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High Temperature and Elevated CO_2 Modify Phenology and Growth in Pepper Plants

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Abstract: The aim of this study was to determine the effect of temperature and CO_2 on seed emergence, seedling quality, and phenological stage of *Capsicum chinense* and *Capsicum annum* cultivated in four controlled growth chambers (C1: 30 °C and 400 µmol CO_2 mol^{-1}; C2: 40 °C and 1200 µmol CO_2 mol^{-1}; C3: 30 °C and 1200 µmol CO_2 mol^{-1}; C4: 40 °C and 400 µmol CO_2 mol^{-1}). Neither temperature nor elevated CO_2 influenced seed emergence, although differences were observed in seedling mortality, with high temperature affecting seedling survival in both species; the mortality rate at 40 °C was 20 and 53% in *C. annuum* and 45 and 58% in *C. chinense* at 400 and 1200 µmol CO_2 mol^{-1}, respectively. Differences were also observed in growth parameters, where positive effects were observed on leaf area, which reached 45.9 cm^2 in *C. annuum* and 23.9 cm^2 in *C. chinense* with elevated CO_2 at 30 °C, but negative effects were observed with high temperature. CO_2 enrichment increased flower and fruit production per plant. However, high temperature delayed flower phenology, increased flower abortion and inhibited fruit set. Elevated CO_2 counteracted the detrimental effects of high temperature on growth parameters and flower number, but this was not sufficient to prevent flower abortion and the detrimental morphological characteristics of fruit caused by a temperature of 40 °C.

Keywords: *Capsicum* spp.; climate change; CO_2 enrichment; elevated temperature; emergence; plant growth

1. Introduction

In recent decades, global warming caused by the increase in the concentration of greenhouse gases (CO_2, carbon dioxide; CH_4, methane; N_2O, nitrous oxide; HFCs, hydrofluorocarbons; PFCs, perfluorocarbons; and SF6, sulfur hexafluoride), mainly attributed to anthropogenic activities, has led to a change in climate conditions, altering precipitation patterns and intensifying desertification in many regions of the planet [1,2]. Climate change represents an important threat to agricultural production in the tropics, where the major factors limiting crop productivity are high temperatures and drought [3], given that they cause the loss of over 49% of world food production, subjecting the agricultural sector to the negative effects of climate change [4,5].

The increase in global temperatures could have a considerable effect on the phenology, anatomy, morphology and physiology of plants and have negative effects on CO_2 assimilation, respiration, growth and reproductive processes [6–8], causing crop damage related to temperature stress and endangering food security [3,9].

As temperature regulates plants' physiological processes, acting as a determining factor for germination, seed formation, flowering and fruiting, it is important to consider the adverse effects of climate change [10], especially in widely consumed tropical crops with economic and agricultural significance such as vegetable species, due to their high nutritional value and important contribution to everyday diets [11].

Changes in temperature and atmospheric CO_2 concentration (C_a) produce important modifications to seasonal rainfall patterns, climate, and the frequency and duration of raised temperatures [12]. There has been a clear increase in C_a, with levels of the gas currently fluctuating around 416 μmol mol^{-1} [13], and this trend will continue for many years [14]. Nevertheless, it has been demonstrated that CO_2 has a beneficial effect on plants, especially in CO_2-enriched environments, by improving photosynthetic efficiency, reducing transpiration losses, stimulating general growth [15], and turning on adaptive mechanisms in plants such as biomass generation and physiological and morphological changes, thus strengthening plants' thermotolerance to achieve better adaptation to climate conditions [16–19].

Cultivable plants will be the most affected by the adverse effects of climate change because a decrease in net production yield is expected in many agricultural zones of the planet. Moreover, ecophysiological research has focused on evaluating individual climate variables with a limited approach to the interaction between them [20], meaning that understanding how the interaction of these variables (temperature and C_a) influences phenological, physiological and growth characteristics is considered of significant importance. In this regard, the genus *Capsicum*, one of the most widely cultivated crops in the world due to the economic and nutritional value of its species, is expected to be affected by temperature [21] and benefited by the increase in C_a [22], so understanding how these variables interact would permit a more accurate prediction of how *Capsicum* spp. will respond to climate change. In this sense, we hypothesized that the high temperature will affect phenology and growth in pepper plants, but elevated CO_2 concentration will mitigate the negative impact caused by high temperature. Therefore, the aim of this study was to determine the phenological and physiological responses (seed emergence, seedling quality, phenological stage: growth and flowering, seed set and fruit production) of *Capsicum annuum* and *Capsicum chinense* under the effect of high temperature and CO_2 enrichment, cultivated in four growth chambers controlling temperature (30 and 40 °C) and CO_2 (400 and 1200 μmol CO_2 mol^{-1}).

2. Materials and Methods

2.1. Location, Plant Material, Crop Management

The present research was conducted in the experimental area of Tecnológico Nacional de Mexico, Campus Conkal, Yucatan, Mexico. As plant material, two of the most commercialized species of the genus *Capsicum* in the region were used, a sweet pepper (D40 variety, *Capsicum annuum*) and a hot pepper (habanero pepper, Jaguar variety, *Capsicum chinense*). To evaluate seed emergence and seedling growth during the nursery stage, 400 seeds of each species were sown in polystyrene trays using peat moss as substrate (Sunshine, Proveedora Agrícola, Guadalajara, Mexico). Once the seeds emerged, seedlings were kept at field capacity by daily watering, and once the plants reached the first pair of true leaves, they were fertilized with Steiner nutrient solution (50%) twice a week until 45 days after sowing (das). The seeds and seedlings were cultivated in growth chambers and subjected to different temperatures (30 and 40 °C) and CO_2 concentrations (400 and 1200 μmol CO_2 mol^{-1}) during the experiment (some seedlings evaluated during the nursery stage died as a consequence of high temperatures applied in the evaluated treatments).

