 and the control treatment were close to FC (36 %) and during the rainy period, all treatments presented an average soil moisture level above FC.

Table 1. Volumetric moisture (θ) levels in the soil associated with the treatments (ET_0), during the assessment period

Treatments	Volumetric soil moisture, θ (%)	
	Dry Season	Rainy Season
0.3 ET_0	29.17 a	39.15 a
0.5 ET_0	34.00 b	39.77 a
0.7 ET_0	37.70 c	41.28 a
0.9 ET_0	39.42 c	45.98 b
Control	35.30 b	39.95 a

Values in the same column with the same letters do not differ significantly, according to Duncan's test ($P \leq 0.05$)

By analyzing the soil moisture condition (excess or deficit), and considering the physical conditions of the soil based on the FC, it can be verified that the 0.3ET_0 treatment would be subject to a hydric restriction condition in dry conditions. While the control and 0.5ET_0 treatments are close to FC with only two percentage points of difference. So these treatments are equal and could be close to providing an adequate hydric and aeration level to the crop at this time of the year (dry season). It is important to mention that the recommended treatment according to FAO (2019) is 0.7ET_0, and in the research it provided a soil water status higher than FC, which could be detrimental due to a higher water accumulation in the crop, just like the 0.7ET_0 treatment.

Photosynthesis (*A*), stomatal conductance (g_s), transpiration (*T*) and Ci/Ca ratio: Under dry season conditions, *A* rate was higher in the control treatment ($P \leq 0.05$), while the 0.3ET_0 and 0.9ET_0 treatments that had lower and higher soil moisture, presented lower *A* rates (Figure 2A). On the other hand, the control treatment had higher g_s and *T*

(Figure 2A,B); the lowest g_s was for the $0.9ET_0$ treatment. This last one accumulated a 39.42 % water volume, higher than FC for this soil, which could be influencing a decrease in the *A* rate.

