



Article

Effects of Light Intensity and Water Stress on Growth, Photosynthetic Characteristics and Plant Survival of *Cistus heterophyllus* Desf. Subsp. *carthaginensis* (Pau) M. B. Crespo & Mateo

María José Gómez-Bellot ¹, María Jesús Sánchez-Blanco ^{1,*}, Beatriz Lorente ¹, María José Vicente-Colomer ² and María Fernanda Ortuño ¹

¹ Department of Irrigation, Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), P.O. Box 164, 30100 Espinardo-Murcia, Murcia, Spain; mjgb@cebas.csic.es (M.J.G.-B.); blorente@cebas.csic.es (B.L.); mfortuno@cebas.csic.es (M.F.O.)

² Department of Agricultural Engineering, UPCT—Technical University of Cartagena, 10, 30203 Cartagena, Bolivar, Spain; maria.vicente@upct.es

* Correspondence: quechu@cebas.csic.es

Abstract: The objective of this study was to identify the physiological mechanisms used by *Cistus heterophyllus* Desf. Subsp. *carthaginensis* (Pau) M. B. Crespo & Mateo, a species in critical danger of extinction, to cope with two typical abiotic conditions. During the summer of 2021, plants were cultivated under a shade mesh that intercepted 50% of the incident solar radiation (SHADE) and in natural conditions (SUN). Three irrigation treatments were also applied: control, T1, moderate water deficit; T2, 60% of the control, severe deficit; and T3, 30% of the control. Therefore, there were six treatments (SUN-T1, SUN-T2, SUN-T3, SHADE-T1, SHADE-T2, SHADE-T3). Plants were more affected by solar radiation than by deficit irrigation. Although leaf water potential, stomatal conductance and root biomass decreased by up to 45%, 63% and 65%, respectively, as deficit irrigation increased, plants were able to develop a leaf osmotic adjustment and an improved intrinsic water-use efficiency to maintain their growth and survival rate. Shade conditions improved gas exchange, reduced leaf temperature and induced the synthesis of chlorophylls, regardless of the irrigation level imposed. This indicated that radiation was the most limiting factor in our experiment. Applying 50% of the radiation and a moderate water deficit would help to obtain a good plant development and high survival rate in future recovery and conservation programs for the species.

Keywords: biomass; gas exchange; leaf mineral content; Mediterranean shrub; osmotic adjustment; shading; soil water deficit; species conservation; water relations



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1. Introduction

Climate change is affecting Mediterranean regions with extreme weather events such as prolonged droughts. These conditions can affect the ability of plants to grow and survive, even for species that are adapted to these regions; as a consequence, the risk of extinction for about 20% of plant species could increase by up to 39% [1–3]. In this sense, the Mediterranean basin ranks third in the world in terms of plant biodiversity and is one of the most important areas on Earth for endemic plants. Human activity such as overpopulation, intensive agriculture and deforestation is, fundamentally and irreversibly, responsible for the loss of vegetal biodiversity in the Mediterranean region. In addition, climate change, pollution and the introduction of the intentional or indirect control of invasive species contribute to a large extent to the reduction, degradation and loss of plant biodiversity. In particular, climate change is expected to increase the impact that a change in land use has on plant biodiversity, since it reduces the availability of areas with suitable

environmental conditions for species, among those that have not yet been converted to human uses [4].

The plant species *Cistus heterophyllus* Desf. Subsp. *carthaginensis* (Pau) M. B. Crespo & Mateo, also known as Jara of Cartagena, is an endemic plant of the region of Murcia that is currently in critical danger of extinction according to the IUCN. It was cited for the first time at the beginning of the 20th century in the Sierra Minera of Cartagena. At this time, it was a very common species. However, episodes such as fires and mining activity in the area, among others, reduced the population. In 1973 it was even considered extinct. According to the latest known data, they are distributed into two groups; only two small spots were located in Europe these being a population of one self-incompatible specimen which was found in Valencia and another small population found in Cartagena, disturbed by human impact and hybridization with close relatives. Recently, Cardona and Capó [5] discovered a new population of this taxon located at the isle of Cabrera with 59 individuals of *C. heterophyllus*.

The small number of wild specimens of the Cartagena rockrose in the Iberian Peninsula has made it necessary to carry out studies aimed at learning the ecological preferences, ecophysiological traits, reproductive viability and survival of the plant throughout its development. These data are essential to create an effective recovery plan that contains both in situ and ex situ measures to be able to reinsert the plant in its natural environment [6,7]. Several genetic relationships of the populations have been carried out which show that the three populations of the Spanish territory seem to come from different colonisations from the African continent, the population of Cartagena being the one that shows the greatest genetic uniqueness. Most of the research is focused on promoting knowledge of the genetics of the species and its application in the recovery of populations, particularly due to hybridization problems and possible genetic poverty [7–10]. There have also been some studies on germination and propagation in the nursery with different results, in which the survival rate of plants showed a wide spectrum [11–13]. However, no attention has been paid to other factors, such as environmental factors, which may endanger the growth and establishment of this species, its water needs and the threshold above which these factors can even cause its death. In this sense, the adverse environmental conditions that occurred during summer in the region of Murcia (high temperatures, intense solar radiation, low rainfall, etc.), together with the lack of an optimal irrigation protocol, hinder the growth and development of *Cistus heterophyllus*, showing very low survival rates. According to Navarro-Cano and Robles [14], the success of its reintroduction depends on the combination of factors such as temperature, water availability and the existence of fires. Water stress can lead to reduced plant growth, leaf wilting and even death in severe cases. Plants respond to water stress by reducing their stomatal openings to conserve water, which can also reduce their ability to photosynthesize and grow [15–17]. Since the increased frequency of drought negatively affects plant growth and development [18], analysing the effects of water deficit on plants is important to hypothesize the influence that future climate change will have on the growth of a particular plant species [19].

On the other hand, the species seem to have a better growth and development in places with a Mediterranean thermotype, in those areas there is an abundance of other tree species, such as *Pinus halepensis*, that provide shade to the population of this species. In this sense, sun and shade plants can differ in their relative composition of photosynthetic pigments, electron carriers, their chloroplast ultrastructure and their photosynthetic rates [20]. Leaf and chloroplast adaptation to either high or low irradiance or to direct sun-light or shade proceed during leaf development and comprise special morphological and biochemical adaptations [21].

