

Irrigation with Controlled Water Stress and its Effects on Physiology and Phenology of 'Navelate' Oranges

ABSTRACT

Aims: The aim of this research was to study physiological responses and changes on phenology of 'Navelate' orange trees submitted to different intensities of water stress in greenhouse.

Study design: The experimental design was completely randomized, with three replications in each experimental unit. Values of each parameter was submitted to analysis of variance, compared by Tukey's test at 5% significance and showed as average.

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), internal CO₂ concentration (Ci), 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: It was observed reductions on photosynthetic rate (10.74% for T-50, and 20.66% for T-25, both compared to Control), indicating that CO₂ assimilation rate were affected by water stress 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: Water stress caused alterations in physiological and phenological responses of 'Navelate' orange plants, especially those submitted to stress with 25% of field capacity, which presented limitations compared to plants under full water availability. Deficient irrigation showed no changes in water use efficiency (normal and intrinsic), which means a good parameter of adaptation for stress condition. Water stress, at any level, reduced both plant growth and fruit production.

Keywords: water stress; *Citrus spp.*; gas exchange; photosynthesis; fruit production.

1. INTRODUCTION

Orange is a fruit of great importance in Brazil, the country is the world's largest orange producer, producing 16,240,000 tons, other countries show high orange production, such as China which has the second largest production 7,823,550 tons, and India which holds the third largest production 7,313,610 tons [1]. Approximately 70% of orange production in Brazil belongs to São Paulo state, which produces about 11,628,150 tons and Rio Grande do Sul is the fifth largest producer, state orange production is 399,296 tons [2].

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

Study 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 both production and quality of fruits. Citrus under several water stress close their stomata in order to reduce water loss through transpiration. Those plants can also limits CO₂ diffusion to substomatal cavity, resulting in reduction photosynthetic rate and increase of foliar temperature [4]. Southern Brazil, which is one of production areas of oranges, have subtropical climate with low temperatures during winter season and both warm and rainy during summer season [5]. Under these conditions, it seems to be necessary to study about citrus crop response to water stress, analyze how to choose a better water management during drought season and because of worldwide climate

34 changes. Evaluation of gas exchange and water state of citrus plants can indicate the best conditions
35 to keep both water and carbon balance during drought season in this region.

36 The objective of this work was to analyze physiological responses and phenology changes on
37 'Navelate' orange trees submitted to water stress conditions.

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39 2. MATERIAL AND METHODS

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

44 Evaluations were done between July 2014 and May 2015 in 'Navelate' orange trees. Young plants
45 were obtained from commercial nurseries and cultivated in 26 L pots, and received same irrigation
46 amount during acclimation period of 45 days. After this period, plants received distinct water
47 management: control (substrate humidity corresponding 100% of field capacity), T-50 (substrate
48 humidity corresponding 50% of field capacity), and T-25 (substrate humidity corresponding 25% of
49 field capacity).

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

52 Gas exchange analyzes were done according to following parameters: photosynthetic rate (A),
53 transpiration rate (E), stomatal conductance (gs), and internal CO₂ concentration (Ci). Evaluations
54 were done by using an infrared gas analyzer model Li-6400 (Portable Photosynthesis System LICOR,
55 Nebraska, USA), with a photosynthetic active radiation intensity of 1200 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, measured in
56 previously selected completely expanded leaves. The evaluations were recorded when the coefficient
57 of variation (CV) was less than 0.5% and in temporal stability. Water use efficiency ($\text{WUE } \mu\text{mol mol}^{-1}$)
58 and intrinsic water use efficiency ($\text{WUE}_{\text{intr}} \text{ mmol mol}^{-1}$) were calculated according to follow equations:
59 $\text{EUA } (\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}) = \text{Photosynthesis} / \text{Transpiration}$, and $\text{WUE}_{\text{intr}} (\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}) =$
60 $\text{Photosynthesis} / \text{Stomatal Conductance}$, respectively.

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

64 The experimental design was completely randomized, with three replications in each experimental
65 unit. Values of each parameter was submitted to analysis of variance, compared by Tukey's test at
66 5% significance and showed as average.