To evaluate reproductive phenology, seedlings at 40 das were transplanted into polystyrene black bags with a capacity of 10 L using a mix of dark soil with peat moss

as substrate (3/1: *v*/*v*) disinfected with formaldehyde (10%). Plants were rotated once a week to avoid border effects. Plant nutrition throughout the experiment was provided with Steiner nutrient solution (50, 75, 100%) according to the phenological development stage and plant nutritional requirements (from seedling to fruiting). Insecticides, acaricides and fungicides were applied preemptively.

2.2. Growth Chambers and Treatments

Four growth chambers were used for the experiment, placed under a white greenhouse plastic roof structure permitting the passage of a photon flux density of up to 1100 μmol m^{-2} s^{-1}. Natural light was used throughout the experiment, with an approximate photoperiod of 12/12 (light/dark), the first photons were recorded at 6:30 h, with a gradual increase until reaching 1100 μmol m^{-2} s^{-1} at noon, which was maintained between 12:00 and 15:00 h; later, the photon flux density gradually decreased until dark (around 18:30 h). Each chamber was completely enclosed, structured with transparent glass, measuring 3 m long by 2.5 m wide and 2.2 m high. To control temperature, each chamber had a 12,000 BTU air conditioner (Split Mirage, model X2, Merida, Mexico) modified with an external thermostat. The thermostat sensor was located above the plant canopy. C_a was regulated with a Telair sensor (T6713) connected to an Arduino microprocessor (Telaire 7001, St. Marys, PA, USA) activating a solenoid valve connected to the hose of the CO_2 cylinder. Relative humidity was controlled with a 25 L capacity dehumidifier (Hisense, DH50K1W, Mexico City, Mexico), and a 12-inch rotating fan homogenized the air inside the chambers. Climate conditions were monitored inside each chamber with dataloggers (HOBO H08-004-02, Onset Computer Corp., Bourne, MA, USA). The conditions of the chambers were set as follows: Chamber 1, 30 °C and 400 μmol CO_2 mol^{-1} (Control); Chamber 2, 40 °C and 1200 μmol CO_2 mol^{-1}; Chamber 3, 30 °C and 1200 μmol CO_2 mol^{-1}; Chamber 4, 40 °C and 400 μmol CO_2 mol^{-1}. At night, all chamber conditions were kept the same for all treatments (average temperature = 26 °C, RH = 70%). Chamber 1 was the control, because 30 °C and 400 μmol CO_2 mol^{-1} are similar to diurnal average temperature and atmospheric CO_2 concentration in Yucatan. In all treatments, both temperature and atmospheric CO_2 had an error range of $\pm$2 °C and $\pm$50 μmol CO_2 mol^{-1}, respectively.

2.3. Seedling Emergence and Seedling Growth Parameters

Seedling emergence was evaluated according to Hernández-Pinto et al. [23] with daily counting for 7 days from sowing, where the emergence percentage (%E) equaled the total number of germinated seeds at the end of the experiment (n) divided by the total number of sown seeds (N) multiplied by 100. Seed emergence was evaluated for 15 days from sowing. From day 16, the mortality percentage was counted from the total emerged seeds.

Evaluations of the seedling growth parameters were carried out at 35 das according to Garruña-Hernández et al. [21]. The height was measured with a measuring tape from the base of the stem to the apex, the stem diameter was measured with a digital caliper at the stem base, leaf area was measured with an area meter (LI-3100, LI-COR, Inc. Lincoln, NE, USA), root volume was determined by displacement using a graduated cylinder, and to obtain the organs' biomass, the tissue was dried in a convection oven at 70 °C for 72 h.

2.4. Flowering and Fruiting Parameters

The presence of flower and fruit buds was recorded daily to evaluate flowering and fruiting until the plants reached 100% of flowering and fruiting, respectively. The number of flower abortions was estimated as the number of flowers minus the number of fruits, according to Garruña-Hernández et al. [24].

To evaluate the weight, diameter, and length of fruits, 100 fruits per treatment collected in the first two harvests were used. A digital balance (Ohaus Adventurer-420, Mexico City, Mexico) and a caliper were used to measure them. It was not possible to carry out the measurements in chamber 4 (C4 = 40 °C with 400 μmol CO_2 mol^{-1}) due to a lack of fruits.

2.5. Experimental Design and Statistical Analyses

The experimental design was completely random with a bi-factorial arrangement, using air temperature (30 and 40 °C) and atmospheric concentration of CO_2 (400 and 1200 μmol CO_2 mol^{-1}). In the evaluation of seed emergence and seedling growth, 100 seeds per species were used as an experimental unit, using five repetitions per treatment. To evaluate reproductive phenology, 20 plants were used as the experimental unit. Percentage data were transformed with the arcsine of the square root. A two-way analysis of variance (two-way ANOVA, $p \leq 0.05$) was performed for all data. Where significant differences were found, a means comparison test (Tukey, $\alpha = 0.05$) was conducted. Statistical analyses were conducted with Infostat (2019) and plotted with SigmaPlot 11.0.

