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Article

Assessment of Plant Responses to Simulated Combination of Heat Wave and Drought

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Abstract: Background: Among the consequences of global climate change, one of the most significant yet understudied is the increased frequency and intensity of heat waves. This article evaluates the responses to combined heat wave and drought in several crops and a non-crop species using an improved methodology to control temperature using IR lamps. **Results:** The effectiveness and precision of simulated heat waves with the system presented were verified at the experimental field of the University of the Balearic Islands. Using IR lamps, an artificial leaf was used to precisely control environmental temperature, a key aspect in simulating heat wave conditions. Concerning plant physiology, the effects of combined heat wave and drought on leaf relative water content (RWC) and photosynthetic parameters presented different patterns between species. Two remarkable particularities were (1) the observation that photosynthesis was sustained under RWC values well below those previously reported to cause complete photosynthesis cessation in C_3 species and (2) the photosynthetic linear electron transport rate (ETR) was stimulated after retrieval of drought and heat wave far above their own initial values and those for control plants, also in some species. **Conclusions:** The use of an artificial leaf as major temperature sensor is key to provide highly realistic simulated heat waves. Using this technical setup, it was possible to determine that there is a large variability among species and some particularly intriguing observations strongly support the view that systematic experiments should be developed on different species and conditions to grab a significant knowledge on how will heat waves affect crop and vegetation in the near future.

Keywords: climate change; heat waves; drought; affordable commercial infrared heaters; plant ecophysiology; photosynthesis; relative water content

1. Introduction

Global climate change is one of the biggest challenges that humanity faces in the 21st century (Feulner, 2017). Among the numerous effects of climate change, heat waves stand out as extreme phenomena with the potential to devastate ecosystems, agriculture, and human health (Intergovernmental Panel on Climate Change, 2021; Shivanna, 2022; Qu et al., 2024). Heat waves consist of periods (typically days to weeks) with warmer temperatures than the average for the same site and period over years. The impacts of heat waves on agricultural yields have been reported to induce major negative consequences, leading to numerous direct and indirect social problems in many regions of the world (Hatfield et al., 2011; Lobell, Schlenker and Costa-Roberts, 2011). Although studies on the effects of heat waves on plants follow very diverse methodologies and are difficult to compare, it has been suggested that heat waves have strong negative impacts on plants, with photosynthesis rates and RWC being major targets, as well as inducing alterations in plant development and hormonal levels (Haworth et al., 2018; Xie et al., 2020; Ostria-Gallardo et al., 2023; Rashid et al., 2023; Tokić et al., 2023), germination (Orsenigo et al., 2015), fruit quality (Tomás, Viegas and Silva, 2020), senescence, and others (Qu et al., 2024).

In this context, although it has been predicted that drought would exacerbate the effects of heat waves on photosynthesis and plant water relations—and vice versa—(López, Ramírez-Valiente and Pita, 2022), there is limited and not very conclusive evidence available at present. Thus, Rashid et al. (2018) found, studying two cultivars of wheat, that only in one of them, photosynthesis was more depressed by drought under hot conditions, and only after recovery and not during its imposition. Moreover, Haworth et al. (2018) observed, in olive trees, that a heat wave strongly reduced photosynthesis in irrigated plants, but not under drought, resulting in non-significant differences in photosynthesis between irrigated and drought plants during the heat wave. Conversely, in natural ecosystems, there is evidence that heat waves alone produce only small and transient effects, while when combined with drought they amplify the negative effects of the latter on carbon balance and productivity (De Boeck et al., 2011, 2016). Regarding to RWC, Davies et al. (2018) observed, in native Australian C3 and C4 grasses, that the combined effect of heat wave and drought was a larger decrease of leaf water content. Although RWC was not reported in that study, the observed values of leaf water content in the three C3 species studied point to RWC values well below 65%, i.e., below the value defined by Lawlor and Cornic (2002) as the threshold for complete photosynthesis inhibition in C3 species, which contrasts with the scarce effects of heat waves on photosynthesis observed in the aforementioned works by Rashid et al. (2018) and Haworth et al. (2018). In contrast, Xie et al. (2020) found no differences in RWC between wheat plants subject to drought alone or combined with heat.

As heat waves become more frequent, prolonged, and intense in various parts of the world, understanding and mitigating their effects is becoming increasingly crucial (Marx, Haunschild and Bornmann, 2021). In order to address this issue, scientists have developed several methods, both passive and active, to simulate these extreme conditions in controlled environments, both inside laboratories and out in the field (De Boeck et al., 2010). Despite decades of research, accurate replication of heat waves remains a significant challenge due to the technical and economic limitations of current systems (Schulze et al., 1999; Rich et al., 2015). Passive methods, such as greenhouses, passive nocturnal warming and open-top field chambers are often used in studies with limited infrastructure, such as those performed in remote areas away from the laboratory. These systems present a lower precision and control, resulting in less accuracy in simulating natural heat waves. In contrast, active systems like phytotrons, active warming chambers, heating cables or tubes, and infrared (IR) heaters, although more costly in terms of energy and implementation, are better suited for studies that require precise and constant temperature control (Aronson and McNulty, 2009; De Boeck et al., 2010). These systems can replicate extreme heat wave conditions more accurately, providing reliable simulations of specific climate scenarios (Shen and Harte, 2000). Still, most of these methods have additional limitations, such as, among others, light quality and intensity largely different from natural conditions, or the inability to reproduce outdoors wind conditions.

