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Effect of deficit irrigation on *Helianthus annuus* L. plants in containers

Efecto del riego deficitario en plantas de *Helianthus annuus* L. en contenedores

Efeito da irrigação deficitária nas plantas de *Helianthus annuus* L. em contentores

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Abstract

In order to evaluate changes related to plant physiology, infrared thermography has been chosen as a non-invasive complement. The research objective was to analyze the effect of deficit irrigation on *Helianthus annuus* L. plants in containers by means of IR thermography in a controlled experimental population at the University of Seville. The experiment consisted of three irrigation treatments to sunflower plants; one treatment received full irrigation (C-100) and two treatments received deficit irrigation: 70 % (R-70) and 50 % (R-50). A randomized block design was used. In the initial stage, polystyrene seedling trays of 54 cells (square) were used for the cultivation of sunflowers. The dimension of the trays was 700 x 400 x 70 mm. The cell size was 65 x 70 mm and the capacity was 135 cm^3. For the development stage, plastic pots with a capacity of 4 L and a dimension of 21 x 16.4 cm were used. The sunflower plants used in this study did not exhibit significant differences in temperature and physiological analyses as a function of the irrigation treatment applied. However, there was a strong tendency for the plants to better resist water stress under a restrictive irrigation of 70 %.

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Resumen

Para evaluar los cambios relacionados con la fisiología de la planta, se ha elegido la termografía infrarroja como complemento no invasivo. El objetivo de la investigación fue analizar el efecto del riego deficitario en plantas de *Helianthus annuus* L. en contenedores mediante termografía IR en una población experimental controlada en la Universidad de Sevilla. El experimento consistió en tres tratamientos de riego a plantas de girasol; un tratamiento recibió riego completo (C-100) y dos tratamientos recibieron riego deficitario: 70 % (R-70) y 50 % (R-50). Se utilizó un diseño de bloques al azar. En la etapa inicial, se utilizaron bandejas de poliestireno de 54 celdas (cuadradas) para el cultivo del girasol. La dimensión de las bandejas era de 700 x 400 x 70 mm. El tamaño de las celdas era de 65 x 70 mm y la capacidad de 135 cm^3. Para la fase de desarrollo se utilizaron macetas de plástico con una capacidad de 4 L y una dimensión de 21 x 16,4 cm. Las plantas de girasol utilizadas en este estudio no mostraron diferencias significativas en temperatura y análisis fisiológicos en función del tratamiento de riego aplicado. Sin embargo, se observó una fuerte tendencia de las plantas a resistir mejor el estrés hídrico bajo un riego restrictivo del 70 %.

Palabras clave: estrés hídrico, termografía, fisiología de las plantas.

Resumo

A fim de avaliar as alterações relacionadas com a fisiologia vegetal, a termografia infravermelha foi escolhida como um complemento não invasivo. O objectivo da investigação foi Analisar o efeito da irrigação deficitária nas plantas de *Helianthus annuus* L. em recipientes utilizando termografia IR numa população experimental controlada na Universidade de Sevilha. A experiência consistiu em três tratamentos de irrigação a plantas de girassol; um tratamento recebeu irrigação completa (C-100) e dois tratamentos receberam irrigação deficitária: 70 % (R-70) e 50 % (R-50). Foi utilizado um desenho de blocos aleatórios. Na fase inicial, foram utilizados tabuleiros de poliestireno de 54 células (quadrado) para o cultivo de girassóis. A dimensão dos tabuleiros era de 700 x 400 x 70 mm. A dimensão da célula era de 65 x 70 mm e a capacidade era de 135 cm^3. Para a fase de desenvolvimento, foram utilizados vasos de plástico com uma capacidade de 4 L e uma dimensão de 21 x 16,4 cm. As plantas de girassol utilizadas neste estudo não apresentaram diferenças significativas de temperatura e análises fisiológicas em função do tratamento de irrigação aplicado. No entanto, houve uma forte tendência para as plantas resistirem melhor ao stress hídrico sob uma irrigação restritiva de 70 %.