Based on the above, the main objective of this study was (1) to evaluate how the different levels of water applied affected the water status of *Cistus* plants under different solar radiation conditions; (2) to identify the morphological and physiological mechanisms used by *Cistus heterophyllus* to cope with two adverse conditions typical of the Mediterranean climate (water deficit and solar radiation) during the summer period; and (3) to determine the most limiting abiotic factor (water deficit and solar radiation) that reduces plant growth

and survival rate. For this purpose, leaf water relations, photosynthetic capacity, leaf temperature, leaf mineral nutrition, biomass and survival rate were evaluated. All this was carried out in order to facilitate the production and ex situ conservation of this threatened species and to allow the establishment of long-term maintenance protocols for plants in nurseries.

2. Materials and Methods

2.1. Plant Material and Experiment Conditions

The experiment was carried out on the CEBAS experimental farm (Santomera, Murcia, Spain). Plants of *Cistus heterophyllus* (n = 360) from the nursery 'El Valle' (Murcia) were transplanted into square black pots (3.5 L) on 24 March 2021. The substrate used was a mixture of blonde peat, a small proportion of black peat and other alternative and sustainable raw materials (recipe 31562-ES Topf, Gramoflor, Valencia, Spain) with corrected pH (5.8) and bottom fertilization with NPK and microelements. The irrigation water came from the Tajo-Segura transfer and was applied to a trough drip irrigation system with a lateral pipe per row of plants. Each pot had a 2.2 L h⁻¹ emitter to favour the moistening of the substrate according to the treatment applied. The amount of water used was controlled weekly both by online water meters and gravimetrically (Table 1). The plants were fertilized manually once by applying a fertilizer with Osmocote Plus (14:13:13 N, P, K plus microelements) after transplantation.

Table 1. Quantity of water applied during the experiment.

Irrigation Treatments	L Week ⁻¹ Plant ⁻¹			
	24 March	1 July	20 Sept	29 Sept
T1	1.02	2.05	1.54	1.02
T2	1.02	1.17	0.88	0.59
T3	1.02	0.59	0.44	0.29

2.2. Treatments and Experimental Design

After the transplant, half of the plants (n = 180) were placed on three cultivation tables (60 plants/table) under a shade house with a metal structure (2.8 m high and 9 m × 3 m at the base) completely covered with a shading mesh. The mesh was made by a polyethylene grey net of 80 g m⁻² and strand diameter of 0.28 mm, which intercepted 50% of the incident solar radiation (SHADE treatment). The other half of the plants were distributed on cultivation tables, but in natural conditions of solar radiation (SUN treatment).

On 1 July 2021, three irrigation treatments were applied in plants from both shady and sunny conditions using different water supplies: T1, plants irrigated every day for 4 min, 2 times/day (approx. 2.00 L/week); T2, plants watered 4 days/week for 4 min, 2 times/day (approx. 1.20 L/week); T3, plants irrigated 2 days/week for 4 min, 2 times/day (approx. 0.60 L/week). Therefore, there were a total of 6 treatments (60 plants/treatment) derived from the combination of the two factors: irrigation regime and solar radiation (SUN-T1, SUN-T2, SUN-T3, SHADE-T1, SHADE-T2, SHADE-T3) in the experimental plot. These treatments were maintained for 11 weeks approximately, until 20 September 2021. The amount of water applied to plants from T1 (control treatment) was estimated using online water meters and gravimetrically by weighing the pots, using a balance (Analytical Sartorius, Model 5201, Gottingen, Germany; capacity 5.2 kg and accuracy of 0.01 g).

The climatic data inside the shade house were obtained by using a temperature and relative humidity sensor, Hobo H8 Logger (Onset Computer Corporation, Bourne, MA, USA) and a PAR sensor (Ha-Li, LI-COR, Lincoln, USA), while the climatic conditions outside were obtained from the data from the automatic meteorological station of the experimental farm (Figure 1).

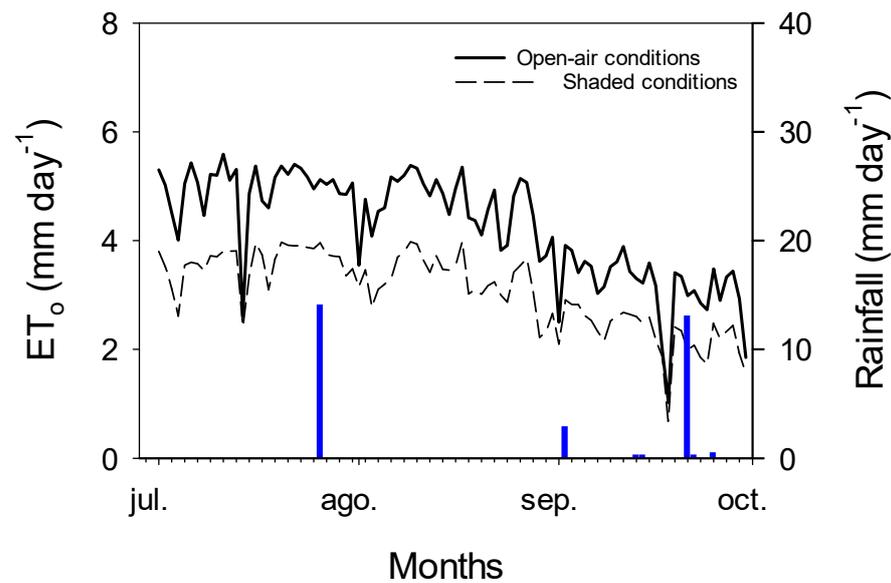


Figure 1. Daily reference crop evapotranspiration (ET_0) in open-air and shaded conditions, and rainfall (blue bars).

2.3. Water Relations Measurements

Leaf water relations were measured at midday every 2 weeks in six plants per treatment (two plants per replication). Leaf water potential (Ψ_{Leaf}) was measured collecting a mature leaf according to Scholander et al. [22] using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA). Leaves were placed in the chamber within 20 s of collection and pressurized at a rate of 0.02 MPa s^{-1} . Adjacent leaves were also collected, frozen immediately in liquid nitrogen ($-196 \text{ }^\circ\text{C}$) and subsequently stored at $-30 \text{ }^\circ\text{C}$. After thawing, the leaf osmotic potential (Ψ_s) was measured in the extracted sap using a WESCOR 5520 vapor pressure osmometer (Wescor Inc., Logan, UT, USA) according to Gucci et al. [23]. The leaf osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_s , and then was placed in distilled water overnight to reach full saturation.