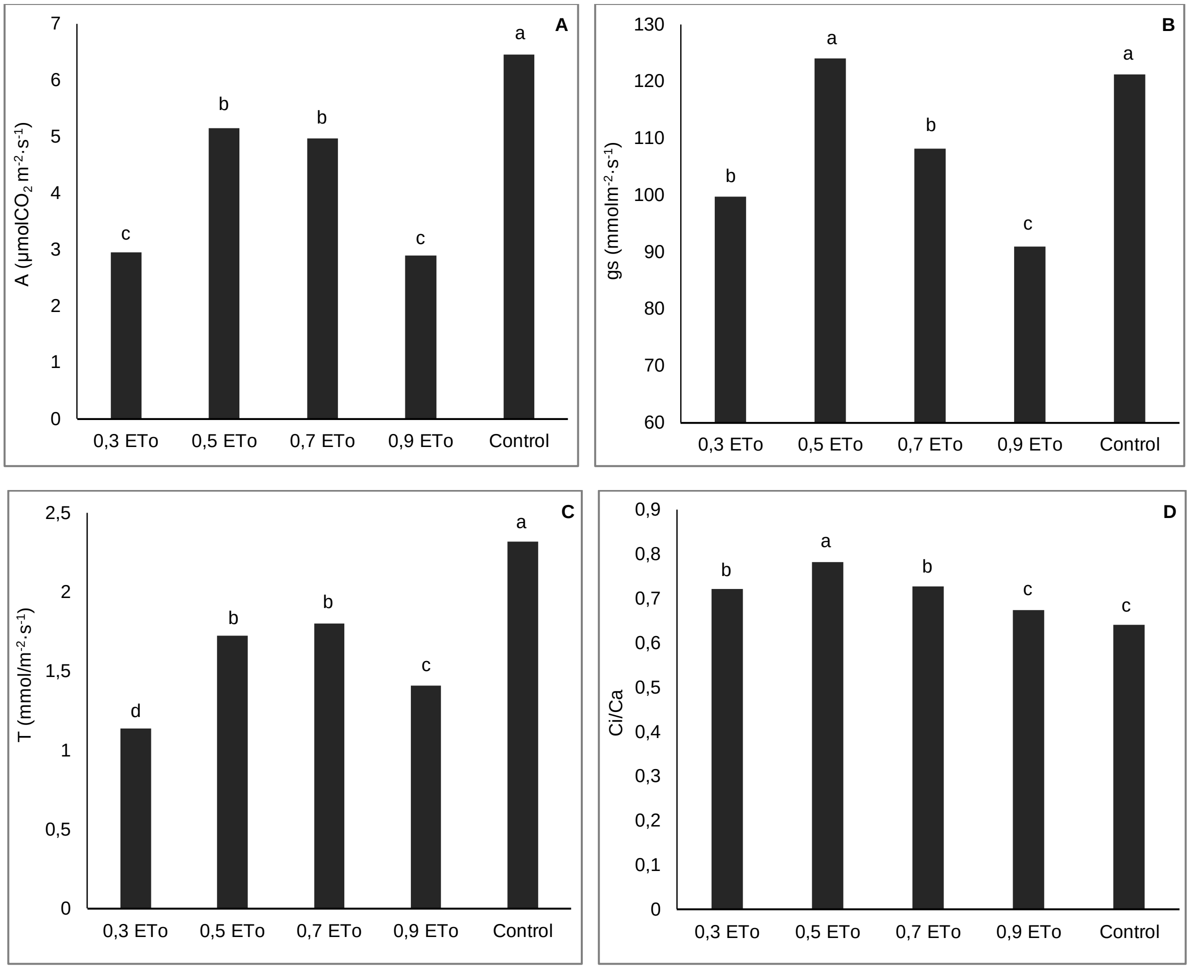

Figure 2. A) Photosynthesis (*A*), B) Stomatal Conductance (g_s), C) Transpiration (*T*), and D) Intra-extracellular cellular CO_2 ratio (C_i/C_a), under different de ET_0 treatments, in dry season conditions at the Maconta site

In dry season conditions, the $0.3ET_0$ treatment, which showed a θ of 29.17%, lower than FC, resulted in a decrease in transpiration (*T*) that was 1.14 mmol·m^{-2}·s^{-1} lower among all the treatments (Figure 2C). Meanwhile, the control treatment showed the highest *T*, as well as a high g_s (similar to $0.5ET_0$) (Figure 2B). This behavior would explain the increase in the rate of CO_2 assimilation (Figure 2A), explained by an adequate irrigation supply and continuity in the stomatal opening. The behavior of the C_i/C_a ratio (Figure 2D), shows that the water sheet $0.5ET_0$ did not present the best *A* and *T*, which could be due to the efficient water management, explained by a low *T* and a high g_s, making a greater CO_2 exchange.

Figure 3 shows the behavior of g_s under dry season conditions, at different soil moisture levels. It is observed that after the water supply applied in treatments 0.9 and y $0,3ET_0$ these increased their g_s, as did the rest of the treatments that received water supply provided by irrigation programming. Then, the crop took maximum advantage of soil moisture (42%), opening their stomata. Upon reaching 36 % (FC) stabilization was observed in all treatments. However, it is the $0.5ET_0$ treatment the one that kept a lower g_s, in spite of having high levels of volumetric soil water (θ). On the other hand, when there is a decrease in the water sheet in soil, this same treatment considerably lowers its g_s, which is similar in all treatments.

Consequently, this behavior may explain the water saving mechanism of the crop under conditions of water deficit.

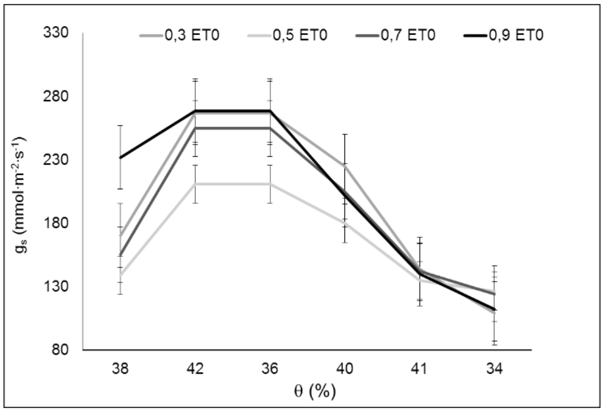

Figure 3. Stomatal conductance in 'Sutil' lime under different soil moisture levels in the dry season at the Maconta site

During the rainy season (mainly February and March) all treatments were above the FC. In fact, the $0.9ET_0$ treatment was 45.98 % (9.98 % above the FC), showing an excess of soil moisture. It could be inferred that this is due to the change in the magnitudes of the variables in the rainy season, where irrigation was not applied. And this would be responding more to a state of acclimatization of the crop during the dry conditions, when g_s dropped considerably but still remained above 100 $mmol·m^{-2}s^{-1}$ (Figure 3).