Among all active methods, heating with IR lamps stands out for its ability to provide heat through infrared radiation, directly warming soil and vegetation similarly to the sun (Kimball, 2005). Further, IR lamps constitute the only active method that can be used in situ, in the field, under the natural dynamic variations of light quantity and quality, wind, precipitation and other environmental factors. Previous studies have shown that IR heaters can have a significant impact on simulating global warming and its effects on ecosystems (De Boeck et al., 2010; Kimball, 2015), while offering significant advantages in terms of thermal precision and speed, thus allowing for specific and consistent temperature increases that accurately mimic heat wave conditions, with minimal disturbance to the ecosystem (Kimball et al., 2008). Nevertheless, the adoption of large-scale IR heaters faces significant challenges, including high installation costs and substantial energy consumption (Kimball, Conley and Lewin, 2012). Moreover, the operational complexity of configurations that require modifying heat intensities and distribution to accurately simulate heat waves in different ecosystems represents another significant implementation hurdle (Kimball, 2015). Harte et al. (1995) presented the first heat wave experiment using infrared heaters, in which the device emitted radiation over a linear space with fixed power throughout the experiment. Later, Nijs et al. (1996) introduced proportional control to maintain a fixed temperature increase in the heated plot compared to a reference plot. Kimball (2005) further improved the methodology by introducing a proportional-integral-derivative (PID) controller. In the present work, some of these and other pioneering implementations of IR-based heatwaves simulations, based on versatile and economical IR heaters, i.e., those often used in bars and terraces, have been used. Overall, one of the crucial issues regarding the use of IR heaters is that they exert a direct effect on the bodies within reach, but not on the surrounding air, while heat waves are defined based on air temperature. This is a crucial issue that constitutes the focus of the present research. A redefinition of heat waves is needed when studying their effects on plants given that leaf temperature is much more dependent on factors beyond air temperature.

These factors are related to (a) leaf or canopy size and morphology with strong influence on the boundary layer, (b) leaf physiology (e.g., transpiration) and (c) additional variable climatic conditions (e.g., wind speed). Consequently, relating the increase in temperature over an artificial leaf which is not influenced by the above-mentioned factors is key to finely controlling temperature changes.

The objectives of the present study are: (1) assessing the suitability of using IR heaters for simulating heat waves on plants; and (2) applying this methodology to study the combined effect of drought and simulated heat waves on leaf RWC and photosynthesis in one native and three crop species.

2. Material and Methods

2.1. Device for simulating heat waves

Commercial, low-cost infra-red based heaters were adapted for simulating natural heat waves to study their effects on plant ecophysiology. A custom control system and dedicated software were specifically implemented for this purpose. A brief description of the main features of the method is provided below with further details in Supplementary Material S1.

Widely distributed and easily accessible commercial IR heaters in Europe (Tristar KA5287, Orbegozo PHF31, Tresko THSL004, Liliana CIPIE2000, and TroniTechnik TT-TH2020) were used. These devices are designed with a circular support to facilitate their placement surrounding those plants under study. Their height is adjustable, ranging from 130 cm to 210 cm, and inclination can be adjusted from 0° to −45°. Heaters are governed by a bus carrier board control system embedded in a Raspberry Pi Compute Module 4 I/O board with a Raspberry Pi Compute Module 4 SBC (Single Board Computer) card that features a quad-core Cortex-A72 processor at 1.5 GHz, also embedded in the I/O board. Temperature was measured using negative temperature coefficient (NTC) thermistors that operate over a range of −30 °C to +105 °C and can function properly in ambient conditions with relative humidity (RH) between 5% and 95%.

Given that IR heaters only affect the temperature of surfaces, temperature sensors were placed in cardboard, simulating a non-exposed artificial leaf without transpiration in two setups (control and simulated heat wave). Two sets of plants were studied, one without IR heaters (control) and another with IR heaters (simulated heat wave). The control system works to increase the temperature reading of the second sensor to a targeted temperature above that registered by the control sensor, using an on/off control with a user-programmable hysteresis within a defined range of ±0.25 °C to ±2 °C (±0.5 °C was selected).

2.2. Assessment of thermal homogeneity across an area surrounded by the heaters

Since the area surrounded by heaters that displays a similar air temperature will depend on the number and spatial disposition of heaters, it is important to assess the limits of what the researchers consider acceptable thermal homogeneity for their experiments before positioning the study plants and subjecting them to a simulated heat wave. Having a given number and array of heaters, this test may allow one to discern the maximum usable area size and, hence, the maximum number of plants that can be assessed in a single run–which would obviously depend on the size of the target plants. Alternatively, if the size and number of plants to be subjected to a simulated heat wave is fixed a priori, a similar assessment may allow one to know how many heaters might be needed, and to adjust their distribution to reach a homogeneous targeted temperature course around all the studied plants.

Thermal homogeneity of the system was evaluated by analysing the sensor temperature data collected when a minimum change of 0.1 °C occurred or when one minute passed without changes greater or equal than 0.1 °C. This data was provided by an array of 15 NTC sensors distributed at three different heights (see Figure 1 for a detailed description of the sensors situation).

Using this sensor array, three basic configurations were studied, all involving the placement of six heaters at the vertices of an imaginary hexagon, either surrounding or being surrounded by the experimental plants. Figure 2A–C illustrate some of the basic configurations that were examined.

More than 150 variants were explored, modifying variables such as the distance of the heaters (IHD) and sensors (TSD) from the centre of the plot, the height (IHH) and inclination angle (IHA) of the heaters, and the power output (Pw).