2.4. Gas Exchange Measurements

The leaf photosynthetic rate (P_n) and the stomatal conductance (g_s) were measured at the same time and in the same plants as leaf water relations were measured using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). The reference CO_2 , photosynthetically active radiation (PAR) and speed of the circulating air flow inside the system were set at 400 ppm, at $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and at $500 \mu\text{mol s}^{-1}$, respectively. The intrinsic water use efficiency (WUE_i) was determined as the P_n/g_s ratio.

2.5. Leaf Temperature Determinations

Infrared (IR) images were taken from the sunlit side of the canopy [24] at midday every 2 weeks, in six plants per irrigation treatment (two plants per replication). Thermal images from the canopy were obtained from a constant distance (d) of 0.5 m. The selected plants were the same on which water relations and stomatal conductance were immediately measured. Thermal images were obtained manually with a thermal imager (ThermaCam FLIR-e50 System, Inc., Danderyd, Sweden). The background temperature ($T_{\text{background}}$) was determined as the temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest with the emissivity set at 1.0 [24]. Emissivity for leaf measurements was set at 0.96 [25–27]. Thermal images were processed with ThermaCam Explorer software (FLIR Quick Report, FLIR Systems, Danderyd, Sweden). Canopy temperature (T_c) for each thermal image was obtained as the average of delimited portions of mature leaves.

2.6. Leaf Chlorophyll Content and Colour Parameters

The chlorophyll content was assayed three times during the experiment according to Inskeep and Bloom [28] in the leaves of six plants per treatment (two plants per replication). The extraction was made from 50 mg of fresh material in 5 mL of 80% dimethyl sulphoxide in the dark at 4 °C. The extract was read at 647 nm for chlorophyll-*a* and 664.5 nm for chlorophyll-*b* in an Uvikon 940 spectrophotometer (Kontron Instruments AG, Zürich, Switzerland).

Leaf colour was measured three times during the experiment in six plants per treatment (two plants per replication) with a Minolta CR-10 colorimeter, giving the colour coordinates lightness (L^*), chroma (C^*) and hue angle (h°) [29].

2.7. Leaf Mineral Content and Biomass Parameters and Survival Rate

At the end of the experiment, the inorganic mineral content of dry leaves was determined in six plants per treatment (two samples per replication) by means of emission spectrophotometry. The leaves were oven dried at 80 °C, ground, and sieved through a 2 mm nylon mesh before analysis. A chemical analysis of water irrigation treatments was performed. The nutrient concentrations were determined in an extract digested with $\text{HNO}_3:\text{HClO}_4$ (2:1, *v/v*) using an inductively coupled plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL, Thermo Fisher Scientific, Waltham, MA, USA).

At the end of the experiment, the substrate was gently washed from the roots of ten plants per treatment. The plants were divided into leaves, stems and roots. Then, they were oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW).

2.8. Survival Rate and Damaged Plants

At the end of the experiment the survival rate was also calculated as follows:

$$\text{Survival rate (\%)} = (\text{n}^\circ \text{ of alive plants} / \text{total n}^\circ \text{ of plants}) \times 100$$

The percentage of damaged plants (plant tissues with chlorosis or necrosis) was also determined as follows:

$$\text{Damage plants (\%)} = (\text{n}^\circ \text{ damaged plants} / \text{total n}^\circ \text{ of alive plants}) \times 100$$

2.9. Statistics

In the experiment, all plants ($n = 360$) were randomly assigned to each treatment, with three replications for each treatment. The data were analysed by one-way ANOVA and two-way ANOVA using IBM SPSS Statistics 25. The independent variables were irrigation water and radiation. Treatment means were separated with Duncan's multiple range test ($p \leq 0.05$).

In order to simplify results, data displayed belong to the beginning (Start, July), half (Mid, August) and end of the experiment (End, September) for most of the parameters.

3. Results

3.1. Water Relations

In general, leaf water potential decreased in plants subjected to deficit irrigation and in both radiation conditions, plants from treatments SUN-T3 and SHADE-T3 showed the lowest values at the end of the experiment (-1.57 MPa and -1.62 MPa, respectively), while plants from treatment SHADE-T1 showed the highest value (-0.9 MPa) (Figure 2A).