3. Results and Discussion

3.1. Seedling Emergence

The emergence of *C. annuum* was faster during the initial days in the treatments at 40 °C (C2 and C4) regardless of the CO_2 concentration, with statistically significant differences present at 6 das. However, from day 7, there were no statistically significant differences in emergence among any treatments of this species (Figure 1A). This was reflected in the cumulative emergence, where all treatments reached at least 95% emergence at 11 das (C1 = 95, C2 = 98, C3 = 96 and C4 = 98%). There were no significant differences observed in the emergence of *C. chinense* (Figure 1B). Cumulative emergence was between 81 and 89% (C1 = 89, C2 = 86, C3 = 81 and C4 = 89%).
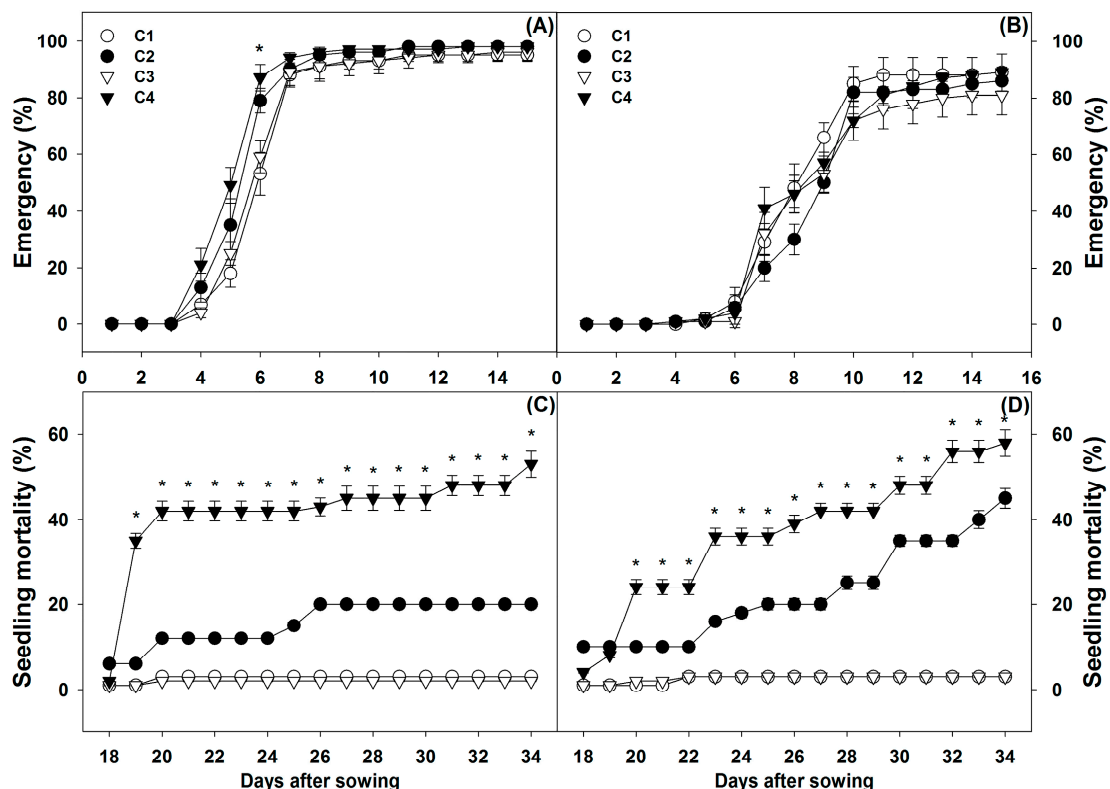

Figure 1. Emergence and mortality of seedlings of *C. annum* (**A**,**C**) and *C. chinense* (**B**,**D**) cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 μmol CO_2 mol^{-1}, C2 = 40 °C and 1200 μmol CO_2 mol^{-1}, C3 = 30 °C and 1200 μmol CO_2 mol^{-1}, C4 = 40 °C and 400 μmol CO_2 mol^{-1}. Data are means $\pm$ SE; * = statistically significant differences (2-way ANOVA $p \leq 0.05$, $n = 100$).

Therefore, the interaction between temperature and CO_2 did not have a significant effect on the final emergence of either species of *Capsicum* evaluated. In contrast, a significant increase in the percentage of emergence was observed in *Bouteloua gracilis* with the

combination of seven temperatures with alternating 12/12 h periods (10/0, 12.5/2.5, 15/5, 20/10, 25/15, 30/20, 35/25 °C) and CO_2 (385 and 600 µmol CO_2 mol^{-1}) [18]. Moreover, [25] mention that CO_2 concentration did not have a significant effect on *Arabidopsis thaliana* seed emergence, with seeds subjected to high temperatures exhibiting the fastest germination rate. In the present work, temperature was essential in the regulation of seed emergence; when the temperature increased, so did the emergence rate, due to temperature acting as a determining factor for enzyme activation or deactivation processes [26,27]. The physiological response of seeds to this factor is crucial for emergence and plant development, and once the optimum temperature point is reached, the emergence process diminishes. Therefore, temperatures under or over the maximum optimum can inhibit emergence, risking the life and survival of seedlings [18,27,28].

Mortality of some plants started from 18 das, which coincided with the appearance of the second pair of true leaves. In *C. annuum*, only 3 and 2% of seedling mortality was observed at 400 (C1) and 1200 µmol CO_2 mol^{-1} (C3), respectively (Figure 1C). However, at 40 °C, seedling mortality was 20 and 53% at 1200 (C2) and at 400 µmol CO_2 mol^{-1} (C4), respectively (Figure 1C). A similar trend to *C. annuum* was observed in *C. chinense*, where mortality reached 3% in C1 and C3. At 40 °C, mortality of plants reached 45 and 58% in C2 and C4, respectively. The results show that in these species, high temperature and CO_2 enrichment do not have any effect on the emergence or the first days of life of seedlings (when they are still dependent on the cotyledon). Nevertheless, when the first pair of photosynthetic leaves appeared, we could observe damage (delayed growth, color changes, flaccid tissue) due to the effect of high temperatures. The appearance and development of the second pair of photosynthetic leaves coincided with the onset of seedling mortality regardless of the CO_2 concentration.