Palavras-chave: estresse hídrico, termografía, fisiologia vegetal.

Introduction

To evaluate changes related to plant physiology, infrared thermography has been chosen as a non-invasive complement, because temperature is an important environmental parameter (Tattersall, 2016). Being important to employ this system as it allows timely detection of diseases, dehydration, in plants (Yang *et al*., 2019); therefore, infrared thermography can be widely applied in the non-invasive examination of seed vigor and allows fast and efficient seed detection for agricultural and silvicultural purposes in the future (Liu *et al*., 2020), in this sense, thermography has provided great advances in field applications and agriculture.

Due to what has been raised, surface temperature measurement through thermographic cameras has been widely used in various fields in recent years (Budzier and Gerlach, 2018). Among the various benefits provided by thermography, its easy handling at the time of capturing the thermal image stands out, as well as its wide spatial resolution in one or several objects simultaneously (Harrap *et al*., 2018), this involves detecting plant water stress in time, a situation that helps to optimize irrigation with the intention of enhancing the plant to an optimal production, this in the medium term, leads to the economic performance of the plantation, as well as sustainability until the harvest is achieved (Al Aasmi *et al*. 2022).

In agreement, Shehzad *et al*. (2020), indicates that drought stress is one of the extreme effects of climate change that causes great losses in crop plant production, applying for sunflower plants, foliar spray K + Ct, (spray with chitosan nanoparticle properties to control stress in plants), helping to significantly improve the gas exchange characteristics of the leaf, which increased proline, soluble proteins and free amino acids and increased the antioxidant defense system, helping to maintain the water status of the plant in sunflowers exposed to drought stress, being functional in this case, to apply IR thermography for the early detection of water stress in sunflowers with the intention of provoking a timely intervention where intervening treatments can be applied for the repair of the plant.

It is important to note that water stress decreases growth rates, photosynthetic pigments, osmoprotectants, yield components, oil and carbohydrates (%) compared to 100 % irrigation requirements (Abdallah *et al*. 2020). Being that the use of IR thermography contributes to obtain accurate information on plant biology during the process of water stress, being an important tool to know the impact and possible consequences, being highly recommended its use, especially in populations under controlled environments (Harrap and Rands, 2021).

Based on the above, the research objective is to analyze the effect of deficit irrigation on *Helianthus annuus* L. plants in containers by means of IR thermography in a controlled experimental population at the University of Seville.

Materials and method

Experimental design

In 2020, an experiment was carried out under controlled conditions in the plant physiology laboratory of the Faculty of Biology of the University of Seville, Spain. The experiment consisted of three irrigation treatments to sunflower plants; one treatment received full irrigation (C-100) and two treatments received deficit irrigation: 70 % (R-70) and 50 % (R-50). A randomized block design was used.

Experiment procedure

Sunflower seeds (*Helianthus annuus* L.) of the dwarf variety were planted under controlled conditions of humidity and temperature. The culture chamber had an initial temperature of 16.02 ºC during the first week, when planting took place. In the following weeks it was maintained at an average temperature of 24 ºC with slight variations of ± 2 ºC. The average relative humidity was between 73 % - 84 %.

Peat was used as substrate for the cultivation of sunflowers, due to its chemical characteristics such as nutrient content, cation exchange capacity (CEC), pH, electrical conductivity (EC) and C/N ratio. Considering the importance of maintaining EC and pH in optimal ranges to avoid low water potential, which could result in water loss of the plant. The content of salts presents in the peat used was ≤ 2 $dS.m^{-1}$ and pH of 6.8 respectively.

In the initial stage, polystyrene seedling trays of 54 cells (square) were used for the cultivation of sunflowers. The dimension of the trays was 700 x 400 x 70 mm. The cell size was 65 x 70 mm and the capacity was 135 cm^3. For the development stage, plastic pots with a capacity of 4 L and a dimension of 21 x 16.4 cm were used.