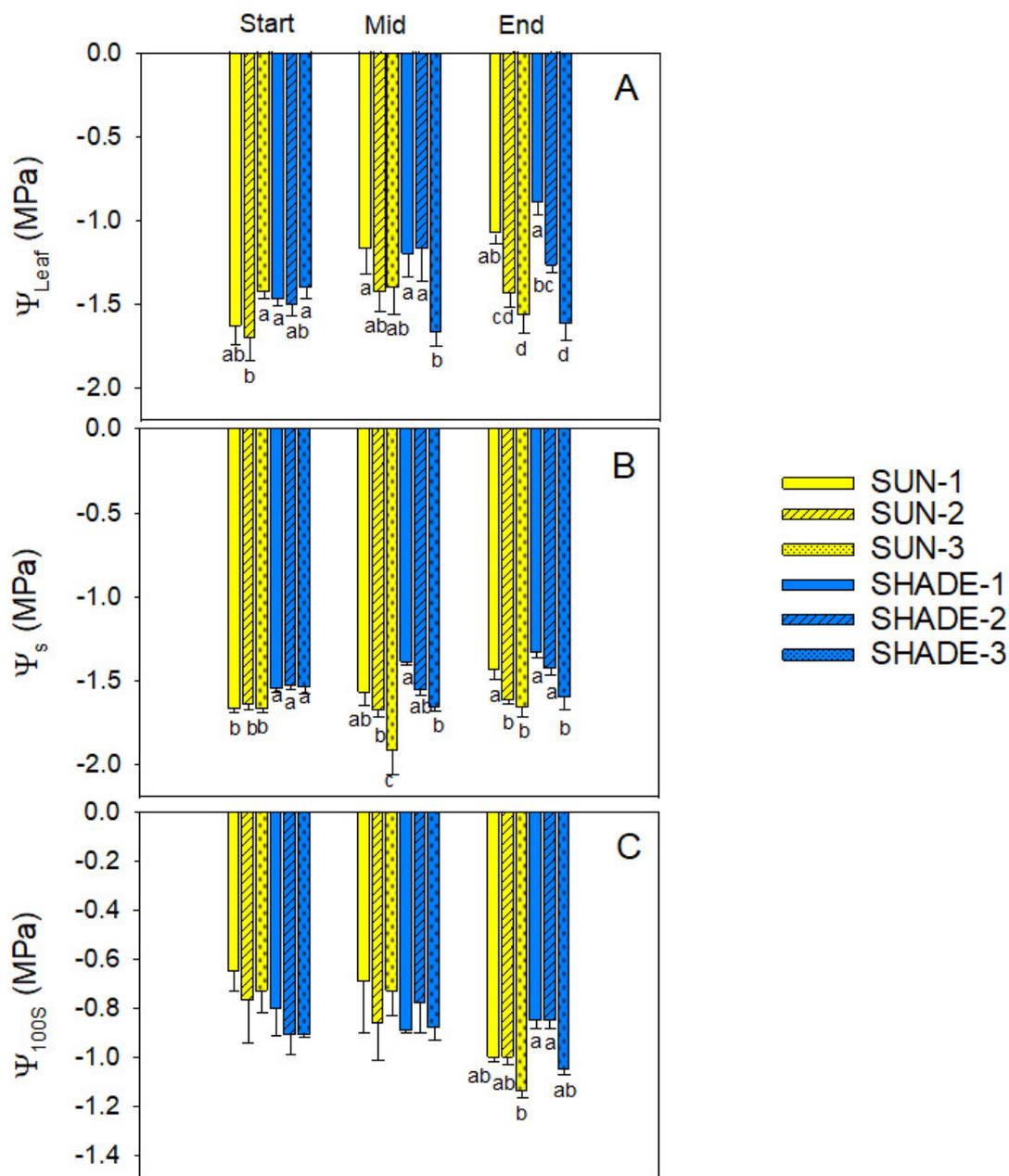


Figure 2. Leaf water potential (Ψ_{Leaf}) (A), osmotic water potential (Ψ_s) (B) and osmotic water potential at full turgor (Ψ_{100s}) (C) *C. heterophyllus* growing under shade and sunny conditions irrigated by three irrigation levels during the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

From half of the experiment, it was found that the actual osmotic potential in plants also decreased as the deficit irrigation applied increased, with plants from treatments SUN-T3 and SHADE-T3 reaching values of up to -1.66 MPa and -1.6 MPa, respectively, these being the most negative values at the end of the experiment (Figure 2B). In general, the leaf osmotic potential at full turgor showed that plants growing in full radiation conditions showed lower values than those growing under low radiation conditions (Figure 2C). At the end of the experiment, plants from SUN-T3 and SHADE-T3 showed the lowest values of Ψ_{100s} with a reduction of 14% and 23%, respectively, compared to those subjected to treatments SUN-T1 and SHADE-T1.

3.2. Gas Exchange

From half of the experiment, it was found that leaf stomatal conductance (g_s) values were reduced in plants growing under full radiation conditions as the level of deficit irrigation increased. Plants from the SUN-T3 treatment showed the lowest values, reaching values up to $45 \text{ mmol m}^{-2} \text{ s}^{-1}$. However, plants growing in shadow showed an unclear trend when deficit irrigation treatments were applied throughout the experiment (Figure 3A), although it can be observed that SHADE-T2 treatment presents the highest absolute values of g_s , reaching $178 \text{ mmol m}^{-2} \text{ s}^{-1}$ at the end of the experiment.