The appearance of functional leaves, capable of performing gas exchange with the environment, probably favored the negative effects of high temperature on seedlings, due to the fact that an increase in leaf area raised transpiration capacity, and also due to the warm environment, causing the onset of heat stress problems caused by temperature in the seedlings. In *Capsicum*, it has been observed that, in high-temperature atmospheres, plants increase their transpiration rate in order to reduce leaf area temperature and be able to maintain an optimum temperature range in order to protect their photosynthetic mechanism [21]. It is likely that two-to-three-week old seedlings do not yet possess suitable characteristics to survive this kind of stress. Furthermore, CO_2 enrichment did not prevent seedling mortality due to the effect of high temperature. Similar cases have been observed in other species. Some authors [29] mention that the emergence in *Asteraceae* genus species occurred in a wide range of temperatures, but was inhibited from 35 °C, presenting only 25% emergence. The *Capsicum* species studied here probably show thermotolerance, such as a preference for high temperatures, allowing them to adapt better. However, it is likely that other species present a higher sensitivity to high temperatures, causing an inhibition of germination.

3.2. Seedling Growth

Statistically significant differences were found in both species in all parameters evaluated to determine seedling growth and quality. The leaf area of *C. annuum* seedlings in the chamber at 30 °C and 1200 µmol CO_2 mol^{-1} (C3) was greater than in the other treatments, and a temperature at 40 °C decreased the seedlings' leaf area at both 400 (18.4 cm^2) and 1200 µmol CO_2 mol^{-1} (22 cm^2) (Figure 2A). In *C. chinense*, seedling leaf area in C3 (23.9 cm^2) was also statistically greater than in other treatments, although there were no significant differences in this species between the chamber at 30 °C and 400 µmol CO_2 mol^{-1} and those at 40 °C (19 and 16.2 cm^2 at 400 and 1200 µmol CO_2 mol^{-1}, respectively) (Figure 2B).
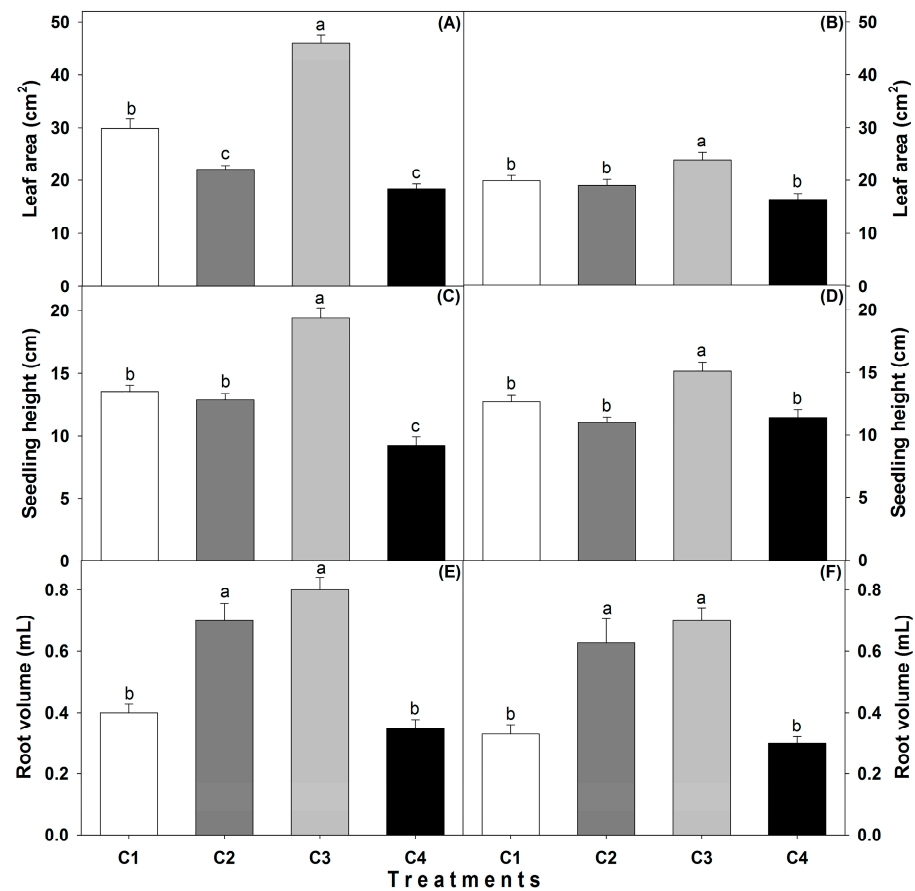
Figure 2. Leaf area, height and root volume of *C. annum* (**A,C,E**) and *C. chinense* (**B,D,F**) seedlings cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 μmol CO_2 mol^{-1}, C2 = 40 °C and 1200 μmol CO_2 mol^{-1}, C3 = 30 °C and 1200 μmol CO_2 mol^{-1}, C4 = 40 °C and 400 μmol CO_2 mol^{-1}. Data are means ± SE. Different letters indicate statistically significant differences among treatments (Tukey, $p \leq 0.05$, $n = 20$).

