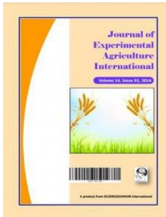

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The Irrigation Deficit and Its Effects on Physiology and Phenology of 'Navelate' Oranges Trees in Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this research was to study physiological responses and changes on phenology of 'Navelate' orange trees submitted to different water stress intensities in a greenhouse.
Study Design: The experimental design was completely randomized, with three replications in each experimental unit. Values of each parameter were submitted to variance analysis, compared by Tukey's test at 5% significance and showed as averages.
Place and Duration of Study: The experiment was carried out in a greenhouse (latitude 31°52'00 "S, longitude 52°21'24" W, 13 m above sea level), during 2014 and 2015.
Methodology: Stressed conditions were based on 50% and 25% of the field capacity. Gas exchange [photosynthesis (A), transpiration (E), stomatal conductance (gs), water use efficiency (WUE)] were analyzed using an infrared gas analyzer, model Li-6400 (Portable Photosynthesis System LICOR, Nebraska, USA), in addition to growth parameters.
Results: Reductions on photosynthetic rate were observed (10.74% for T-50, and 20.66% for T-25, both compared to Control), indicating that CO_2 assimilation rate was affected by water stress

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conditions. Water stress affected all gas exchange parameters of the exposed orange trees, limiting growth in diameter and height. Fruit yield decreased with the amount of water (100%> 50%> 25%).

Conclusion: Navelate orange plants exposed to water deficit were tolerant in the initial phase of the treatments and during the vegetative phase, being more sensitive in the reproductive period. Plants submitted to stress with 25% of field capacity, presented limitations compared to control plants under full water availability, such as differences in height, diameter and fruit production. Water stress, at any level, reduced plant growth and fruit production. Therefore, due to variations in phenological parameters among the treatments, further studies should be performed on these variables to search for water deficit tolerant varieties and quality fruits production under these conditions.

Keywords: Water stress; Citrus sinensis; gas exchange; photosynthesis; fruit production.

1. INTRODUCTION

Orange is a fruit of great importance in Brazil, as the world's largest orange producer with 16,240,000 tons/yr. Other large orange producing countries are China and India with respectively 7,823,550 and 7,313,610 tons/yr [1]. Approximately 70% of orange production in Brazil comes from São Paulo state with about 11,628,150 tons/yr. Rio Grande do Sul is the fifth largest orange producing state with 399,296 tons/yr [2].

Water stress is one of the most frequent environmental factor which limits orange (*Citrus sinensis*) crop expansion in several places around the world. Water deficit can be resulted from an excessive soil water deficit or an excessive water loss through transpiration in relation to water absorption by the root system. In trees, a high evaporative demand of atmosphere enhances high transpiration rates, influencing water potential of leaves because of low hydraulic conductivity of the root system [3].

Studies of physiological parameters, such as photosynthetic rates, transpiration rates, and stomatal conductance, are very important to evaluate drought tolerance in several plant species by elucidating changes on production and fruit quality. Citrus fruits under several water stress close their stomata in order to reduce water loss through transpiration. Those plants can also limit CO_2 diffusion to sub-stomatal cavity, resulting in the reduction of photosynthetic rate and in the increase of foliar temperature [4].

Southern Brazil, as one of orange producing areas, is submitted to a subtropical climate with low temperatures during winter and both warm and rainy during summer [5]. Under these conditions, it seems to be necessary to study about the response of citrus crop to water stress, analyzing how to choose better water management during drought season and because of the worldwide climate changes. The evaluation of gas exchange and water state of citrus plants can indicate the best conditions to keep water and carbon balance during dry season in this region.

The objective of this research was to analyze physiological responses and phenological changes in ‘Navelate’ orange trees submitted to water stress conditions.

2. MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Fruit Crop Sector, at Federal University of Pelotas, in Brazil (Latitude 31°52’00" S; Longitude 52°21’24" W; altitude 13 m). The climate of this region is Cfa, according to Köppen-Geiser climate classification.

Evaluations were done between March 2014 and March 2015 in ‘Navelate’ orange trees. Young plants were obtained from commercial nurseries, cultivated in 26 L pots and received the same irrigation amount during the acclimation period of 45 days. After this period, the plants received different water management treatments: control (substrate humidity corresponding 100% of field capacity), T-50 (substrate humidity corresponding 50% of field capacity), and T-25 (substrate humidity corresponding 25% of field capacity).