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68 3. RESULTS AND DISCUSSION

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70 During this experiment, it was not observed changes on gas exchange and plant phenology until 100
71 days after started treatments of controlled water stress (Figure 1A). After this period, it was observed
72 reduction in this parameter according to the treatments: T-25 < T-50 < Control.

73 Changes were also observed in water use efficiency (Figure 1C), leaf temperature (Figure 1D), and
74 vapor pressure deficit (Figure 1F) in plants submitted to controlled water deficit in comparison to
75 control plants.

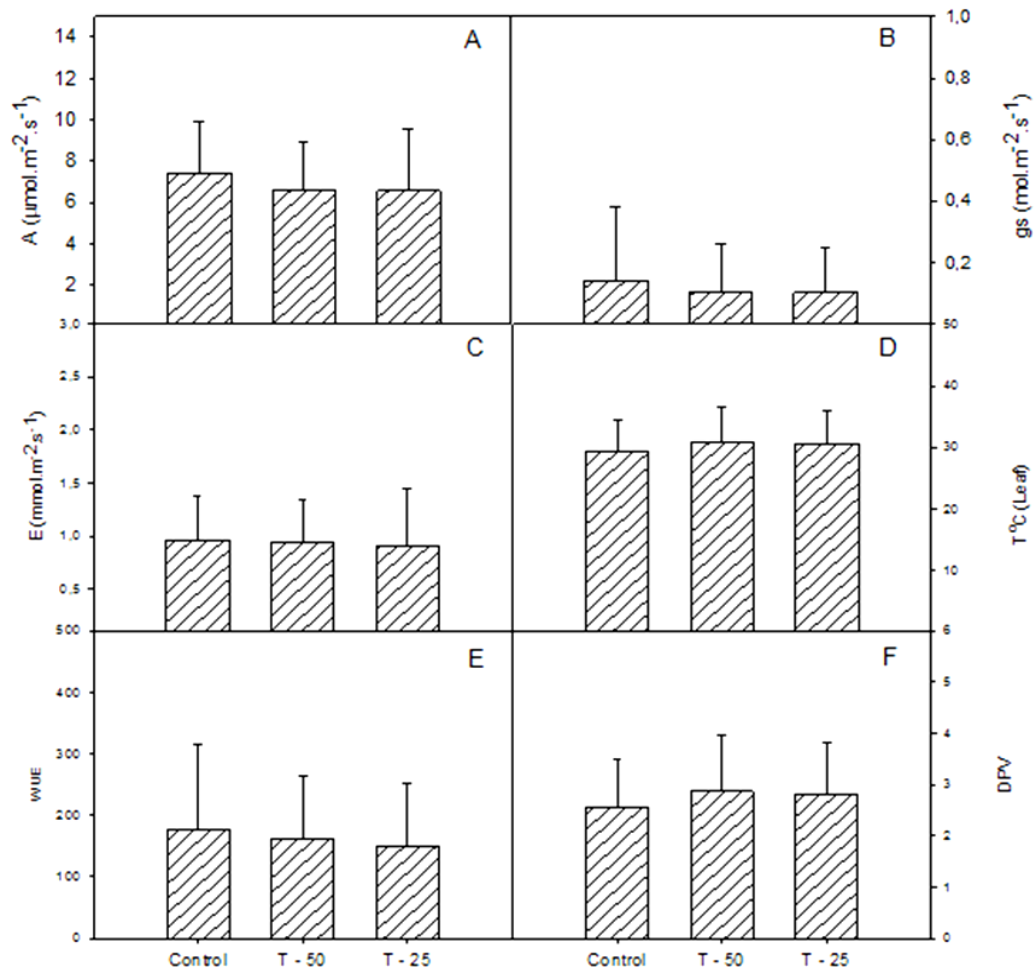
76 Internal CO₂ concentrations, in general, followed gas exchange, showing values lower than the
77 control. Capacity to maintain physiological activity throughout reduction of water availability result in
78 some consequences, such as changes on gas exchange. In fact, it was observed changes on
79 photosynthetic rate and reductions on stomatal conductance in plants submitted to higher deficit of
80 atmospheric vapor pressure. This lower level of stomatal opening, which occurred in plants submitted
81 to water stress, was a consequence of reduction of turgor pressure of cells, a higher deficit of
82 atmospheric vapor pressure, or by chemical signals coming from root system [6,7].