In both species, the positive effect of elevated CO_2 was observed at a comfortable temperature (30 °C). However, in *C. annuum*, the negative effect of high temperature was observed compared to seedlings in the control chamber (C1 = 30 °C and 400 μmol CO_2 mol^{-1}). This was not found in *C. chinense*, probably because these seedlings are less leafy and have a slightly lower growth rate than *C. annum*. Thus, Wullshleger et al. [30] suggest that plants exposed to CO_2-enriched environments are subjected to important trade-offs, either to increase leaf size or to reduce stomatal conductance, which could explain the differences in the leaf area of the two species. On the other hand, in both species, it was found that seedling root volume of plants cultivated in CO_2-enriched chambers (C2 and C3) statistically exceeded that of plants cultivated at 400 μmol CO_2 mol^{-1} (Figure 2E,F). As opposed to what occurred with aerial growth variables (leaf area and height), root volume at 40 °C and 1200 μmol CO_2 mol^{-1} was statistically similar at 30 °C and 1200 μmol CO_2 mol^{-1}. In this case, the substrate watered at field capacity was probably acting as a temperature buffer, with the plant had a major concentration of atmospheric CO_2 available to be used to send photoassimilates to the root zones. CO_2 enrichment allows the plant to better distribute photoassimilates [19]. Furthermore, the two evaluated species (*C. annuum* and *C. chinense*) are tropical species that are well adapted to the regional climate conditions, meaning that they have a wider optimum temperature range than species adapted to mild climates and likely possess tolerance mechanisms for these climate conditions [31]. Additionally, Rahman et al. [32] indicated that high temperatures can modify the composition and structure of cell membranes, pointing out that the ability of species to adapt to temperature stress requires a physiological adaptation to such stress.

In both species, seedling dry biomass in C3 (0.36 and 0.24 g) was statistically greater than in the other treatments. Nevertheless, for this variable, the increased CO_2 concentration in C2 (40 °C and 1200 µmol CO_2 mol^{-1}) (0.16 and 0.19 g for *C. annuum* and *C. chinense*, respectively) counteracted the negative effects of C4 (40 °C and 400 µmol CO_2 mol^{-1}) (0.12 g in both species), and was even higher than that of seedlings of *C. chinense* in C1 (0.15 g) (Figure 3). This was likely due to the increase in root biomass in the chambers with elevated CO_2. In a study conducted on *Eucalyptus* spp., major growth was found in environments with high temperature and CO_2 enrichment, highlighting that biomass allocation differs among species and also among the organs of the plants [33]. In contrast, Marcos-Barbero et al. [19] suggest that temperature and CO_2 are two factors that must be evaluated simultaneously, adding that selection of genetic varieties is a strategy that can be used to select crops well adapted to future climate change conditions, given that species exist that are better adapted to extreme climate conditions and in which temperature can even accelerate seed emergence and increase leaf area and biomass allocation, as well as other phenological and physiological traits of plants [34].
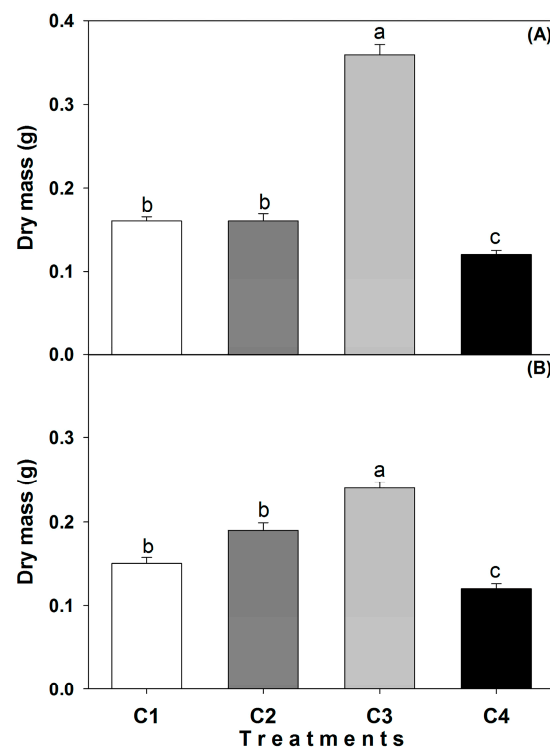

Figure 3. Seedling biomass of *C. annuum* (**A**) and *C. chinense* (**B**) cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 µmol CO_2 mol^{-1}, C2 = 40 °C and 1200 µmol CO_2 mol^{-1}, C3 = 30 °C and 1200 µmol CO_2 mol^{-1}, C4 = 40 °C and 400 µmol CO_2 mol^{-1}. Data are means ± SE. Different letters indicate statistically significant differences among treatments (Tukey, $p \leq 0.05$, $n = 20$).