Gas exchange analyzes, growth measurement and phenology evaluations were done after acclimation period, monthly, during 210 days from August 2014 to March 2015.

Gas exchange analyzes were done according to the following parameters: photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and internal CO_2 concentration (Ci). Evaluations were done by using an infrared gas analyzer, model Li-6400 (Portable Photosynthesis System LICOR, Nebraska, USA), with a photosynthetic active radiation, intensity of 1200 μmol.m-2.s-1, measured in previously selected and completely expanded leaves. The evaluations were recorded when the coefficient of variation (CV) was less than 0.5% and in temporal stability. Water use efficiency (WUE μmol mol^{-1}) and intrinsic water use efficiency (WUE_{intr} mmol mol^{-1}) were calculated according to the following equations: EUA (μmol CO_2 $mmol^{-1}$ H_2O) = Photosynthesis / Transpiration, and WUE_{intr} (μmol CO_2 $mmol^{-1}$ H_2O) = Photosynthesis / Stomatal Conductance, respectively.

The plant height (cm) was evaluated, adopting, as criterion, the graft distance to the terminal shoot of the main branch; the trunk diameter of the plants was determined 5 cm above the graft, using a scale and a digital caliper, respectively.

The experimental design was a randomized complete block with three replications in each experimental unit. Values of each parameter were submitted to analysis of variance, compared by Tukey's test at 5% significance and showed as averages.

3. RESULTS AND DISCUSSION

During this experiment, changes on gas exchange and plant phenology were observed up to 100 days after starting the treatments of controlled water stress (Fig. 1A). After this period, a reduction in this parameter according to the treatments was observed: T-25 < T-50 < Control.

Changes were also observed in water use efficiency (Fig. 1E), leaf temperature (Fig. 1D), and vapor pressure deficit (Fig. 1F) in plants submitted to controlled water deficit in comparison to control plants.

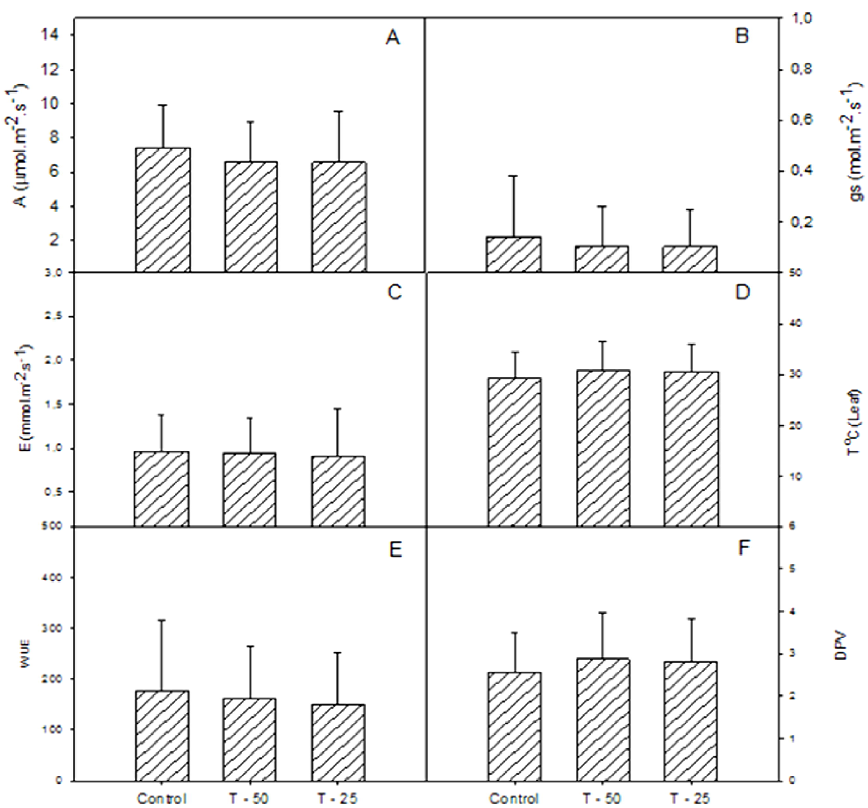

Fig. 1. Photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), leaf temperature (D), water use efficiency (E), and vapor pressure deficit (F), in 'Navelate' oranges submitted to different levels of irrigation [substrate humidity corresponding 100 % (Control), 50 % (T-50), and 25 % (T-25) of field capacity], evaluated monthly from August 2014 to March 2015