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84 Ramos and co-workers [8] observed that metabolism of orange plants were strongly affected by
 85 thermal regime, resulting in physiological changes related to photosynthesis, exportation of
 86 photoassimilates, and photosynthetic pigments, which changed both fruit development and
 87 composition. Therefore, exposition of orange plants to temperatures higher than optimal can result in
 88 reduction of both photosynthesis rate and carbohydrate metabolism. Reduction in CO₂ assimilation
 89 can be resulted as a consequence of closing the stomatal pores, and also because of possible
 90 photochemical damages in photosynthetic membranes [9]. Increase of air temperature, and
 91 consequently increase of leaf temperature, promoted a reduction on photosynthetic rate because of
 92 increase on respiration rate [10,11].

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 94 Results obtained from this work corroborated with Pedroso and co-workers [12], which found
 95 substantial suppression on gas exchange in plants submitted to water stress. According to this author,
 96 water deficit resulted in proline accumulation in leaves, reduction on water potential, stomatal
 97 conductance, respiration rate, CO₂ assimilation, and mass accumulation. This reduction on
 98 photosynthetic activity caused by water stress occurred at same time of reduction on decrease of
 99 turgor pressure [13].

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 102

103 **Figure 1. Photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), leaf**
 104 **temperature (D), water use efficiency (E), and vapor pressure deficit (F), in 'Navelate' oranges**
 105 **submitted to different levels of irrigation [substrate humidity corresponding 100% (Control),**
 106 **50% (T-50), and 25% (T-25) of field capacity], evaluated monthly from August 2014 until March**
 107 **2015.**

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 tendency of reduction 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 were expected because of vapor flux reduction on 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. It was observed significant differences in photosynthetic rate, stomatal conductance, transpiration rate, and internal CO₂ concentrations (Table 1).

Table 1. Average values of photosynthetic rate (A), stomatal conductance (E), transpiration rate (gs), internal CO₂ concentration (Ci), and intrinsic water use efficiency (WUE_{int}) in 'Navelate' oranges submitted to different treatments and levels of irrigation [substrate humidity corresponding 100% (Control), 50% (T-50), and 25% (T-25) of field capacity], evaluated monthly from March 2014 until August 2014.

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

* Values followed by 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.

Under water stress conditions it was observed highest values of water use efficiency, probably because of reduction on stomatal conductance and consequently reduced the photosynthetic rate when compared to control (Table 1). Ma and co-workers [16] concluded that high photosynthetic rate associated with lower stomatal conductance and transpiration rate are typically observed in plants which tolerate lower water availability, but it was not clearly observed in this experiment.

It was observed reductions on photosynthetic rate (10.74% for T-50, and 20.66% for T-25, both compared to Control), indicating that CO₂ assimilation rate were affected by water stress conditions (Table 1). The intrinsic water efficiency levels did not differ significantly between treated plants and control, but it was higher under water stress conditions. It could be related to lower levels of stomatal conductance. The CO₂ absorption and water consumption occurred when the stomatal pores are partially open in the beginning of water stress condition. 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 and the 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 are closely related to water availability. Since the photosynthetic rate are affected by water deficit, a reduction on both carbohydrate levels and fruit weight, which can affect fruit quality [18,19].

The organic matter accumulated in a plant came from photosynthesis, and represented 95% of its dry matter [20, 21], and around 60% are in root system in orange seedlings [22]. In this experiment, the highest growth rate (height and trunk diameter) occurred in plants with appropriate water supply (Control), which had higher photosynthetic rate, when compared to plants grown under water stress

(T-50 and T-25). With water restriction, both T-50 and T-25 plants showed reduction on trunk growth (diameter) and leaf expansion. These responses to acclimation limited water consumption by different tissues, helping to keep water status of those plants [6].

With the water supply reduction, the plants of treatments T-50 and T-25 present stem growth, 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]. Figure 2 shows differences in growth among control treatment plants and those submitted to controlled water deficit.