2.3. Defining and simulating heat waves

Although this is somehow out of the scope of this manuscript, it is worth mentioning that there are various definitions of heat waves at the European and global levels (De Boeck et al., 2010), which should be considered for designing simulating experiments. Given that the present study is focused on Mediterranean environments, heat waves were defined as provided by the Spanish State Meteorological Agency (AEMET). This agency defines a 'heat wave' as an episode of at least three consecutive days during which, at least 10% of the considered stations record maximum temperatures above the 95th percentile of their series of daily maximum temperatures for the months of July and August during the period 1971–2000 (Área de Climatología y Aplicaciones Operativas, 2023). It should be noticed that heat waves are defined considering air temperature. On the other hand, IR heaters are designed to heat solid objects—e.g., plants—rather than their surrounding air, which is heated indirectly. Therefore, when simulating the effects of heat waves on plants, it would be legitimate to consider the need for re-defining the heat wave on a leaf or canopy temperature basis. However, this approach has been discarded because the leaf-to-air temperature is a function of (a) leaf or canopy size and morphology (which strongly influences the boundary layer), (b) leaf physiology (e.g., transpiration), (c) additional variable climatic conditions (e.g., wind speed) and, (d) most especially, because empirically-determined leaf temperatures are extremely heterogeneous on an experimental plot as used here, hence making it difficult to define a 'target' temperature for simulating the heat wave. Thus, given that this experiment compares different species (i.e., different leaf and canopy sizes and morphologies) under naturally variable conditions (e.g., different wind velocities among days and times of the day); and that heat waves are based by definition on air temperature, we developed the solution of placing an 'artificial leaf' in the middle of the two plots (control and heat wave treatment), i.e., a cardboard suspended within the canopies. This artificial leaf was used to register ambient temperature at the control plots as well as on the heat wave plots, the latter serving as the indicator to the heaters for adjusting their intensity as a function of the programmed temperature mismatch between control and heat wave plots. Because using an artificial leaf implies a 'non-transpiring' leaf, hence altering the whole energy balance, theoretical considerations on this and a complete simulation of how leaf temperature can vary at any given air temperature are provided in Supplementary Material S3. It is concluded that for the leaf sizes and environmental conditions used in this experiment, the differential temperatures between leaf and air under the simulated heat wave are sufficiently small as to be considered correct. However, care should be taken when using the present heat wave system with very large leaves, such as tobacco or banana, or under windless environments, such as those in growth chambers.

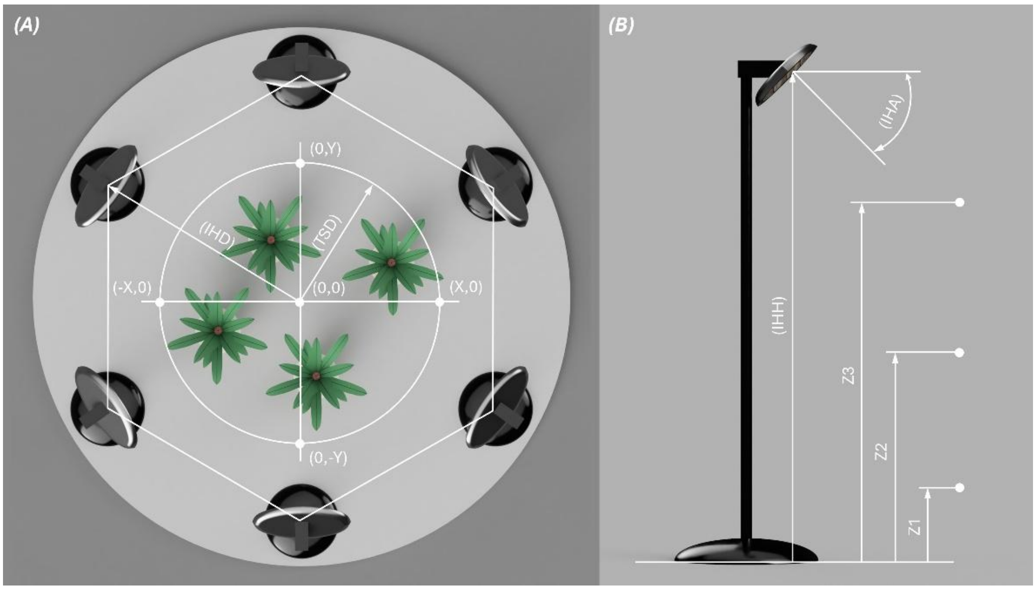


Figure 1. Variable parameters examined for the study of thermal homogeneity in different configurations. Figure 1**A** shows a plan view of the variables located on the X,Y axes: Temperature Sensor Distance (TSD) for homogeneity measurement and Infrared Heater Distance (IHD), both measured from the centre of the plot. Figure 1**B** shows the variables in elevation on the Z axis: Infrared Heater Height (IHH), Infrared Heater Angle (IHA), and height of the temperature sensors for homogeneity measurement, Z1: 25 cm, Z2: 80 cm, and Z3: 135 cm.

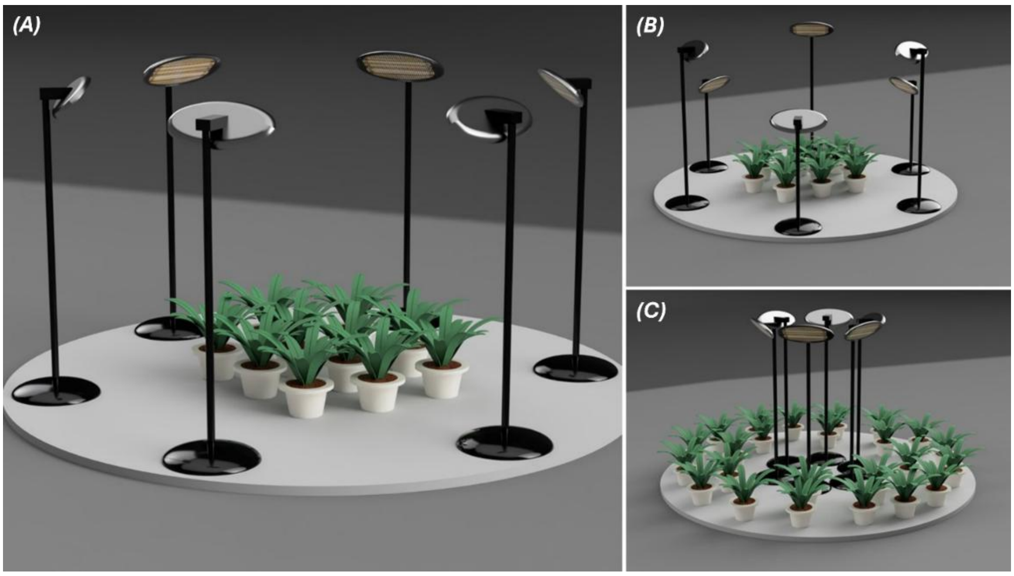


Figure 2. Basic configurations examined to study the combination of parameters that resulted in better thermal homogeneity. Configuration 2**A** shows a plot of plants surrounded by 6 heaters located at the same height over the vertices of a hexagon. Configuration 2**B** shows the same plot surrounded by 6 heaters placed at staggered different heights over the same vertices. Configuration 2**C** shows the heaters in the centre, surrounded by plants forming a circle.