Internal CO_2 concentrations, in general, followed gas exchange, showing values lower than the control. The capacity to maintain physiological activity throughout reduction of water availability result in some consequences, such as changes on gas exchange. In fact, changes on photosynthetic rate and reductions on stomatal conductance in plants submitted to higher deficit of atmospheric vapor pressure were observed. This lower level of stomatal opening, which occurred in plants submitted to water stress, was a consequence of reducing turgor pressure of cells, a higher deficit of atmospheric vapor pressure, or by chemical signals coming from the root system [6,7].

Ramos et al. [8] observed that the metabolism of orange plants was strongly affected by thermal regime, resulting in physiological changes related to photosynthesis, exportation of photoassimilates, and photosynthetic pigments, which changed fruit development and composition. Therefore, exposition of orange plants to temperatures higher than optimal can result in the reduction of photosynthesis rate and carbohydrate metabolism. The reduction in CO_2 assimilation can be a result from a consequence of closing the stomatal pores, and also because of possible photochemical damages in the photosynthetic membranes [9]. The increase of air temperature, and consequently the increase of leaf temperature promoted a reduction on photosynthetic rate because of an increase on respiration rate [10,11].

Results obtained from this research corroborated with findings of Pedroso et al. [12] related to substantial suppression on gas exchange in plants submitted to water stress. According to these authors, water deficit resulted in proline accumulation in leaves, reduction on water potential, stomatal conductance, respiration rate, CO_2 assimilation, and mass accumulation. This reduction on photosynthetic activity due to water stress occurred at the same time as the decrease in turgor pressure [13].

One of the first reaction of orange plants grown under severe stress can be to close the stomatal pores, in order to minimize water loss [14]. The possible reduction tendency on transpiration rate observed in all treatments coincided with significant reduction on stomatal conductance throughout the experiment (Table 1). Direct interdependence between transpiration and stomatal conductance was expected because of vapor flux reduction on the atmosphere caused by closing the stomatal pores. According to Lawlor and Tezara [15], during the periods of water shortage, plants keep their stomata closed in order to maintain favorable turgor pressures, an important characteristic for drought tolerance.

Water management affected gas exchange in plants submitted to water stress. Variations in photosynthetic rate, stomatal conductance and transpiration rate were observed (Table 1).

Ma et al. [16] concluded that photosynthetic rate associated with lower stomatal conductance and transpiration rate is typically observed in plants which tolerate lower water availability, but it was not clearly observed in this experiment.

Reductions on photosynthetic rate (10.7% for T-50, and 20.7% for T-25, both compared to Control) were observed, indicating that CO_2 assimilation rate were affected by water stress conditions (Table 1). The intrinsic water efficiency levels did not differ significantly among treated plants and control. At this time, diffusion processes are promptly reduced, and the intrinsic water efficiency levels reaches the highest values. So, under severe water stress, mesophyll cells dehydration strongly inhibits plant metabolism and photosynthesis [14]. Variations in physiological factors were not accentuated in this period probably due to the winter period with low temperatures and days with a small solar radiation incidence, a fact that may have limited the stomata opening, generating less pressure on the plant.

Orange plants had morphological characteristics and physiological mechanisms which give considerably tolerance to water stress when compared to other perennial plants [17], but productivity is closely related to water availability. As photosynthetic rates are affected by water deficit, a reduction in both carbohydrate levels and fruit weight will affect fruit quality [18,19,20].

The highest growth rate (height and trunk diameter) occurred in plants with appropriate water supply (Control) and higher photosynthetic rate compared to plants grown under water stress (T-50 and T-25). With water restriction, plants from both T-50 and T-25 treatments showed reduction on trunk growth (diameter) and leaf expansion. These responses to acclimation limited water consumption by different tissues, helping to maintain plant water status [6,21].

With water supply reduction, the plants of treatments T-50 and T-25 present stem growth, and diameter and leaf expansion inhibitions. These acclimatization responses end up limiting the expenditure of water by the tissues, helping to maintain the plant water status [6,22]. Fig. 2 shows differences in growth among control treatment plants and those submitted to controlled water deficit.