3.3. Flowering and Fruiting

In *C. annuum* plants, the presence of flower buds started at 59 das (19 days with high temperature and CO_2-enrichment treatment). Two days later (61 das), 90% of plants had flower buds, and no statistically significant differences were found among treatments (Figure 4A). On the other hand, *C. chinense* plants did exhibit statistically significant differences among treatments, with plants at 30 °C (C1 and C3) presenting the first flower buds at 58 das (18 das with high temperature and CO_2-enrichment treatment), whereas the plants of C2 (40 °C and 1200 µmol CO_2 mol^{-1}) and C4 (40 °C and 400 µmol CO_2 mol^{-1}) showed the presence of flower buds at 65 and 63 das, respectively. In C1 and C3, 90% of plants presented flower buds at 64 and 66 das, respectively, while those in C2 and C4 reached a similar value at 72 and 80 das, respectively (Figure 4B).
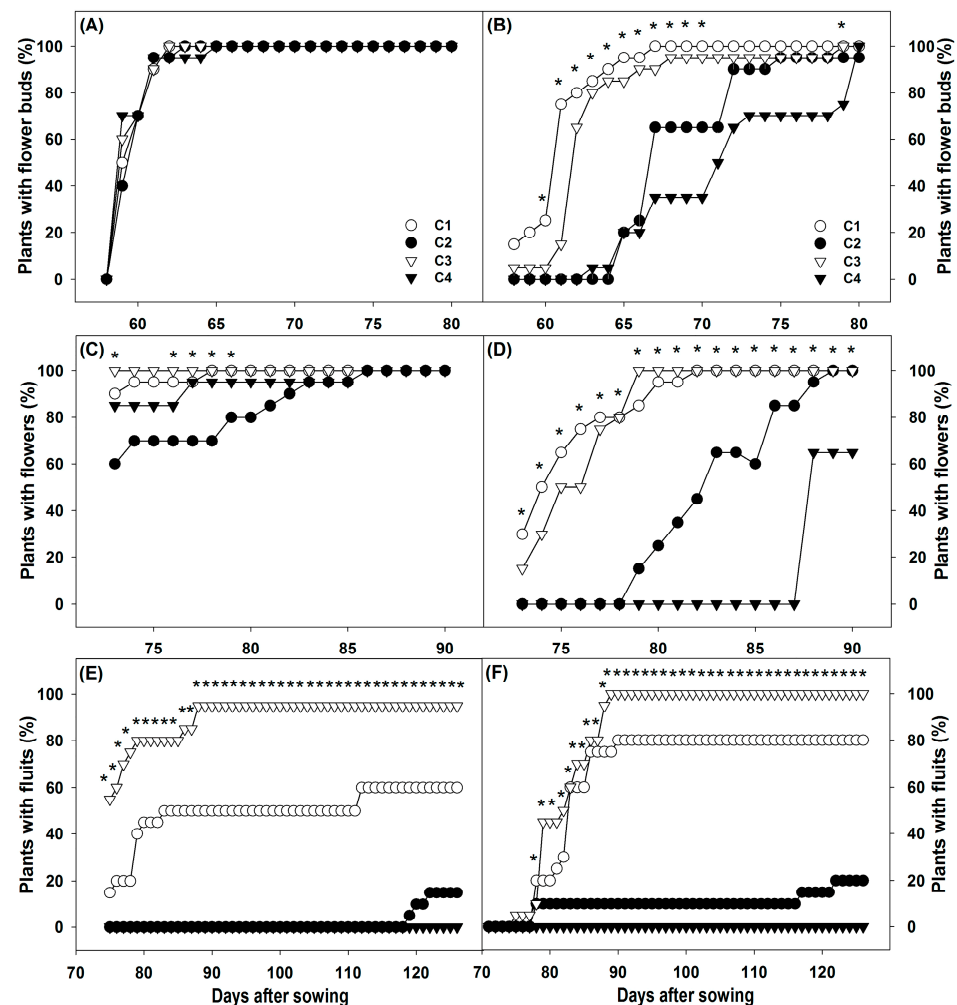
Figure 4. Plants with flower buds, plants with flowers and plants with fruits of *C. annuum* (**A**,**C**,**E**) and *C. chinense* (**B**,**D**,**F**) cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 μmol CO_2 mol^{-1}, C2 = 40 °C and 1200 μmol CO_2 mol^{-1}, C3 = 30 °C and 1200 μmol CO_2 mol^{-1}, C4 = 40 °C and 400 μmol CO_2 mol^{-1}. Data are means $\pm$ SE. * = statistically significant differences (two-way ANOVA $p \leq 0.05$, $n = 120$).

C. annuum plants showed 100% flowering at 78, 86, 73 and 86 das in C1, C2, C3 and C4, respectively (Figure 4C). Whilst statistically significant differences were found in *C. chinense* throughout the experiment, the negative effect of high temperature on this species was more pronounced, since a delay of 5 and 15 days, respectively, was found in flower phenology compared to the chambers at 30 °C (Figure 4D).

Fruit appearance in *C. annuum* started at 75 das in C1 and C3 and at 119 das in C2, but there was no formation of fruits in C4. At 112, 122 and 88 das, 60, 15 and 95% of plants had a presence of fruits in C1, C2 and C3, respectively (Figure 4E). In *C. chinense*, fruits appeared from 78 (C1 and C2) and 75 (C3) das. As in *C. annuum*, there was no presence of fruits in C4. Fruiting at 100% was only shown in C3, while C1 and C2 showed only 80 and 20%, respectively (Figure 4F). In this regard, Kim et al. [35] mention that *Phalaenopsis* plants exposed to over 800 μmol CO_2 mol^{-1} before flower spike induction had higher biomass production. However, they also mention flower production and the number of flower buds could be reduced in plants subjected to long CO_2 exposure. Additionally, Chaturvedi et al. [36] point out that in rice cultivars, the use of species with high temperature tolerance could reduce the negative impact of heat stress during flowering.