The dry mass in grams of stems and leaves of the three treatments was obtained, for which the fresh weight (FW) of the sample leaves was recorded and then the leaves were immersed for 1h in distilled water. The leaves were removed, and excess water was removed, and the turgent weight (WW) was recorded. The samples were placed in an oven at 70 ºC for 48 h to obtain the dry weight (DW). The same procedure was used to calculate the values obtained in the treatments, C-100 showed root dry mass values higher than R-70 but lower than R-50.

According to Córdova-Téllez, (2018) between 60,000 and 70,000 seeds can be sown in one hectare, with a loss percentage of 10 %. Due to the above, the water consumption was established based on the water need of 55,000 $L.ha^{-1}$ considered 50,000 plants in 10,000 m^2. The water demand per plant was established based on the calculated field capacity of 11 L in a complete cycle.

The initial seedling stage in the seedbed lasted 28 days, until a minimum of three true leaves were obtained. Once the sunflowers were transplanted to the pot, the development stage lasted 35 days, where the plants acquired more than 8 unfolded leaves. In the intermediate stage of 24 days, the inflorescence was produced and 26 days lasted the final stage where black seeds were evidenced inside the flowers. At the beginning of the experimental phase the sunflowers were 85 days old and once concluded 113 days.

The photosynthetic activity (A) was measured through the quantification of CO_2 and atmospheric water vapor using an open-circuit infrared gas analyzer (IRGA) and to calculate the water use efficiency (WUE), the photosynthesis values obtained per treatment and the stomatal conductance data (gs) were used in the formula: WUE $A.gs^{-1}$

Five plants were used for each experimental unit, for a total of 15 plants in the experiment. The selected sunflowers had between 9-12 leaves of similar shape and size. From the experimental phase onwards, irrigation was programmed every three days. The sunflowers were supplied with water and fertilizer using a watering can. The volume supplied was 300 mL for full irrigation (R-100). Deficit irrigation of 70 % to 210 mL of water per sunflower and deficit irrigation of 50 % to 150 mL respectively.

Allen *et al*. (1998), established the Kc of sunflower at 0.35 in the initial stage. With the development of the plant, the Kc increases until it reaches a value of 1.15. After some time, the crop ages and withers, producing a decrease in Kc to 0.35.

Viridis N30 mixed nitrogen fertilizer was used. With a formula characterized by its high content of nitrogen, fulvic acids and microelements that benefits plant growth. With a pH of 7.5 according to the manufacturer's recommendations, 30 mL of fertilizer were used for every 10 L of water (table 1).

Table 1. Fertilizers used for evaluation of effect of deficit irrigation on *Helianthus annuus* L. plants in containers.

Viridis N30	% weight.weight^{-1}	% weight.volume^{-1}
Total nitrogen	24.00	29.56
Ammonia nitrogen	6.00	7.31
Nitric nitrogen	5.00	6.27
Urea	13.5	14.9
Also contains: Boron (B); Copper (Cu); Manganese (Mn); Molybdenum (Mo) and Zinc (Zn).	≤ 0.1%	
pH	7.8	

Note: Fertilizers used in % weight and volume during the experiment.

Thermal Imaging

Thermographic images were recorded per individual of each treatment, using a portable infrared thermal camera that was incorporated into a Samsung Tablet model Galaxy Tab A (2019, 10.1"). The camera model used was FLIR ONE (Flir Systems, Wilsonville, OR, USA). With resolution of 80 x 60 pixels, whose spectral range is 8-14 µm. The temperature range measured by the camera is -20 °C to 120 °C with a thermal sensitivity of 150 mK and frame rate of 8.7 Hz. The thermal images were taken between 11:00 - 13:00 and were examined using the free software package "Flir One" and then the data was analyzed by means of the Flir Tools application for smartphones with Android operating system.

For temperature calculation, the emissivity was set to 1.0 based on the radiation temperature of a crumpled aluminum foil sheet. The emissivity for the sunflower canopy was set to 0.95. The distance between the camera and the target was less than 1 meter. Three areas of different circumference were selected within each thermal image to determine the average temperature per leaf. The selected areas were exported to Excel to calculate the average temperature per treatment, as well as the standard error of the data. The sampling days were February 06, 12, 14, 18 and 27. The thermal images were taken under the same conditions, except for February 12, when the images were obtained prior to irrigation.