2.4. Plants and treatments

After temperature homogeneity was considered correct (see the Results section), 30 plants in 3.8 L pots were placed in the simulated heat wave area. In both experiments, between 6 and 10 plants per species were used: control, irrigated plants in the absence of heat wave (IC), and plants subjected to a combined drought + heat wave (DHW). Single treatments were skipped as they have been reported to have a much lower effect (De Boeck et al., 2011, 2016; Davies et al., 2018).

In the first experiment, plants of sugar beet (*Beta vulgaris* var. cycla), sunflower (*Helianthus annuus*) and *Limonium gibertii* (an evergreen semi-shrub endemic to the Balearic Islands) were used. Sugar beet and sunflower plants germinated 8th November 2021 and were grown for about two weeks in a growth chamber at 23 °C and 350 µmol m^{-2} s^{-1} PAR, under a 12/12 h photoperiod. Once the first true leaf emerged, plants were transferred to 3.8 L pots filled with a 4:1 mix of commercial horticultural substrate (Prohumin, Projar, Quart de Poblet, Spain) and perlite and placed in a greenhouse at the University of the Balearic Islands (UIB). *Limonium gibertii* seeds were germinated in a growth chamber at 20 °C and grew outdoors under shade in 3 L pots containing a mix of 61.5% coconut fibre, 33% moss peat and 5.5% expanded perlite. They were fertilized with slow release 4.40 mg L^{-1} *Osmocote* NPK 19:10:19 (ICL, The Netherlands) and joined with sugar beet and sunflower plants in the greenhouse two weeks before the onset of the treatments. At this point, all plants were irrigated at field capacity every two days and supplied once with slow release *Multigreen Classic* NPK (Haifa, Israel). A week before the experiment, each plant received 6 g of Poly-feed NPK (Haifa, Israel).

In the second experiment, sugar beet (*Beta vulgaris* var. cycla), sunflower (*Helianthus annuus*) and tobacco (*Nicotiana tabacum* var. Wisconsin) plants were used. Germination and growth conditions were similar to those described for Experiment 1.

Two weeks before simulating heat waves, plants were transferred to a plastic semi-greenhouse (transparent plastic walls covered the top and half the distance between the ceiling and the floor, allowing wind to flow around plants). Irrigation was stopped in plants under DHW treatment until 30% substrate water content was reached. Thereafter, water deficit was sustained by daily supplying the amount of water equivalent to pot weight loss. These plants were kept under these irrigation conditions for a week before they were subjected to a simulated heat wave.

Experiment 1 and 2

Simulated heat waves were slightly different in the two experiments. Thus, in experiment 1, plants were placed on working tables 70 cm above ground, and HOBO temperature sensors (see Simulated heat waves section) were suspended among the leaves forming the canopy. Heat wave was applied for 7 days, using the following differential temperatures: from 10:00 AM, DHW plants would experience a 2 °C increase over control (IC) plants; from 12:00 PM, a 5 °C increase; and from 4:00 PM, a 2 °C increase again. At 6:00 PM, the infrared heaters were turned off until 10:00 AM the next day (Figure 3). In experiment 2, plants were placed directly on the ground with HOBO temperature sensors also suspended within the canopy. Plants were subjected to a 5-days heat wave. The heat wave was stablished as follows: starting at 10:30 AM, the DHW plants should experience an increase of 2.5 °C above IC plants; from 11:00 AM, the increase would be 3 °C; from 12:00 PM, 4 °C; from 1:00 PM, 5 °C; from 4:00 PM, 4 °C; from 5:00 PM, 3.5 °C; from 6:00 PM, 3 °C; and at 7:00 PM, 2 °C, which remained until 10:30 AM the next day, when the cycle started again (Figure 3).

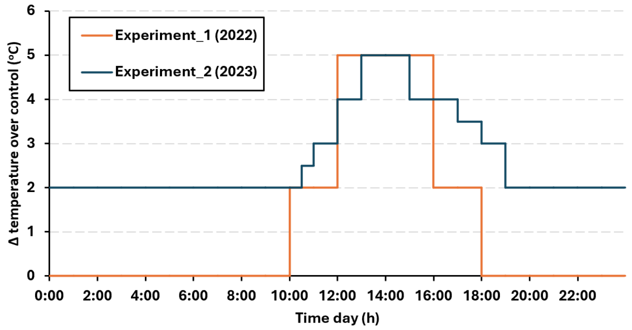


Figure 3. Warm-up regimen based on the time of day. Treated plots were heated by a theoretical air temperature differential (see Supplementary Material S3 for details) with reference to the control plots, depending on the time of day. Temperature regimes varied for each experiment according to the heat wave intended to be simulated.

2.5. Physiological measurements

Gas exchange and chlorophyll fluorescence parameters, and leaf relative water content, were measured between 10:00 AM and 2:00 PM on six different individuals of each species and treatment, on three days: T1 (plants under irrigation or drought the day before the onset of the heat wave), T2 (after four days under combined drought and heat wave), and T3 (three days after re-watering and heat wave simulation removed). All measurements were performed on sun-oriented, young, fully expanded leaves.

Leaf discs were taken and immediately weighed to determine their fresh weight (FW). Then, the discs were rehydrated in distilled water for 24 h under dark conditions at 4 °C to obtain the turgid weight (TW). Finally, leaves were placed in an oven at 70 °C for 72 h to determine dry weight (DW). RWC was calculated as:

$$\mathrm{RWC}\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

Gas exchange and chlorophyll fluorescence parameters were assessed at ambient temperature and vapour pressure deficit (VPD) using an infrared gas analyser (IRGA) LI-6400XTR coupled with a fluorimeter (Li-6400-40; Li-Cor Inc., Lincoln, NE, USA). Photosynthetic photon flux density (PPFD) was fixed at 1500 µmol m^{-2} s^{-1} (90% red, 10% blue light). Net CO_2 assimilation (A_N), stomatal conductance (g_s), and the rate of linear electron transport rate (ETR) were analysed.