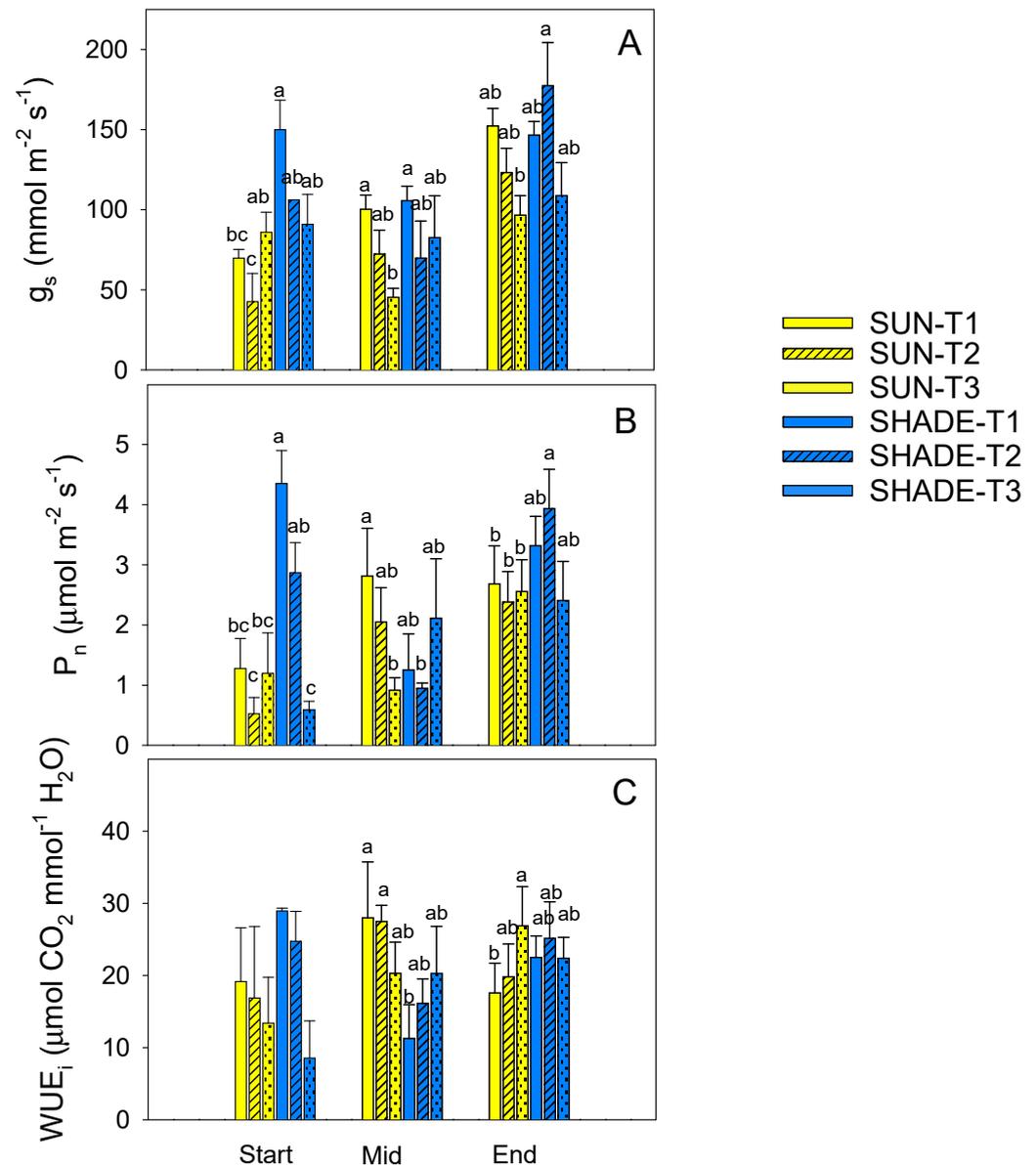


Figure 3. Stomatal conductance (g_s) (A), net photosynthetic rate (P_n) (B) and intrinsic water use efficiency (WUE_i) (C) of *C. heterophyllus* growing under shade and sunny conditions, irrigated by three irrigation levels during the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

Net photosynthetic rate (P_n) behaved similarly to g_s in all *Cistus* plants during the experiment, although at the end of the experiment plants growing at full radiation conditions reached similar P_n values (around $2.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$) regardless of the irrigation

level applied (Figure 3B). By the middle of experiment, the intrinsic efficiency of water use in plants growing in shade increased as water deficit irrigation increased, while at the end of the experiment, this behaviour was observed in plants growing at full radiation. Plants from treatment SUN-T3 showed the highest WUE_i values, with an increase of 52% compared to plants from treatment SUN-T1 (Figure 3C).

3.3. Leaf Temperature

From the middle of experiment, it was found that plants growing at full radiation showed higher leaf temperature than plants growing at 50% of radiation, regardless of the irrigation level (Figure 4). This increase was more evident at the end of the experiment when plants growing at full irrigation reached the highest values (around 32.6 °C) of leaf temperature in the whole experiment. Plants growing at 50% of radiation reached an average temperature of 30.7 °C.

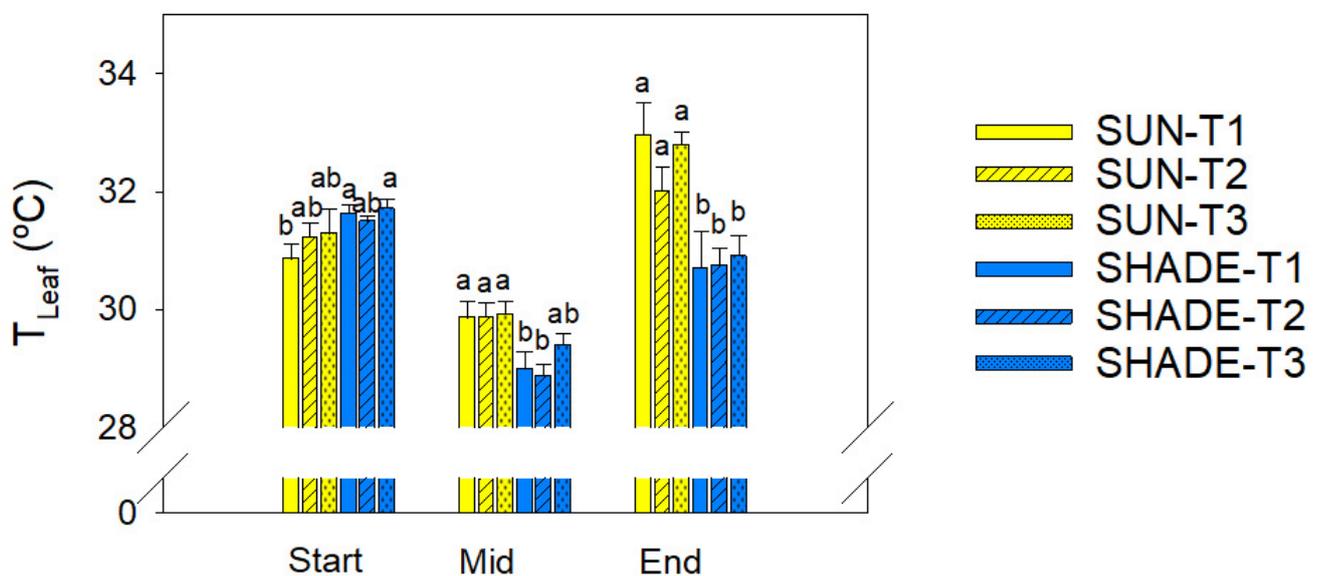


Figure 4. Leaf temperature of *C. heterophyllum* growing under shade and sunny conditions, irrigated by three irrigation levels during the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

3.4. Leaf Chlorophyll Content and Colour Parameters

From the beginning of the experiment, the leaf chlorophyll content (chl *a*, chl *b* and total chl) was higher in plants growing at 50% of the radiation than in plants growing at full radiation (Figure 5A–C). By the middle of experiment, the highest values of leaf chlorophyll content were found in plants from SHADE-T2, which showed twice the total chlorophyll content than plants from SUN-T1 and SUN-T2. At the end of the experiment, the highest values corresponded to plants from SHADE-T2 and SHADE-T3 (with values around 11 mg g⁻¹ of total chlorophyll content), while the lowest values corresponded to plants from SUN treatments regardless of the irrigation levels (with values around 6.5 mg g⁻¹ of total chlorophyll content (Figure 5A–C)).

At the end of the experiment, colour parameters showed no statistical differences in the chroma and luminosity of leaves between treatments (Figure 6A,B), while angle hue was approximately 7% higher in plants that were subjected to shade conditions, specifically in plants from treatments SHADE-T2 and SHADE-T3, than in plants under full radiation (Figure 6C).