Statistical analysis

SPSS Statistics version 25.0 (IBM Corp., 2017) was used to calculate the statistical analysis. They were processed by statistical design of comparison of means, by application of Duncan's method at a margin of error of ($p<0.05$), which allowed comparison of all pairs of means used in the experiment, supported by an ANOVA analysis, after a review of the normal distribution of the data series and homogeneous variance.

Results and discussion

From the temperatures obtained by thermography, no significant differences ($p=0.054$) were found between the different treatments by means of the analysis of variance. However, there was a tendency to increase the temperature from the first measurement in the treatments subjected to deficit irrigation. To which Iseki and Olaleye, (2020), point out that through thermal imaging it is possible to determine canopy and leaf surface temperatures.

Likewise, recent studies show that the plant hormone abscisic acid (ABA) plays a central role in the regulation of stomatal movements under water deficit conditions (Hsu *et al*. 2021), ABA is actively synthesized in the vascular tissues of roots and leaves and transported to the protective cells. Consequently, when there is water stress, stomatal closure occurs in the sunflower leaf, which generates an increase in its temperature, in addition to the fact that plants detect the water deficit signal mainly through the leaves and roots. In consideration, figure 1 is presented, where the leaf temperature obtained by IR thermography is identified. Mean temperature per treatment per day on the x axis given to the measurement periods.

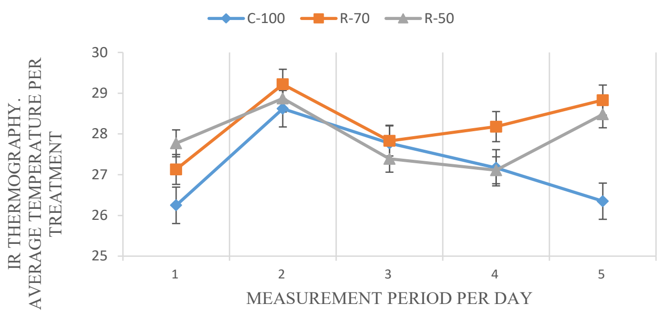

Figure 1. Leaf temperature obtained by IR thermography. C-100: full irrigation (100%), R-70: 70 % of irrigation, R-50: 50 % irrigation. Irrigation was carried out every three days. The sampling days were in February 1:06, 2:12, 3:14, 4:18 and 5:27.

The R-70 treatment, despite not being the most restrictive irrigation, leads to a greater increase in leaf temperature on all days sampled. This could indicate the tendency of sunflowers to regulate water loss by closing the stomata for a deficit irrigation of 70 % and resist water stress, which is similar to the study conducted by Mostafa and Afify, (2022), where it was shown that sunflower plants are resistant and productive in the presence of an intermediate irrigation. The plants that were subjected to the R-50 treatment presented temperatures between 1 and 2 ºC below the R-70 treatment, but despite this difference, two of the five plants died, which could indicate the low tolerance of sunflowers to a restrictive irrigation of 50 %.

Jones (2004), points out that, in the plant, the increase or decrease of temperature are not limiting for biochemical interactions (around the optimum temperature), but can become determinant under severe restrictive conditions, although they are reflected in small variations of temperature.

At the beginning of the experimental phase, the sunflower plants were robust, with an intense green color in leaves and stem, with a length of more than 40 cm. They had several leaves of appropriate shape and size. Some plants had small flowers, the first temperature data taken from the beginning of the experimental phase, under the conditions mentioned above. Almost at the end of the experimental phase, the plants had lost their vigor, which could be described as the characteristic intense green color of leaves and stems. Leaf spots and necrotic areas on the edges of the leaves were clearly visible in the treatments subjected to deficit irrigation.