In changes of trunk diameter and plant height, it was also observed the reduction and changes in weight and diameter of fruits produced. T-25 plants had reduction on both fruit size (50%) and weight (90%) compared do Control. Some authors concluded that the reduction on productivity occurred in plants under water stress during both flowering and fruit set stages [23,24]. In our experiment, water deficit was applied since vegetative growth stage, and reduction on fruit production was observed in plants grown under water deficit conditions (Fig. 2).

Table 1. Average values of photosynthetic rate (A), stomatal conductance (E), transpiration rate (gs), and intrinsic water use efficiency (WUE_{intr}) in 'Navelate' oranges submitted to different irrigation treatments, evaluated monthly from March 2014 to August 2014

Treatments	A ($\mu mol \cdot m^{-2} s^{-1}$)	E ($mmol \cdot m^{-2} s^{-1}$)	*gs* ($mol \cdot m^{-2} s^{-1}$)	WUE_{intr} ($mmol \cdot mol^{-1}$)
Control	6.05^{a}	3.06^{a}	0.06^{a}	1.98^{a}
T-50	5.40^{a}	2.64^{b}	0.05^{a}	2.01^{a}
T-25	4.80^{a*}	2.41^{b}	0.04^{a*}	1.99^{a}

** Values followed by the same letter did not differ at the 5% probability level by Tukey's test.*
** Means by comparison in a column and indicates differences among treatments in different times*

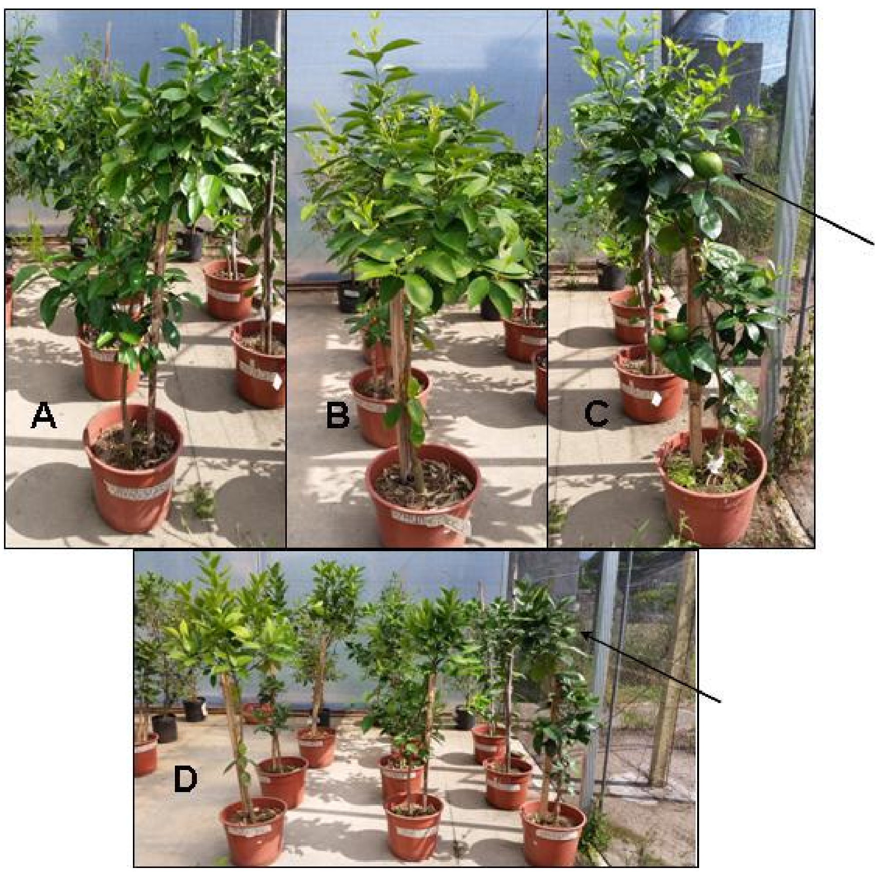

Fig. 2. A - treatment T-25 plants, B - treatment T-50 plants and C - control plants. The arrow shows that the control plants have already produced fruits. D - overview of the experiment and presence of fruits in the control, as indicated by the arrow

Oliveira et al. [25] found significant differences in the size and yield of 'Cabula' orange fruits submitted to water deficit when compared to control plants. The plants exposed to extreme water shortages produced fruits, but without presenting commercial characteristics.