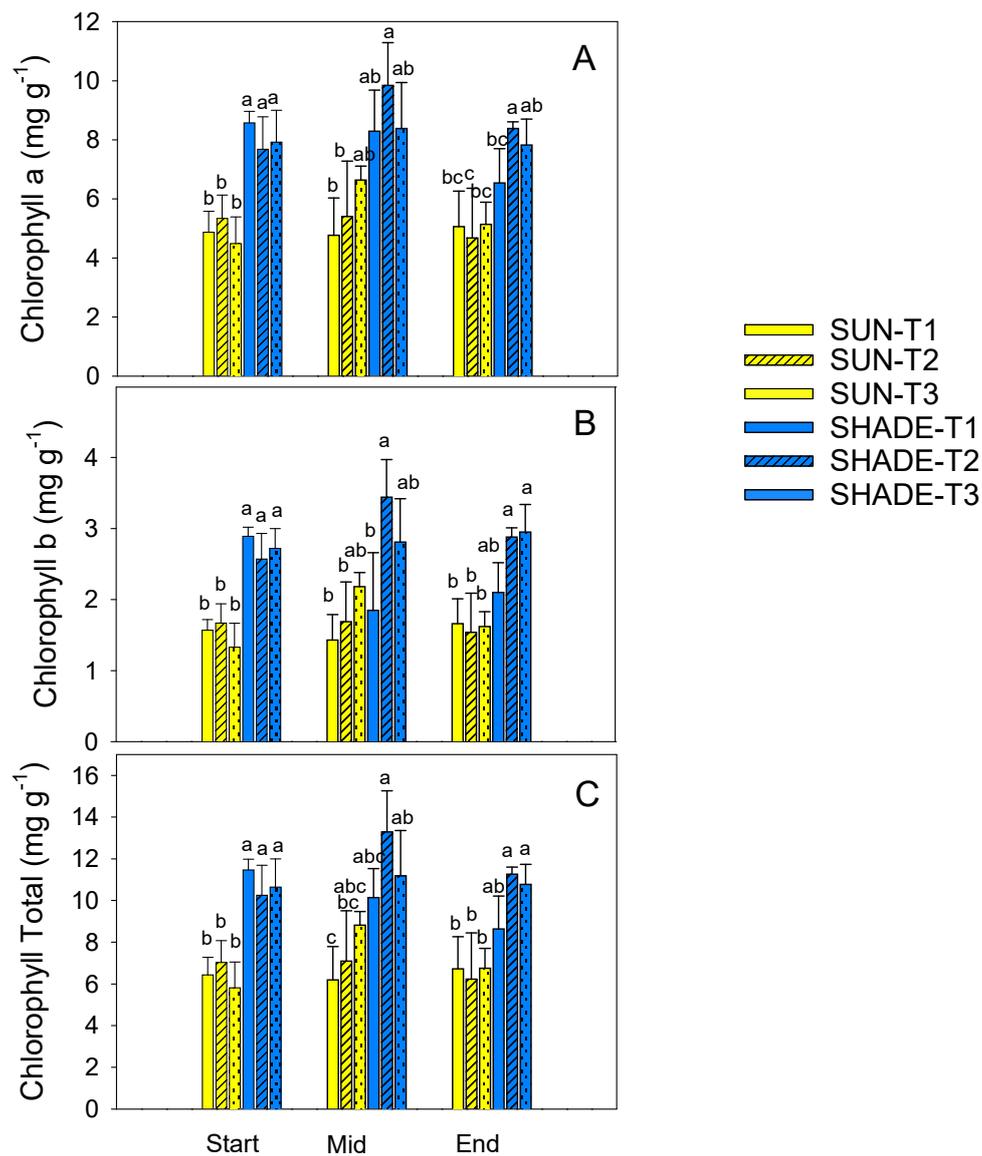


Figure 5. Leaf chlorophyll *a* content (A), leaf chlorophyll *b* content (P_n) (B) and leaf total chlorophyll content (C) of *C. heterophyllum* growing under shade and sunny conditions, irrigated by three irrigation levels during the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

3.5. Biomass Parameters and Growth

In spite of the dry weight of leaves and stems tending to decrease when the irrigation level was lower, no statistical differences were observed between treatments (Figure 7A,B). Nevertheless, aerial dry weight tended to increase in plants growing under shade conditions compared to those growing under full irrigation conditions (Figure 7C). Root dry weight was reduced up to 42% by the irrigation treatments in plants growing under both radiation conditions (Figure 7D). The highest value of root dry weight was found in plants from treatment SHADE-T1 (showing a root dry weight of 10.58 g), while the lowest values were found in plants from treatments SUN-T3 and SHADE-T3, reaching 6.26 g and 6.16 g, respectively.

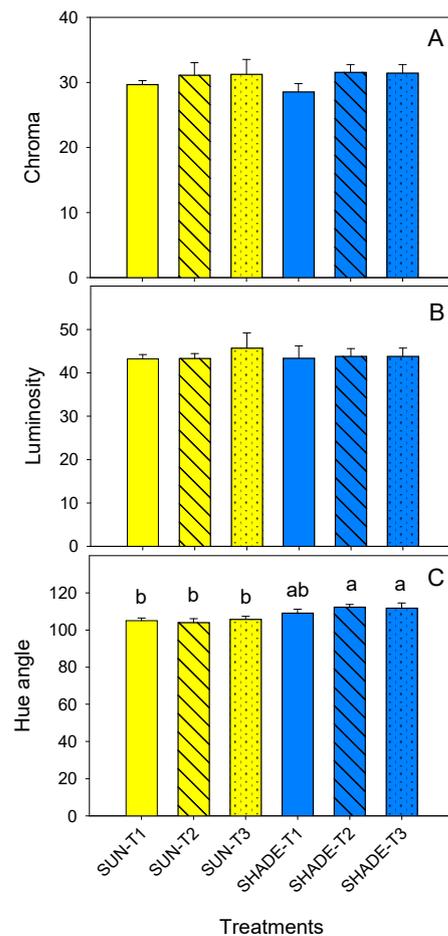


Figure 6. Leaf colour parameters: chroma (A), luminosity (B) and hue angle (C) of *C. heterophyllus* growing under shade and sunny conditions, irrigated by three irrigation levels at the end of the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