Andrade *et al*. (2017), it is always related stomatal conductance to leaf water status. As well as the opening and closing of the stomata are regulated through the integration of environmental signals and stimuli.

In this research, no significant differences were obtained for photosynthesis among the different treatments (p=0.59). As leaf temperature increased with deficit irrigation in R-70 and R-50, so did photosynthesis values, which may have been due to reductions in CO_2 solubility. The above is presented in figure 2.

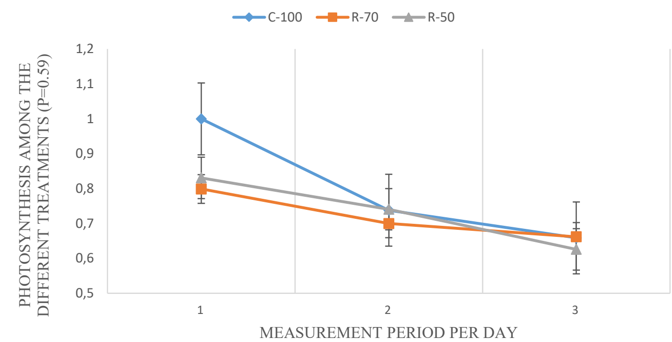

Figure 2. Differences were obtained for photosynthesis among the different treatments. C-100: full irrigation (100%), R-70: 70 % of irrigation, R-50: 50 % irrigation. Irrigation was carried out every three days. The sampling days were in February 1:06, 2:12 and 3:14. 5:27.

View complemented by the study of Killi *et al*. (2017), who reported similar affectation in photosynthesis and growth of sunflowers directly by water deficit and temperature. Although temperatures were higher at R-70 photosynthesis is higher at R-50. A peak is observed in the data for the third measurement and then a steep drop for deficit irrigation (both cases), with time as water stress develops it becomes more accentuated. This may have contributed to the degradation of physiology in the sunflower plants of the R-50 treatment.

Water use efficiency for this experiment was not statistically significant (p=0.53). WUE was higher for the R-70 treatment, which may be associated with the high temperatures exhibited by this deficit irrigation, possibly due to osmotic adjustment, as sunflower are consistent with an ABA-mediated hormonal stomatal response to vapour pressure deficit (VPD) rather than a hydraulically driven stomatal response to VPD (Cardoso et al. 2020). The above is presented in figure 3.

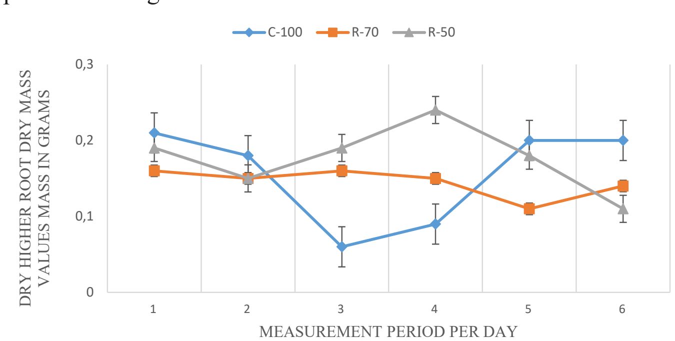

Figure 3. Water efficiency. C-100: full irrigation (100%), R-70: 70 % Irrigation was carried out every three days. The sampling days were in February 1:06, 2:12 and 3:14.

Generating the best utilization of water content by plants exposed to water stress under the R-70 treatment. Although the values fall in the fourth sampling (R-70), they return to be the highest at the end of the experiment. WUE for C-100 from the second data collection is reported below the two deficit irrigation treatments, which may have been influenced by external factors such as the location of the plants in the growth chamber. Both WUE and biomass of the R-70 treatment were the highest recorded, which relates to the research of Killi *et al*. (2017), where he points out that WUE is associated with biomass production in sunflowers and can be seen to influence it directly.

Once the experiment was concluded, the dry mass in grams of stems and leaves of the three treatments was obtained. In the statistical analysis, there were no significant differences (p=0.971) in the root biomass of the different irrigations applied. It is possible that the number of individuals per treatment may have caused non-determinant results. The above is presented in figure 4.