2.6. Statistical analysis

To evaluate the effectiveness of the treatments (DHW vs. IC) on each of the physiological parameters (RWC, A_N and ETR) for each species and experiment, data were analysed independently. First, normality for each dataset was assessed using the Shapiro-Wilk test, and the homogeneity of variances was verified using the Levene test. In cases where both assumptions were met, an independent samples t-test was applied; otherwise, the non-parametric Mann-Whitney U test was used. A significance level of $p < 0.05$ was adopted.

3. Results

3.1. Thermal homogeneity assessment using different configuration variants

Three basic configurations were studied, each involving the placement of six heaters at the vertices of an imaginary hexagon, either surrounding or being surrounded by the experimental plants. Thermal homogeneity of these configurations was assessed using an array of 15 NTC sensors distributed at three different heights. More than 150 variants were explored, modifying variables such as the distance of the heaters (IHD) and sensors (TSD) from the centre of the plot, the height (IHH) and inclination angle (IHA) of the heaters, and their power output (Pw). In general, all measurements obtained from each sensor offered readings close to the target temperature value (Figure 4) with low maximum absolute errors.

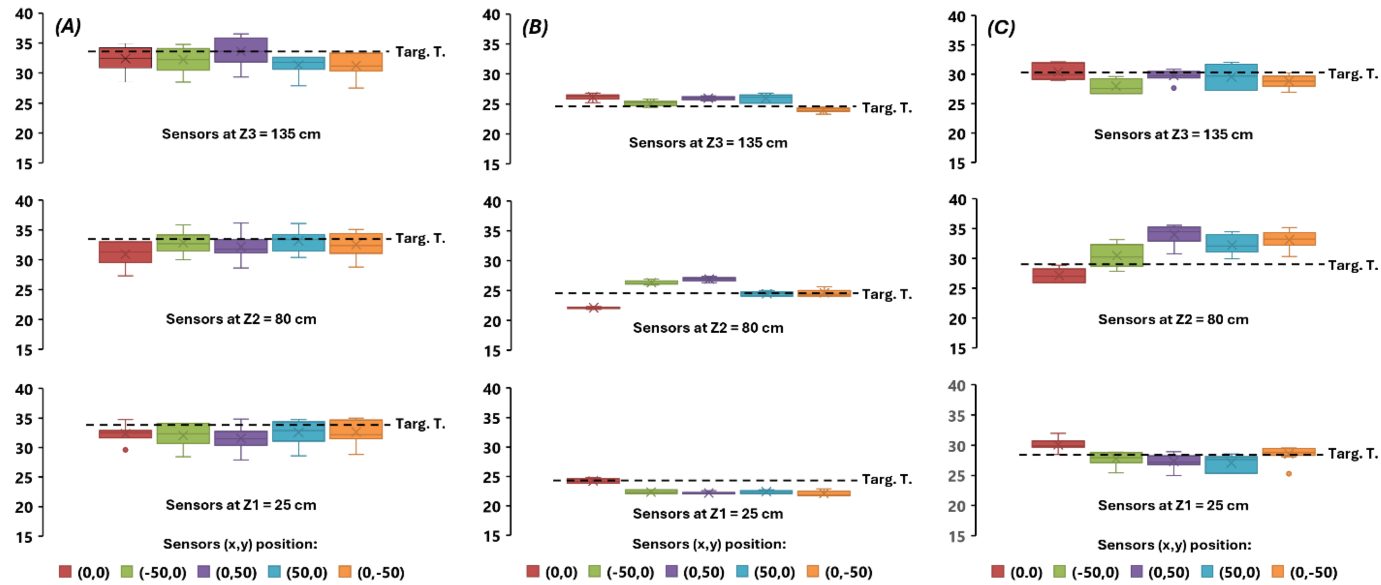


Figure 4. Temperature dispersion across each of the 15 sensors arranged to measure thermal homogeneity. Figure 4**A** corresponds to test 160, which in turn is related to the configuration in Figure 2A. Figure 4**B** corresponds to test 49, which in turn is related to the configuration in Figure 2B. Figure 4**C** corresponds to test 7 which in turn is related to the configuration in Figure 2C. Targ. T, is the target temperature that the system had to reach in the treated plot; refer to Figure 1 for details on the (x,y) and Zn positions.

3.2. Simulated heat waves

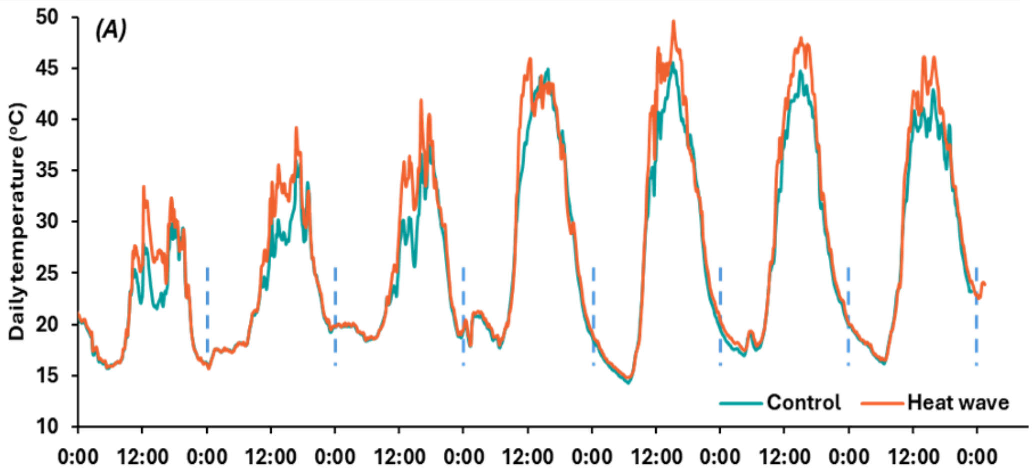
The effectiveness and precision of the generated heat waves were measured using temperature and humidity sensors Onset HOBO UX100-003 (470 MacArthur Blvd., Bourne, MA 02532, USA), placed in the centre of the canopy of both control (IC) and treatment (DHW) plots. Real temperatures of control and heat wave plots during several days are represented in Figure 5 showing the viability and fine control of temperature gradients as scheduled.