Water use (WUE) and carboxylation efficiency (CE): Table 2 shows that there were significant differences in WUE ($P≤0.05$) between treatments $0.5ET_0$ and $0,9ET_0$, with $0.5ET_0$ being the most efficient in water use and $0.9ET_0$ the most inefficient. As for the carboxylation efficiency (CE), significant differences ($P≤0.05$) were also found; however, a similar behavior was observed in treatments 0.3 and $0.9ET_0$ which presented θ 29.17 and 39.42 %, respectively, values that are either lower or higher than FC. It means that the plant responded to the stress generated by these two humidity states with a low rate of CO_2 input, confirming, at least, a partial stomatal closure.

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Table 2. Water use efficiency (WUE) and carboxylation efficiency (CE) in 'Sutil' lime under different soil moisture levels in dry season conditions

Treatment	WUE (*A*/T) ($\mu mol\ CO_2/mmol·H_2O·m^{-2}·s^{-1}$)	CE (*A*/C_i) ($(\mu mol\ CO_2·m^{-2}s^{1})/(\mu mol\ CO_2·m^{-2}·s^{1})^{-1}$)
0.3 ET_0	2.20 ab	0.008 b
0.5 ET_0	3.22 a	0.017 a
0.7 ET_0	2.45 ab	0.017 a
0.9 ET_0	1.06 b	0.004 b
Control	2.36 ab	0.022 a
X	2.26	0.013
Pr > F	0.0202	0.0001
DMS (5%)	1.62	0.03

Values within the same column with the same letters do not differ significantly according to Duncan's test ($P≤0.05$)

Quantum Efficiency (F_v/F_m): The crop photosynthetic activity or quantum efficiency showed that in dry season conditions there were significant differences ($P≤0.05$) in the treatments $0.3ET_0$ and $0.9ET_0$, with respect to the other irrigation treatments, which did not show differences among them. The F_v/F_m ratio of the treatment $0.3ET_0$ was lower than that of $0.9ET_0$, and in turn, much lower than those of $0.5ET_0$, 0.7 ET_0 and control (Figure 4A).

In the rainy season the F_v/F_m ratio of $0,9ET_0$ was different ($P≤0.05$) to the rest of treatments and the lowest in magnitude (Figure 4B). Despite this behavior, the values of all the treatments evaluated in this season were relatively low, compared to those from the dry season. This behavior would indicate that the crop during the rainy season may suffer stress from water excess, thus affecting photosynthetic processes.

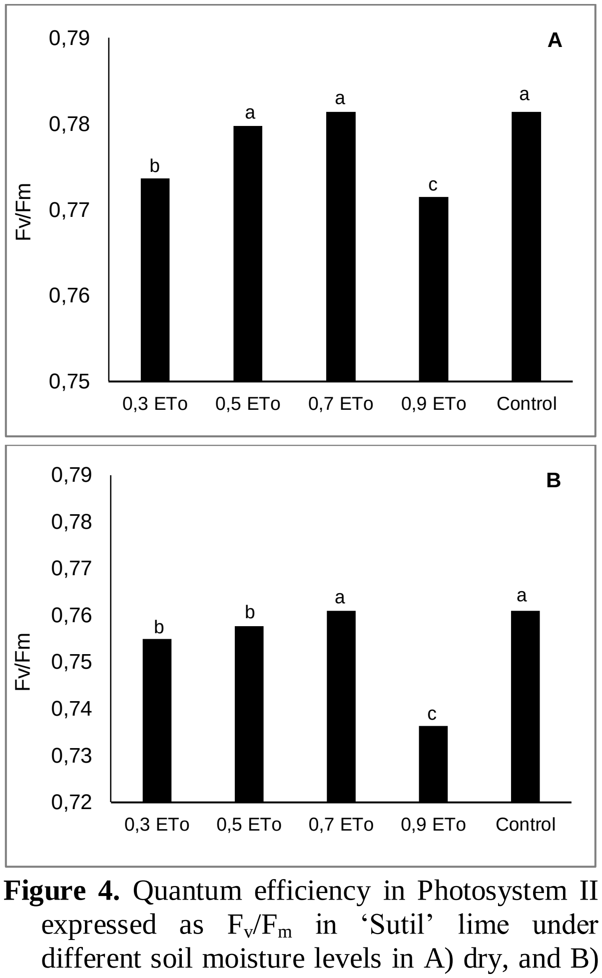

Figure 4. Quantum efficiency in Photosystem II expressed as F_v/F_m in 'Sutil' lime under different soil moisture levels in A) dry, and B) rainy season, at the Maconta site