Generally, *C. annuum* plants had fewer flowers and fruits than *C. chinense* due to the size difference between the two genotypes. In *C. annuum*, elevated CO_2 increased flower

production (C2 = 12 flowers; C3 = 12.88 flowers) (Figure 5A). Nevertheless, plants in C2 (40 °C and 1200 µmol CO_2 mol^{-1}) had just two fruits per plant, whilst plants in C3 (30 °C and 1200 µmol CO_2 mol^{-1}) had nine fruits per plant (Figure 5C). A similar trend was found in *C. chinense*, where C3 was statistically superior to all treatments with 42 flowers and 24 fruits per plant (Figure 5B,D). In both species, flower production in C4 was statistically lower than the other treatments, and the presence of fruits was not observed (Figure 5C,D), indicating that 100% of plants aborted at 40 °C. Plants in C3 had the fewest aborted fruits (*C. annuum* = 42 aborted flowers and *C. chinense* = 30 aborted flowers) (Figure 5E,F). These results agree with those reported by [24], who mention that CO_2 has a positive effect on flowering, and, therefore, on the number of fruits, which implies a yield improvement. In addition, Xu et al. [37] indicated that treatments with CO_2-enriched environments promoted flowering in *Gerbera jamesonii*. Likewise, Meneses-Lazo et al. [38] mentioned that habanero pepper has a good rate of carboxylation, promoting flower and fruit production. In this regard, Marcelis et al. [39] remark on the importance of evaluating the phenological state and the developmental stage of plants, and that fruit abortion could be linked to the sink-source balance of the plant. Furthermore, Garruña et al. [24] point out that temperature affects the reproductive cycle of plants, causing precocity or delays, including the inhibition of flowers and fruits, but they also mention that elevated CO_2 availability decreases the number of aborted flowers.
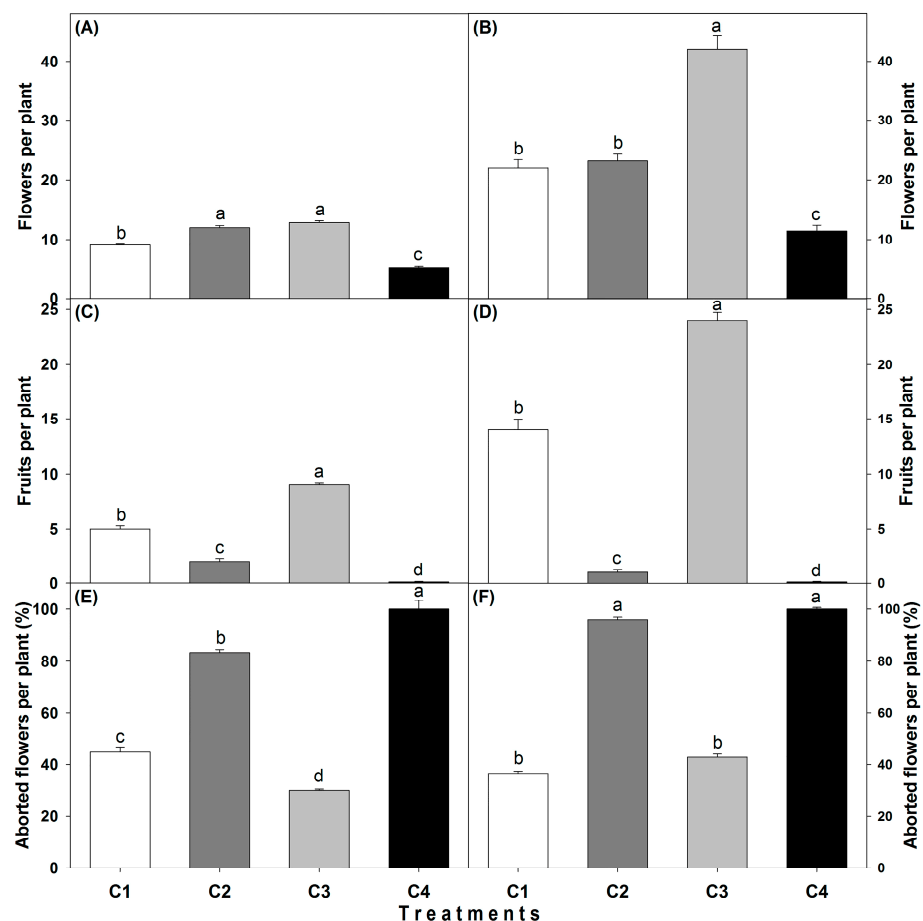

Figure 5. Number of flowers per plant, number of fruits per plant and flower abortion in *C. annum* (**A,C,E**) and *C. chinense* (**B,D,F**) plants cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 µmol CO_2 mol^{-1}, C2 = 40 °C and 1200 µmol CO_2 mol^{-1}, C3 = 30 °C and 1200 µmol CO_2 mol^{-1}, C4 = 40 °C and 400 µmol CO_2 mol^{-1}. Data are means $\pm$ SE calculated at the first harvest of fruits. Different letters indicate statistically significant differences among treatments (Tukey, $p \leq 0.05$, $n = 32$).