3.3. Physiological results

The present study combines two primary objectives. The first objective was to describe a heat wave simulating system and prove its accuracy and usefulness, combining this with a second objective which was to depict the response of RWC and photosynthesis in several different species and conditions, owing to the apparently controversial results of previously published studies. Given the duality of goals of the present work, a combination of drought and heat wave stress was applied simultaneously instead of separately in order to limit the number of species and replicates. Beyond this limitation, the obtained results (Figure 6) elucidate the potential impacts of heat waves on plant physiological responses and the expected heterogeneity depending on the plant and conditions during a Mediterranean heat wave.

As soon as plants were randomly assigned to either IC and DHW treatments, and before applying drought and heat waves, all parameters were measured, finding no significant differences for any of them between the two plant groups. Figure 6A shows that the combination of heat wave and drought often results in a severe decrease in leaf relative water content (RWC). This effect is particularly remarkable in sugar beet and sunflower—sugar beet only in Experiment 1, and sunflower in both experiments—although the decrease in sunflower RWC was not statistically significant in Experiment 1. In both species, RWC dropped well below 65%, a value defined by Lawlor and Cornic (2002) as the threshold for complete photosynthesis inhibition in C3 plants. This agrees with previous findings by Davies et al. (2018) and highlights how extreme the impact of a heat wave can be on plants already experiencing water stress. Net CO_2 assimilation (A_N) and stomatal conductance (g_s) followed almost identical patterns in all cases, for which only the former are shown. Figure 6B shows that, in all cases except sunflower in Experiment 1, A_N was depressed under DHW compared to IC, although this reduction was not statistically significant in sugar beet during Experiment 2. While results in T1 (i.e., plants under drought but before the onset of heat wave) are not shown for simplicity, it is worth saying that A_N in T2 (an additional four days under drought accompanied by heat wave) was significantly decreased compared to T1 except in sunflower in Experiment 1, where it was unchanged, and in Limonium, where it actually increased (data not shown). However, ETR was more stable than photosynthesis in response to DHW, although in some species it was significantly reduced, but to a lesser extent than A_N (Figure 6C). Three days after stress was alleviated (T3), both A_N and ETR of DHW plants increased to values at (Limonium and sunflower in Experiment 2) or even above (sunflower and sugar beet in Experiment 1) control plants (Figure 6D,E). In contrast, in Experiment 2 sugar beet DHW plants kept lower values than control plants upon recovery.

Time	0:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00
ΔT	0	2	2	5	5	5	5	2	2	0	0



Time	0:00	10:30	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00
ΔT	2	2,5	3	4	5	5	4	4	3,5	3	2

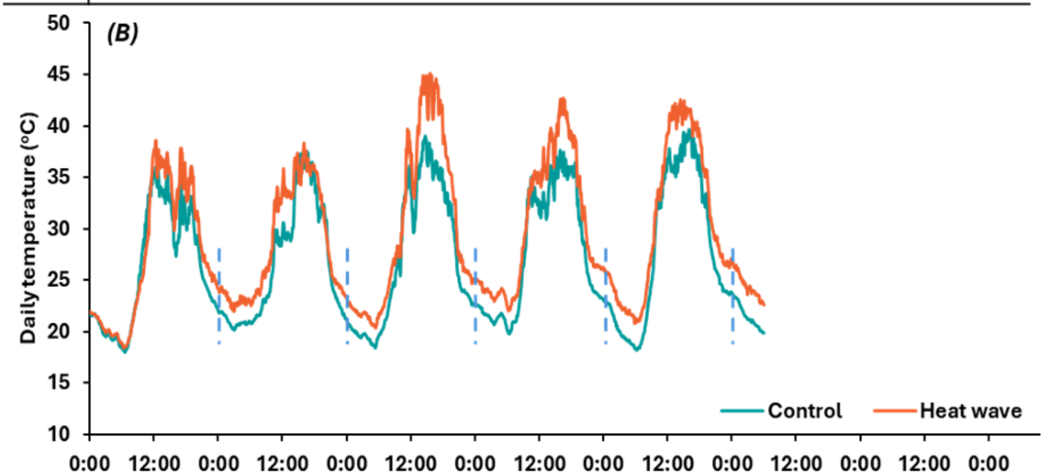


Figure 5. Two experimental heat waves were developed at the University of the Balearic Islands (UIB), simulating typical Mediterranean heat waves. Both Experiment 1 (Figure 5**A**) and Experiment 2 (Figure 5**B**) illustrate the scheduled increases in air temperature over 24-h periods.