Following changes of trunk diameter and plant height, it was also observed 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 reduction on productivity occurred when water stress occurred was applied during both flowering and fruit set stages [23, 24]. In our experiment, water deficit were applied since vegetative growth stage, but reduction on fruit production was observed in plants grown under water deficit conditions (Figure 2).



Figure 2: A - treatment T-25 plants, B - treatment T-50 plants and C - control plants. The arrow points out that control plants already produce fruits. D - overview of the experiment and presence of fruits in the control, as indicated by the arrow.

Oliveira and colleagues [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 and co-workers [26] and Domingo and colleagues [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, the plants with 25% ETc were irrigated with 100% ETc during the period of rapid fruit development, after the june drop, indicating that a controlled deficit can improve the efficiency of water use from water. This fact that does not apply when the deficit is continuous, as observed in this experiment.

Velez and colleagues [28] mentioning different authors, among them Pérez-Pérez and co-workers (2009), Ginestar and Castel (1996) and Romero and colleagues (2006) report that in citrus plants under Mediterranean climate conditions, lack of water is a limiting factor for fruit growth and production. Maotani and co-workers [29] observed that in 'Satsuma' mandarins fruit and trunk growth were more sensitive to stress than in other parts of the plant.

In this 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 up to 90% in relation to the control plants (Figure 2C). In addition, it is important to note that, in case of fruit trees, it is important to observe that there is a significant decrease in plants productivity under water deficit. In this experiment, although the water deficit was applied from the beginning of plants vegetative phase, the results were similar, being observed reductions in fruit production in the 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 [30]. However, those plants were irrigated with 100% of crop evapotranspiration after 'june drop' phase until stage of rapid fruit growth. It can be concluded that a controlled water deficit can improve water use efficiency.

Other parameters related to plant water status such as trunk sap flow, stem diameter shrinkage or reduction [31]. In this context, leaf temperature is another indicator of water stress in plants since transpiration aids leaf cooling. With water content decrease in soil, the transpiration decreases and the leaf temperature increases in relation to the air temperature [32]. Generally, plants submitted to water deficit in the soil present an increase in leaf temperature and, as stress signs, stomatal closure is also observed in order not to lose water due to transpiration [33]. This factor could, when combined with other parameters, demonstrate plant water status as well as a potential indicator of irrigation time 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. Plant height and trunk diameter of 'Navelate' orange plants, evaluated monthly from March 2014 until February 2015.

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

The variables presented in Table 2 correspond to the water deficit intensities studied and the variations in plant height and stem diameter are recommended as indicators of citrus water status given their sensitivity to the substrate moisture variation [34]. As a response to water stress, the plant development is inhibited, the first symptoms begin to appear, like: leaf withering, water potential reduction, stomatal conductance decrease, CO₂ assimilation reduction and hydraulic conductivity of the root depletion [35]. Water stress affects the plant's phenological stages in different ways and intensities, affecting flower formation, fertilization and even fruit abscission, besides causing plant growth and development reductions [36].

The water extracted through the root system is transported by the xylem, being distributed through different plant parts until reaching the atmosphere through the transpiration process that occurs in

response to the energy gradient provided by solar radiation [32]. In fact, plant growth and development is influenced by environmental factors and water availability since it plays a vital role because it intervenes in practically all physiological processes.

Finally, a global climate change can be resulted in adverse conditions to plant growth. Moreover, under such conditions they should acclimate and adapt to survive. Resistance to water deficit in citrus plants, for example, is based on tolerance (osmotic adjustment) and prevention (stomatal control) to water stress, and the stomatal control is very developed in this genus [22, 28].

4. CONCLUSION

Orange plants exposed to the water deficit were tolerant in the initial treatments phase and during the vegetative phase, being more sensitive in the reproductive period. Water stress caused alterations in physiological and phenological responses of 'Navelate' orange plants, especially those submitted to stress with 25% of field capacity, which presented limitations compared to plants under full water availability. Deficient irrigation showed no changes in water use efficiency (normal and intrinsic), which means a good parameter of adaptation for stress condition. Water stress, at any level, reduced both plant growth and fruit production. Therefore, due to variations in the physiological and phenological responses among the trataments, further studies should be performed on these variables to search for water deficit tolerant varieties and production quality fruits under these conditions.

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