Torrecillas et al. [26] and Domingo et al. [27], studying lemon plants during one year, submitted to irrigations based on 100% of crop evapotranspiration (ETc) and irrigated at 25% of ETc, did not find changes in fruit growth and yield. However, for this result to be possible, plants with 25% ETc were irrigated with 100% ETc during the period of rapid fruit development, after June drop, indicating that a controlled deficit can improve the efficiency of water use. This fact does not apply when the deficit is continuous, as observed in our experiment.

Velez et al. [28] mentioned different authors, among them Pérez-Pérez et al. [29], Ginestar and Castel [30] and Romero et al. (2006) who report that in citrus plants under Mediterranean climate conditions, the lack of water is a limiting factor for fruit growth and production. Maotani et al. [31] observed that in 'Satsuma' mandarins fruit, trunk growth was more sensitive to water stress than in other plant parts.

In our study, following the changes in diameter and height, reductions in fruit production were also observed. T-25 plants produced fruits with size and weight reduced in up to 90% as compared to those from the control treatment (Fig. 2C). It is important to note that, in case of fruit trees, there is a significant decrease in plants productivity under water deficit. Although water deficit was applied from the beginning of plants vegetative phase, the results were similar in terms of reductions in fruit production to that of plants under controlled water deficit.

It was found no changes on plant growth rates and production in plants irrigated with both 25% and 100% of crop evapotranspiration [29,32]. However, those plants were irrigated with 100% of crop evapotranspiration after 'June drop' phase until the stage of rapid fruit growth. Therefore, it can be concluded that controlled water deficit can improve water use efficiency.

Other parameters related to plant water status.

Leaf temperature is another indicator of plant water stress as transpiration enhances leaf cooling. With the decrease in soil water content, transpiration decreases whereas leaf temperature increases in relation to air temperature [33]. Generally, plants submitted to soil water deficit present an increase in leaf temperature with stomatal closure as physiological signals in order to maintain their water status [34]. Stomatal closure in combination with other parameters, such as trunk sap flow, stem diameter shrinkage or reduction, regulates plant water status as a potential indicator in irrigation scheduling [25]. Table 2 shows the differences between the height and the diameter of 'Navelate' orange trees submitted to water deficit compared to those submitted to normal irrigation conditions (field capacity).

Table 2 shows the levels of water deficit investigated and variations in plant height and stem diameter as suggested indicators of water status in citrus fruits given their sensitivity to the substrate moisture variation [35]. As a response to water stress, plant development is inhibited, as some of the first symptoms being leaf withering, water potential reduction, decrease in stomatal conductance, reduction in CO_2 assimilation and depletion in hydraulic conductivity of the root depletion [36]. Water stress affects plant phenological stages in different ways and intensities, affecting flower formation, fertilization and even fruit abscission, besides causing plant growth and development reductions [30].

Water extracted through the root system is transported by the xylem and distributed across different plant parts before reaching the atmosphere through transpiration process that occurs in response to the energy gradient provided by solar radiation [33]. In fact, plant growth and development are influenced by environmental factors which, among them, water availability plays a vital role as it intervenes in most physiological and biological processes.

Table 2. Plant height and trunk diameter of 'Navelate' orange plants, evaluated monthly from March 2014 to February 2015

Treatments	Height (cm)	Diameter (mm)	Number of leaves
Control	174^{a}	$12{,}23^{a}$	119^{b}
T-50	168^{ab}	$9{,}74^{b}$	123^{ab}
T-25	163^{b}	$8{,}62^{b}$	127^{a}

** Values followed by the same letter did not differ at the 5% probability level by Tukey's test*

In the context of global warming, it is crucial to select crops which are adapted to growing environmental stress conditions. Resistance to water deficit in citrus plants, for example, is based on tolerance (osmotic adjustment), and prevention of water stress through stomatal control is a process highly developed in this genus [22,28].

4. CONCLUSION

Navelate orange plants exposed to water deficit were tolerant in the initial phase of treatments and during the vegetative phase, being more sensitive in the reproductive period. Water stress did not cause perceptible alterations in physiology; however, phenological parameters changes were observed. Plants submitted to stress with 25% of field capacity presented limitations compared to control plants under full water availability, such as differences in height, diameter and fruit production. Water stress, at any level, reduced both plant growth and fruit production. Therefore, due to variations in phenological parameters among the treatments, further studies should be performed on these variables to search for water deficit tolerant varieties and quality fruits production under these conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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