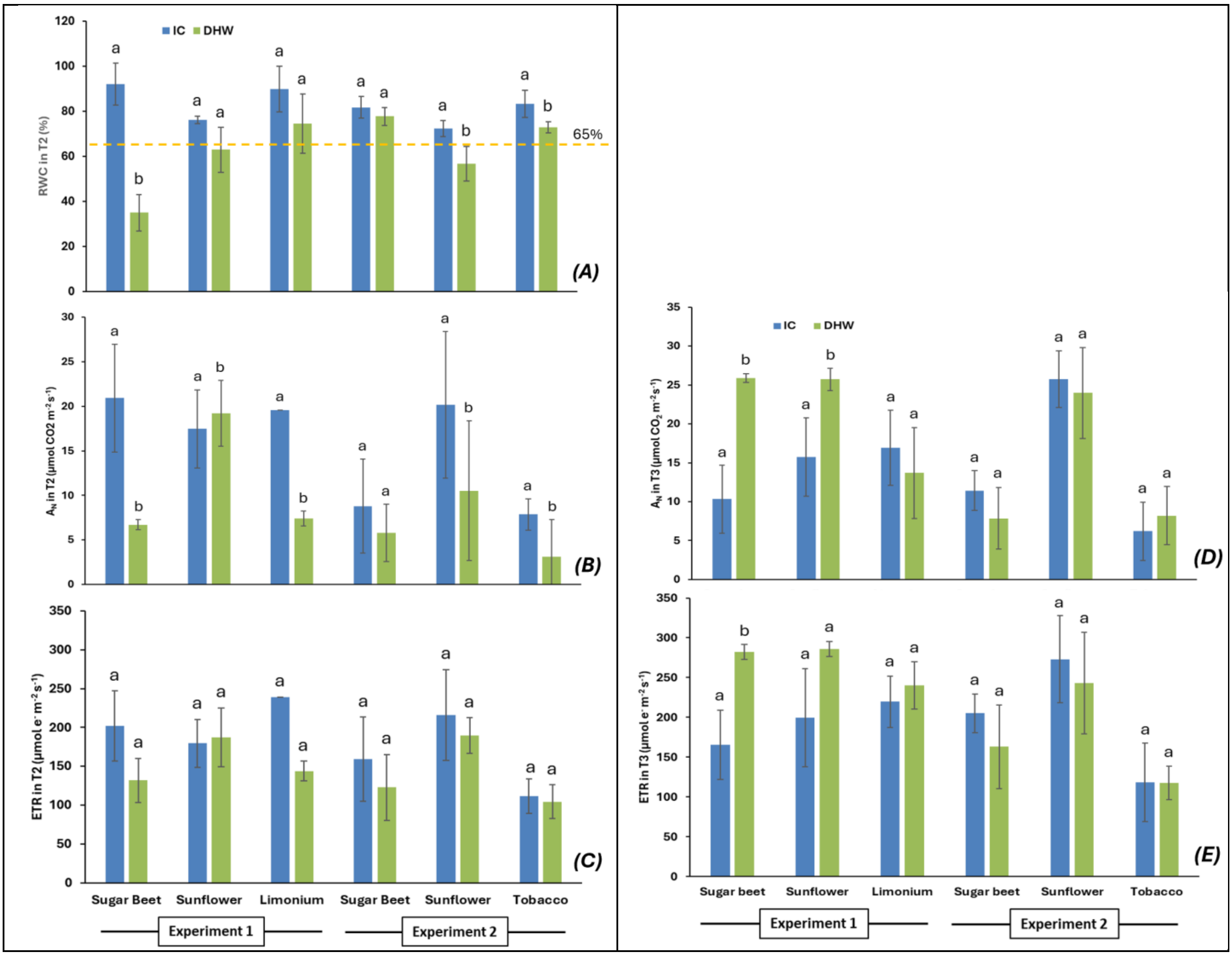


Figure 6. Physiological data indicating the effect of simulated Mediterranean heat waves on experimental plants. Figure 6**A** shows leaf relative water content (RWC) in plants under control conditions (IC) and those subjected to heat wave and drought (DHW), measured immediately after the simulated heat wave (T2). Figure 6**B**,**C** show net photosynthesis (A_N) and the electron transport rate (ETR), respectively, under the same treatments at T2. Figure 6**D**,**E** present A_N and ETR, respectively, for both treatments after a three-day recovery period (T3). The yellow line in Figure 6**A** represents the 65% RCW threshold defined by Lawlor and Cornic (2002) as the point of complete photosynthesis inhibition in C3 plants. Different letters indicate significant differences ($p < 0.05$) between treatments for a given species and/or experiment.

4. Discussion

4.1. An economic commercial device for simulating heat waves

The present paper presents a new system that enables the use of economical commercial infrared heaters, coupled with a microcomputer and dedicated software to accurately simulate natural heat waves under a wide range of conditions, including outdoors. While it is not possible to perfectly simulate past recorded heat waves, especially in the field, due to the inherent differences between current and past weather conditions, our system offers a practical approach.

In experiments simulating heat waves, primarily two types of sensors have been used over the years: thermocouples (contact sensors) and infrared sensors (non-contact sensors) (Bridgham et al., 1999; Nijs et al., 2000; Wan, Luo and Wallace, 2002; Van Peer et al., 2004), each with its own advantages and disadvantages. While IR sensors measure the surface temperature of bodies, contact sensors measure the temperature through direct contact with the body being measured. IR sensors measure temperature by integrating the various temperatures in the coverage area, which can result in unrealistic values on surfaces with low vegetation coverage (Jones et al., 2003; López et al., 2012; Chen, 2015). Additionally, leaf temperature largely depends on transpiration (Nobel, 2020). On the other hand, contact sensors inserted in the canopy are heated by both radiation and convection, which can also influence the measured temperature. Many researchers claim that infrared heaters first heat the surface of plants and then, by convection, the surrounding air (De Boeck and Nijs, 2011; Kimball et al., 2014; LeCain et al., 2015). However, this claim has nuances, as it depends on the efficiency of the heaters, which is linked to the surface working temperature, the emissivity of the material, and consequently, the spectral distribution of the radiation according to Planck's law. The efficiency of IR heaters varies between 40% and 96% depending on these characteristics, indicating how much energy is used for heating by radiation and how much by convection and conduction (Mor Electric Heating Assoc., 2019). Therefore, using more efficient sources makes heating in windy conditions more effective. In the present study, radiators with 60% efficiency were used, resulting in low efficiency in windy conditions, but justifying the use of contact sensors attached to cardboard or plastic to simulate leaf conditions. In fact, the differential temperatures between leaf and air under the simulated heat wave are sufficiently small to be ignored for the type of leaves and environment analysed here (Supplementary Material S3). Nevertheless, it should be noticed that additional controls or target temperature corrections should be considered if the present heat wave system is to be used with very large leaves, such as tobacco or banana, or under windless environments, such as those in growth chambers.