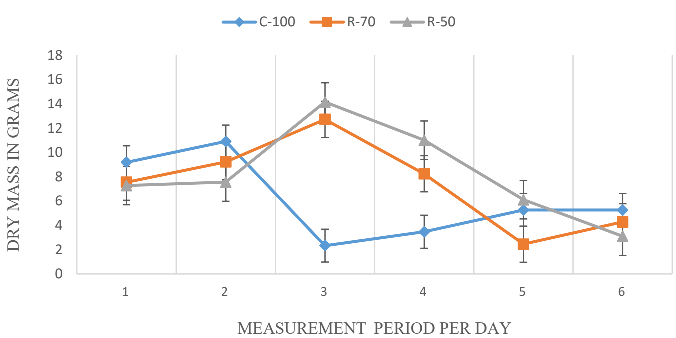

Figure 4. Dry mass in grams of stems and leaves of the three treatments. C-100: full irrigation (100%), R-70: 70 % Irrigation was carried out every three days. The sampling days were in February 1:06, 2:12, 3:14, 4:18 and 5:27.

Of the values obtained in the treatments, C-100 presented higher root dry mass values than R-70 but lower than R-50. According to Killi *et al*. (2017), sunflowers under water stress present an increased investment in root systems, propitiating a greater use of available soil water. What could have generated the increase of the root system in R-50 due to the high deficit irrigation to which it had been subjected. A more extensive root network can cause plants to make better use of the available water. The above is presented in figure 5.

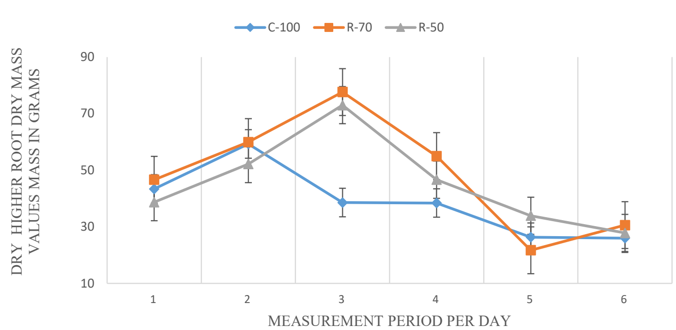

Figure 5. The treatments, C-100 presented higher root dry mass values than R-70 but lower than R-50. C-100: full irrigation (100%), R-70: 70 % Irrigation was carried out every three days. The sampling days were in February 1:06, 2:12, 3:14, 4:18 and 5:27.

Therefore, periods of drought will have a negative impact on the growth of sunflower crops, being that García-Tejero *et al*. (2017), reported relationships between thermal indicators and physiological parameters in plants. What would be correlated in our experiment by manifesting elevated temperatures in sunflower plants and physiological parameters reported in plants. While R-70 had the highest values in stem biomass, followed by C-100 and finally R-50. Differences in stem biomass were not significant (p=0.745) for the three treatments.

Conclusions

The temperatures obtained by thermography; no significant differences (p=0.054) were found between the different treatments by means of the analysis of variance. However, there was a tendency to increase the temperature from the first measurement in the treatments subjected to deficit irrigation. The R-70 treatment, despite not being the most restrictive irrigation, leads to a greater increase in leaf temperature on all days sampled. This could indicate the tendency of sunflowers to regulate water loss by closing the stomata for a deficit irrigation of 70 % and resist water stress.

Generating the best use of water content by plants exposed to water stress under the R-70 treatment, sunflowers under water stress show an increased investment in root systems, leading to a greater use of available soil water. This may have led to an increase in the root system in R-50 due to the high deficit irrigation to which it had been subjected. A more extensive root network may cause the plants to make better use of the available water.

Having an effect that R-70 had the highest values in stem biomass, followed by C-100 and finally R-50. The differences in stem biomass were not significant (p=0.745) for the three treatments.

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