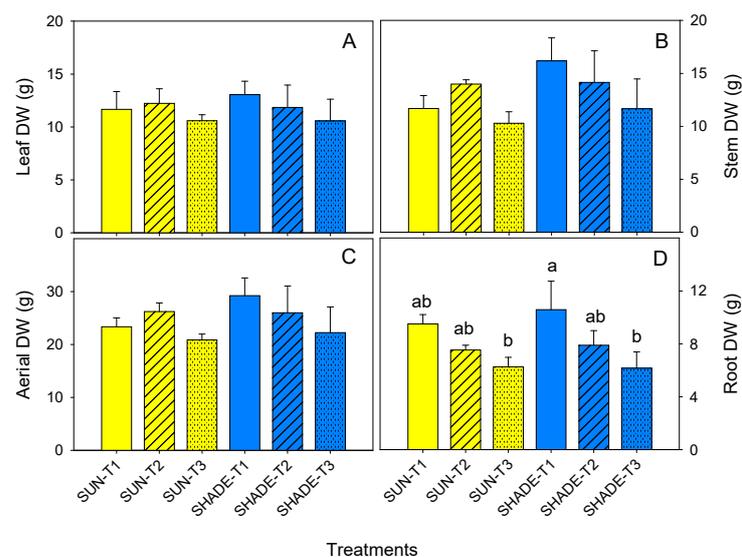


Figure 7. Leaf dry weight (DW) (A), stem DW (B), aerial DW (C) and root DW (D) of *C. heterophyllus* growing under shade and sunny conditions, irrigated by three irrigation levels at the end of the experimental period. Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. The vertical bars indicate standard errors.

3.6. Leaf Mineral Content

The content of most of the macronutrients in leaves were modified by the treatments applied (Table 2). In general, regardless of irrigation level applied, K, P, B and Zn were accumulated 38%, 26%, 23% and 42%, respectively, more in plants growing at shade than in plants growing at full radiation, while the content of Ca, Mg, S, Fe and Ni was 21%, 17%, 33%, 60% and 30% higher, respectively, in sunny plants than in plants growing in shade. Regardless of the radiation level applied, severe deficit irrigation caused an accumulation of S, Fe and Mn (104%, 7% and 38%, respectively) and a reduction of K content (around 7%) in comparison to the control treatment (Table 2).

Table 2. Leaf mineral content of *C. heterophyllus* growing under shade and sunny conditions, irrigated by three irrigation levels at the end of the experimental period.

		Macronutrients (g 100 g ⁻¹)					
		Ca	K	Mg	Na	P	S
	SUN-T1	1.40	0.66 bc	0.45 abc	0.20 ab	0.20 ab	0.28 b
	SUN-T2	1.52	0.72 bc	0.52 a	0.29 a	0.16 b	0.49 a
	SUN-T3	1.44	0.55 c	0.50 ab	0.26 ab	0.21 ab	0.45 a
	SHADE-T1	1.17	0.89 ab	0.40 bc	0.16 ab	0.26 a	0.18 b
	SHADE-T2	1.01	1.07 a	0.35 c	0.04 b	0.22 ab	0.25 b
	SHADE-T3	1.41	0.75 bc	0.51 ab	0.23 ab	0.22 ab	0.48 a
Significance	P	ns	**	*	*	*	***
Radiation	SUN	1.45 a	0.65 b	0.49 a	0.25	0.19 b	0.40 a
	SHADE	1.20 b	0.90 a	0.42 b	0.14	0.24 a	0.30 b
Irrigation	T1	1.29	0.70 ab	0.42	0.18	0.23	0.23 b
	T2	1.27	0.90 a	0.44	0.17	0.19	0.37 a
	T3	1.42	0.65 b	0.45	0.20	0.22	0.47 a
Significance	Radiation	*	***	*	ns	*	*
	Irrigation	ns	**	ns	ns	ns	***
	Interaction	ns	ns	ns	ns	ns	*
		Micronutrients (mg kg ⁻¹)					
		B	Cu	Fe	Mn	Ni	Zn
	SUN-T1	22.24 b	1.61	316.5 ab	52.84 c	0.94 ab	36.25 b
	SUN-T2	21.14 b	1.68	288.9 b	69.98 abc	0.93 ab	35.43 b
	SUN-T3	20.67 b	1.67	354.4 a	74.39 ab	1.14 a	33.63 b
	SHADE-T1	28.21 a	2.12	224.1 c	64.54 bc	0.75 b	51.45 a
	SHADE-T2	24.84 a	1.87	151.4 d	58.97 bc	0.67 b	55.02 a
	SHADE-T3	25.83 a	1.61	224.7 c	87.79 a	0.87 ab	43.50 ab
Significance	P	*	ns	***	*	*	*
Radiation	SUN	21.35 b	1.65	319.9 a	65.7	1.01 a	35.10 b
	SHADE	26.29 a	1.90	200.1 b	70.4	0.76 b	49.99 a
Irrigation	T1	25.22	1.87	270.3 a	58.7 b	0.85	43.85
	T2	23.00	1.78	220.2 b	64.5 b	0.80	45.23
	T3	23.25	1.64	289.5 a	81.1 a	1.01	38.57
Significance	Radiation	***	ns	***	ns	*	***
	Irrigation	ns	ns	*	**	ns	ns
	Interaction	ns	ns	ns	ns	ns	ns

Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. $p > 0.05$ non-significant differences are indicated by "ns".

3.7. Survival Rate and Damaged Plants

The highest survival rate was found in plants from treatments SUN-T1, SUN-T2 and SHADE-T2, reaching values around 96%, while the lowest survival rate was found in plants from SHADE-T1, showing an 88.3% survival rate (Table 3). The highest number of damaged plants were found in plants from treatment SHADE-T1 (25%), and the lowest number of damaged plants were found in plants from treatment SHADE-T2 (13.5%).

Table 3. Survival rate and percentage of damaged plants of *C. heterophyllus* growing under shade and sunny conditions, irrigated by three irrigation levels at the end of the experimental period.

	Survival Rate (%)	Damaged Plants (%)
SUN-T1	96.55	18.97
SUN-T2	96.49	17.54
SUN-T3	94.74	19.30
SHADE-T1	88.33	25.00
SHADE-T2	96.15	13.46
SHADE-T3	92.31	21.15

4. Discussion

In spite of the fact that plants from the genre *Cistus* form part of naturally clear forests under a Mediterranean or sub-Mediterranean climate, which leads to the assumption that they show a similar behaviour under such conditions, it has been demonstrated that species from this genre may differ in their capacity to confront water stress situations [30–32]. In this sense, there is no study on *Cistus heterophyllus* under water deficit conditions to date. In our experiment, plants did not show a difficulty to absorb water from the soil up through several weeks of imposing deficit irrigation treatments. The declined plant water status generated by the deficit irrigation induced the plants to synthesize osmoprotective compounds that could promote water retention in the cytoplasm or stabilize the membranes, preventing the loss of cell turgor [33]. This resulted in the reduction of the osmotic potential of the cell at the end of the experiment, showing a slight osmotic adjustment in plants subjected to severe deficit irrigation, regardless of the level of radiation applied. Towards the middle of the experiment, the plants grown under full radiation experienced a partial closure their stomata as deficit irrigation was higher, behaviour that has been also observed in other *Cistus* species as a mechanism to avoid water losses [34,35]. However, this stomatal closure was not low enough to excessively reduce photosynthesis at the end of the experiment, which allowed improved WUE_i values. An increase in WUE_i indicates that the plants are able to photosynthesize and produce the same amount of biomass while using less water, which is an important adaptation for plants growing in environments with water scarcity [36].