This is not the first use of IR heaters for simulating heat waves. Harte et al. (1995) presented the first heat wave experiment using infrared heaters, in which the device emitted radiation over a linear space with fixed power throughout the experiment. Later Nijs et al. (1996) introduced a proportional control to maintain a fixed temperature increase in the heated plot compared to the reference plot. Kimball (2005) further improved this by introducing a PID-type controller. He modified the reflector of an infrared heater to expand the coverage area, thus effectively heating a sorghum (*Sorghum vulgare* Pers) cover measuring 1 m wide by 1.5 m long at a height of 60 cm. The researchers concluded that this system offered a radiation angle of 67°. In subsequent research, Kimball et al. (2008) conducted a thorough analysis with the goal of uniformly heating a sorghum plot. Using six heaters positioned at the corners of a hexagon inscribed in a circular plot 3 m in diameter, tilted 45° downward and placed at a height of 1.2 m, it was determined that the optimal ratio between the coverage diameter and height was 0.8. However, this study did not mention the radiation angle. Given that our heaters had a radiation angle of 120° and there was uncertainty about their effectiveness, different configurations were explored. These tests revealed that thermal homogeneity was not a critical factor, as many of the configurations evaluated provided satisfactory results. In the interest of improving future experiments, it is important to note that achieving proper homogeneity involves having identical plots with the same physical and environmental factors: orientation of all elements, shadows and therefore dummy heaters, level of lighting, placement of sensors and heaters, etc. Additionally, it would be beneficial to adopt innovative strategies that ensure uniform heating throughout the plot. A possible improvement could be the use of thermal cameras to visualize and adjust the heat distribution, thereby optimizing the uniformity of the thermal treatment on the plants.

4.2. Physiological effects of combined drought and simulated heat waves

In line with Xie et al. (2020), no significant differences in RWC between IC and DHW treatments for some species were found. However, in agreement with previous observations by Davies et al. (2018), in three cases (sugar beet in Experiment 1 and sunflower in both experiments) RWC values dropped well below 65%, i.e., a value defined by Lawlor and Cornic (2002) as the threshold for complete photosynthesis inhibition in C3 species. Nevertheless, while net CO_2 assimilation generally decreased during heat wave and drought, highlighting the severe impacts of these combined stresses, it did not reach zero, as suggested by Lawlor and Cornic (2002), indicating that plant physiology under a heat wave may differ from that during simple drought. In fact, except for sugar beet during Experiment 1 and Limonium, A_N depression due to combined drought and heat wave was moderate, in line with previous findings in other species by Haworth et al. (2018) and Rashid et al. (2018). In sunflower during Experiment 1, A_N under DHW remained at IC levels despite the very low RWC observed. As previously mentioned, in DHW Limonium, A_N even increased at T2 (drought + heat wave) compared to T1 (drought only).

In the case of sugar beet and sunflower, it is remarkable that the same species grown from the very same seed batch displayed such different results between the two experiments. However, it has to be considered that, while most growing conditions were identical, the heat wave cycles in both experiments differed and plant age at the onset of treatments differed as well (sugar beet plants were ca. 6.5 months-old in Experiment 1 and only 2.5 months-old in Experiment 2; while sunflower was 1.4 months-old in Experiment 1 and ca. 3 months-old in Experiment 2; being tobacco 2.8 months-old and Limonium 1.5 years-old). Additional differences were the natural conditions outdoors or in control plots that the heat wave plots tried to follow adding at each moment the programmed temperature increment, and slight differences in fertilization treatments among years. The different responses observed for each of these two single species despite the relatively small differences in their conditions among the two experiments points to the large complexity of genotype x environment interactions in determining the plant's responses to these stresses.

Interestingly, the electron transport rate (ETR), which is a chlorophyll fluorescence-based indicator used to assess the photochemical and biochemical stability of photosynthesis independently of gas exchange estimates of net CO_2 assimilation, was often not greatly affected by the combined stresses. This suggests that stomatal limitations were the most significant, but again, in a species—and experiment—dependent manner. More intriguingly, in some cases, during recovery (i.e., the simultaneous alleviation of heat wave and drought), ETR of the stressed plants reached values above those of the control plants, most notably in the case of sugar beet in Experiment 1, whose RWC had dropped below 40%. An increase in photosynthetic capacity was shown by Galmés, Medrano and Flexas (2006) in response to long-term plant acclimation to drought and by Galle et al. (2011) in response to repeated irrigation-drought cycles. However, to the best of our knowledge, such an increase upon recovery from combined drought and heat wave has never been reported.

It should be mentioned that an inherent problem of heating experiments is that the relative humidity (RH) and vapour pressure deficit (VPD) surrounding the treatment plants is also changed. As an example, such changes were observed during experiment 2 (Supplementary Material S4). It can be observed that this problem is much larger during the night than during the day, likely because plants almost do not transpire during the night, so that air temperature is the major driver of RH and, thus, VPD. During the day, although RH was different among treatments, VPD was almost identical in two out of five days and much less increased than RH in heatwave plants than in controls. Since VPD and not RH is the main driver of plant physiological responses, the fact that VPD changes less than RH among treatments suggest that the major observed physiological effects is the heat-wave-related different temperature, but nevertheless it cannot be ruled out that a fraction of them is due to different VPD rather than to different temperature. To the best of our knowledge, this is a limitation of all the systems described to simulate heat waves. Nevertheless, De Boeck et al. (2010), reported that large increases in VPD are a common feature of real natural heat waves.

The insights from the preliminary experiments presented here on the effects of heat waves on plant physiology, using the instrument presented in this study, reflect the potential impact of heat waves on plants, the high heterogeneity in their responses, and even the possibility that investigating heat wave responses could challenge our current perspectives on plant stress responses. The diversity of physiological responses observed within a very limited range of observations, even within the same species under slightly different experimental conditions, highlight the need for intense research in this area on different conditions and species. The affordable device presented here offers an economic and easy opportunity for addressing such research.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2505160945544071/plantecophys-580-Supplementary-final.pdf.

Author Contributions

FC-M directed the development of the device, its implementation in the experiments, and wrote the manuscript with JF. JF conceived the idea and designed experiments 1 and 2. KL conducted the device field testing. MR-O conducted experiment 1 and ÁV conducted experiment 2, with help from MDC, JC and AJF. LC-C developed the control software and assisted in developing the device. MR-C contributed to writing the manuscript and supervised experiments 1 and 2, as well as the overall manuscript preparation. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare that they have no competing interests.

Peer Review Statement

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