DISCUSSION

Soil moisture condition: Making an analysis of soil moisture condition (excess or deficit), and considering the premises of soil physics as a function of FC, it can be infer that in dry season conditions, plants with the $0.3ET_0$ treatment would be under water restriction, while the control and $0.5ET_0$ treatments are close to FC, with only two percentage points of difference, which indicates that these treatments, besides being similar, could be the closest to provide an adequate water level and ventilation to the crop at this time of the year. The variables for these two treatments present similar magnitudes, even with lower moisture contents than the FC. This allows inferring that 'Sutil' lime in dry season can be irrigated even with lower levels of the FC and still obtain an adequate productive response, as pointed out by Ríos et al. (2018) for Tahiti acid lime in a dry warm climate condition for Colombia. This moisture level is even lower than that proposed by FAO for Kc 0.7 in productive stage. The statistical similarity presented between the $0,5ET_0$ treatment and control, leads to deduce that the experience of the producer is key in his decision making to improve the yield of 'Sutil' lime. However, it should be adjusted to a greater technification in terms of irrigation control (period and frequency); likewise, it should be considered that $0.5ET_0$ in magnitude is less than the producer's treatment, which, in terms of volume and energy, implies irrigating less and being more efficient in the use of water. Ballester et al. (2013), in Navel oranges subjected to water stress with 50 % ETc, found similarities with the control treatment; however, they clarified that with the treatment under study they would save 19 % water. Based on these findings, it is corroborated what was reported by Alves et al. (2007), who emphasize that citrus Kc values are associated with differences between varieties, rootstocks, planting spacing, age, soil and irrigation timing.

The treatment of $0.7ET_0$, maintained the soil above FC, an excess that could be detrimental to the crop at the Maconta site. This condition is similar for the $0.9ET_0$ treatment. An excess of 3.43 % in θ, in relation to FC (36 %), for the $0.3ET_0$ treatment, would result in a decrease in the photosynthetic rate of the 'Sutil' lime crop, that may be explained by the lack of oxygen in the roots. However, other authors report treatments for Tahiti acid lime with Kc 0.82 and 1.18 (Junior et al., 2009), which verifies the importance of the climatic condition of the site, even in the same species. In the case of 'Sutil' lime, the $0.5ET_0$ treatment performed favorably in terms of gas exchange. Also in Navel orange, it was observed that water stress was marked when only 50 % of the water requirements were applied, in relation to 100 % irrigation, which favored the crop (Abdelraouf et al., 2020). In orange trees subjected to different irrigation treatments, it was observed that applying water at 90 % of ET_0 provided adequate water requirements to this crop (Jafari et al., 2021).

Although citrus crops are able to withstand long periods of water restrictions, it is essential to provide an adequate water supply to contribute to productivity and fruit quality (Pires et al., 2011; Moreno et al., 2019). For the case of this experiment, the lowest values of θ (treatment $0.3ET_0$) allow verifying that the physiological response of the crop is affected with these low moisture levels, generating a decrease in gas exchange (*A*, g_s and *T*; as shown in Figure 2), thus affecting productivity (Barboza et al., 2017).

On the other hand, in rainy season conditions, all treatments exceed the FC due to the high moisture retention of the clay loam soil of the experimental site. The $0.9ET_0$ treatment was presented 9.98 % higher than the FC, showing an excess of soil moisture. It could be inferred that this treatment, which maintains high moisture levels in dry season and continues so in rainy season, has executed a physiological adjustment (Pérez et al, 2009), for this moisture condition, since g_s, does not fall to levels below 100 $mmol·m^{-2}s^{-1}$, and it continues tending some productive level, although not an ideal value.

Photosynthesis (*A*), stomatic conductance (g_s), transpiration (*T*) and $C_{i/}C_a$ ratio: For 'Sutil' lime, average values for *A* of 7.2 $\mu molm^{-2}·s^{-1}$ were found, similar to those reported by Da Silva et al. (2005), who found averages between 4-7 $\mu molm^{-2}·s^{-1}$, lower than those reported for Navel oranges which ranged 12.0-14.5 $\mu mol·m^{-2}·s^{-1}$ (Syvertsen et al., 2003). With the values of θ of 29.17 and 39.42 %, obtained using the $0.3ET_0$ and $0.9ET_0$ treatments, it can be concluded that one of the responses of the 'Sutil' lime crop to water restrictions and excess water would be to decrease the rate of *A* and g_s (Figure 3A,B). Similar results for water deficit are reported by Martínez et al. (2021) in their research on *Citrus limon* on flood tolerant 'King' mandarin hybrids observed a decrease in net CO_2 assimilation rate and g_s, as well as a reduction in ABA concentration in roots in most of these hybrids.