When stomata are closed by stress induced by factors such as high radiation, heat or drought, the dissipation of radiation in the form of latent heat is reduced and the temperature of the leaf rises in a vicious circle [37]. If the stress is prolonged over time, the integrity of photosystem II is compromised due to the stacking of thylakoid membranes, thus reducing the photosynthetic efficiency [38]. In our case, the degree of stomatal closure also did not cause an increase in leaf temperature which is in concordance with Ortuño et al. [39] who did not find leaf temperature increases when irrigation suppression was applied to *Cistus albidus* plants for 4 weeks. In addition, there were no significant differences in the leaf chlorophyll content at the different irrigation levels, suggesting that not even the highest level of water deficit caused the destruction of chloroplasts or an increase in the activity of the enzyme chlorophyllase [40]. Contrary to what might be expected, these plants were able to maintain their aerial dry weight, while root dry weight was reduced as deficit irrigation increases. Lorente et al. [41] found that aerial biomass in *C. albidus* was reduced by deficit irrigation while root biomass was not affected. This can be explained because irrigation was suppressed, thus plants had to minimize water losses as much as possible by reducing their leaf area. In our case, water stress was less drastic and prolonged throughout time, therefore plants had time to adapt to those conditions.

Shaded plants were exposed to a lower incident PAR radiation, and such unsaturated conditions would allow the stomata to remain open longer. The highest stomatal conductance was determined in the plants of the SHADE-T2 treatment, which was accompanied by an improvement in CO_2 absorption, increasing the value of net photosynthesis [42,43]. Sebastiani et al. [44] stated that, although leaf temperature differences between sunny and

shaded plants could have a relatively lower impact in the photosynthetic performance, such leaf temperature increases may severely limit PSII photochemistry when coupled with water stress. On the other hand, the higher values of leaf chlorophyll content per g of dry weight in shaded plants than in sunny plants, regardless of the irrigation level applied [45], was related to colour parameters results. The higher hue angle in shaded plants provided a greener colour to leaves. The green colour in shaded plants could be appreciated with the naked eye and gave the plants a higher quality, which has been seen in other studies under lower-level radiation [46,47]. However, all these results are not in concordance with Puglielli et al. [48] and Sebastiani et al. [44] who reported that *C. salvifolius* decreased in leaf chlorophyll content and net photosynthesis rate and *C. incanus* reduced its gas exchange and leaf mass per area in shade conditions, leading to an increased light-harvesting capacity and a physiological acclimation of these species to a low-light environment. Nevertheless, plant acclimation to different levels of radiation depends mainly on the plant genotype, and thus is species-specific. According to Fini et al. [49], excessive radiation may lead to photoinhibition and damage the photosynthetic machinery, whereas excessive shading can reduce photosynthesis rates, decrease root growth and result in a reduced capacity to survive drought, which did not occur in *C. heterophyllus* when they grew at 50% of the radiation. On the other hand, both factors differently influenced the accumulation of minerals in leaves, especially regarding the K element. The highest accumulation of potassium, which is a key nutrient in the water–plant system, may be related to an improvement in leaf turgor and a good stomatal regulation [50]. In shade conditions, leaf P and K content in *Cistus* plants were also higher than in sunny conditions, which is in agreement with what was observed by Semida et al. [51]; leaf P and K concentration in cucumber plants increased when the shade level applied increased. The accumulation of Zn in shaded plants could also be linked to a greater synthesis of chlorophylls. Valladares and Niinemets [52] stated that the tolerance to a given stress is reduced by other common stresses and, when plants are growing under shade conditions, tolerance to other environmental limitations such as drought may be compromised. This is not true in our case, since the results observed in water relations were similar under both radiation conditions. The microclimate produced at low radiation conditions allowed the evaporative demand to be lower, which in turn allowed the plants to show better photosynthetic performance than plants grown at full radiation. The survival rate found at the end of the experiment was more than 90% in plants from all treatments except in plants from the SHADE-T1 treatment, which showed the highest percentage of damaged plants. This was probably because under 50% of the radiation, water supply (a total of 2.05 L week⁻¹ and plant⁻¹ distributed everyday) could be excessive for the evaporative demand conditions during several days under the shade house. Thereby, moisture might be accumulated in the substrate, drowning the roots. Nevertheless, plants from treatment SHADE-T2 showed one of the highest survival rates and the lowest percentage of damaged plants, coinciding with the highest values of gas exchange found in these plants. Thus, an irrigation of 1.2 L week⁻¹ and plant⁻¹ distributed 4 days per week could be recommended under 50% of the radiation to obtain plants with optimal growth, development and quality.

5. Conclusions

Cistus heterophyllus growing in nursery conditions showed a high degree of resilience to a long period of water deficit irrigation. These plants were able to develop several mechanisms of adaptation such as leaf osmotic adjustment and improved intrinsic water-use efficiency, which could help to it to cope successfully against drought in the natural conditions imposed by the Mediterranean climate. In general, the condition of using 50% of the radiation imposed during the summer period improved gas exchange, reduced leaf temperature and induced the synthesis of chlorophyll, improving the quality of the plants as reflected in the greener colour of the leaves. The results show that imposing the 50% radiation condition produced the most favourable outcome when facing moderate water stress conditions, i.e., under the treatment SHADE-T2. These results show the importance

of taking into account the radiation factor as radiation was shown to be the most limiting factor in our experiment. Applying less radiation, either by using shelters or reintroducing the species in areas with sufficient trees in future ex situ and in situ experiments, would help to obtain a good development and stabilization of the species, as well as a high survival rate in future recovery and conservation programs for the species.

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