Figure 3 shows the behavior of g_s versus soil moisture variability. The $0.9ET_0$ treatment presents high g_s values and continues to do so even with the increase of moisture over time; nevertheless, the increase of the wet period generates an abrupt drop in g_s. In this case, the 0.3 treatment improved the behavior of g_s over time and maintained high levels in longer periods of high moisture. These two treatments demonstrate the ability of the plant to adapt to the new moisture condition, increasing homeostasis (Osakabe et al., 2014). For its part, the $0.7ET_0$ treatment had a similar behavior to $0.9ET_0$, but with lower g_s values. The $0.5ET_0$ treatment, presents during the period the lowest and continuous values of g_s, which indicates a greater self-regulation of its physiology, which is why it is the one with the highest WUE. The importance of decreasing g_s in water use is a favorable strategy (Rodríguez and Brodribb, 2020) for saving the resource, maintaining productivity (Jamshidi et al., 2020). Therefore, under productive conditions it is important to design an optimal irrigation strategy.

Water Use Efficiency (WUE): The highest WUE was achieved with the $0.5ET_0$ treatment (Table 2). However, this treatment did not have the highest *A* rates, so it can be inferred that the plant decreased g_s and *T*, and avoided dehydration and kept more CO_2 (Begg and Turner, 1976; Brakke and Allen, 1995). Despite existing information on WUE in crops (Peddinti et al., 2019) and even in *Citrus*, for 'Sutil' lime it is scarce and limited. The quantification of WUE would become an indispensable strategy for water economy, as presented in the results, where $0.5ET_0$ and the producer approach, which present similarity, are within the treatments with higher WUE. Due to the importance of the species in Ecuador and worldwide, its study should be intensified.

Quantum efficiency (F_v/F_m): The decrease in

F_v/F_m in the 0.3 ET_0 treatment with an average θ of 29.17%, (Figure 4A) caused alterations in chlorophyll fluorescence, which could be associated with the decrease in soil moisture. This would be consistent with what is reported by Santos et al., (2019), in a study on physiological responses in citrus subjected to drought, where it is noted that the deficit of water in the soil caused the reduction of photosynthetic electron flow. Stress can cause a change in the chlorophyll content of plant leaves, and thus a change in photosynthetic function (Xu et al., 2020). A notorious decrease in the F_v/F_m ratio given by the $0.9ET_0$ treatment with a θ of 45.98 % was observed by this study in rainy season conditions (Figure 4B); it is concluded that, despite the availability of water in the soil, the crop responds by decreasing its F_v/F_m, to the excess, which would be associated with a stomatal closure (Garcia et al., 2007). Studies carried out on citrus rootstocks subjected to salinity (Bleda et al., 2001) indicate that F_v/F_m values fluctuated between 0.81 and 0.69, the first value referring to the control and the second to a high salt stress condition. The information on this indicator is scarce in citrus and especially in limes.

CONCLUSIONS

The results suggest that the producer does an adequate irrigation during the dry season. However, he uses a water sheet around 0.6 ET_0, higher than the treatment that presented the best results in physiological behavior (treatment $0.5ET_0$), that is, keeping the soil at θ close to 34 %. This moisture is sufficient to maintain the photosynthetic apparatus in operation, ensuring that both g_s and *A* are maintained at adequate levels. Likewise, it is not advisable for the producer to irrigate in deficiency or excess water (under or over 36 %), such as the case of the 0.3 and $0.9ET_0$ treatments, respectively. Likewise, the $0,7ET_0$ treatment (recommendation according by FAO) was not favorable for the site under study, since irrigation with atmospheric demand depends on environmental conditions. On the other hand, under usual rainy conditions it is not necessary to irrigate, as found in the research, because the crop may suffer from excess water, leading to plant stress.

RECOMMENDATIONS

These results are presented to growers in order to improve the recommendations given so far for management the crop of ‘Sutil’ lime in Maconta, Manabí, Ecuador.

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