In *Capsicum annuum*, fruit weight was 21.5 and 22 g in C1 and C3, respectively, whilst a lower weight was found in C2 (6.66 g) (Figure 6A). Stem diameter was 4.2 and 4.4 cm in C1 and C3, respectively, while in C2 it was 2.8 cm (Figure 6C). Fruit length in C1 and C3 was 4.5 and 4.7 cm, respectively (Figure 6D), whilst fruits in C2 reached a length shorter than 2 cm (1.8 cm).
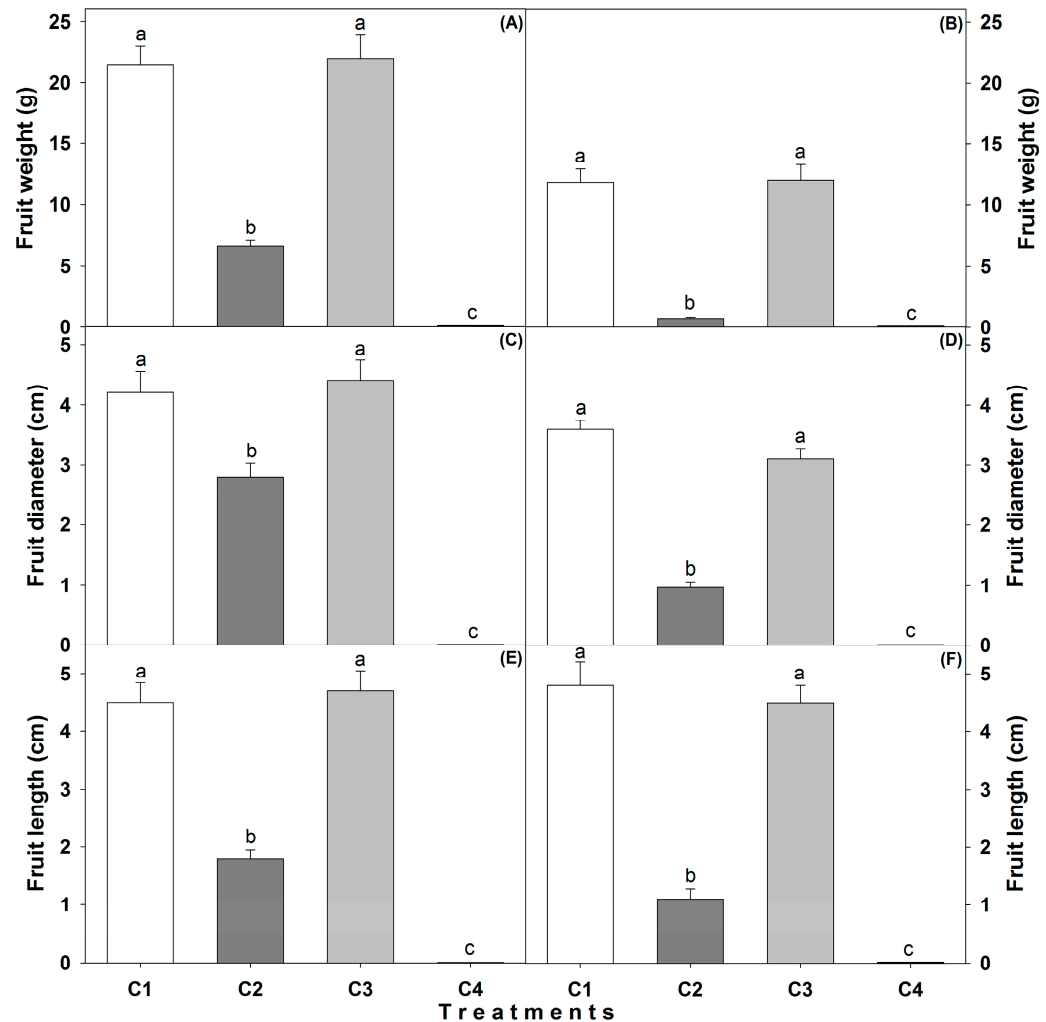

Figure 6. Fruit weight, diameter and length in *C. annum* (**A,C,E**) and *C. chinense* (**B,D,F**) cultivated under different temperatures and CO_2-enriched atmospheres. C1 = 30 °C and 400 µmol CO_2 mol^{-1}, C2 = 40 °C and 1200 µmol CO_2 mol^{-1}, C3 = 30 °C and 1200 µmol CO_2 mol^{-1}, C4 = 40 °C and 400 µmol CO_2 mol^{-1}. Data are means ± SE calculated at the first harvest of fruits. Different letters indicate statistically significant differences among treatments (Tukey, $p \leq 0.05$, $n = 100$).

In *C. chinense*, fruit weight was 11.83 and 12 g in C1 and C3, respectively, whereas the lowest value was found in C2 (0.74 g) (Figure 6B). Stem diameter was similar in C1 and C3 (3.6 and 3.10 cm, respectively), while in C2, it did not reach 1 cm (0.97 cm) (Figure 6D). Fruit length reached levels similar to *C. annuum* in C1 and C3 (4.8 and 4.5 cm) and C2 (1.1 cm) (Figure 6E). The negative effect of high temperature (40 °C) was evident, especially in C4, where the increase in temperature led to inhibited fruit growth. Furthermore, an increase in fruit weight, stem diameter and fruit length was found in seedlings established in a comfortable environment at 30 °C (C1 and C3). The trend observed for both species in C1 and C3 could indicate that the plants presented a better response at a comfortable temperature (30 °C) in spite of CO_2 enrichment, given that although these kinds of crops are adapted to tropical climate conditions, an optimum temperature is able to provide the plants with a better capacity to adapt and develop. The results obtained are similar to those

in [40] in *Solanum lycopersicum* Mill, who obtained the highest number of fruits per plant and the highest fruit weight in environments with supplemented light and CO_2-enrichment.

4. Conclusions

Evidently, modifying the atmospheres led to changes in the phenological rhythm of the plants. Although neither temperature nor CO_2 influenced seed emergence, differences in seedling mortality were found, with high temperature affecting seedling survival, as well as in growth parameters, where positive effects were found in the presence of atmospheric CO_2 enrichment (30 °C and 1200 µmol CO_2 mol^{-1}) and negative effects were associated with high temperature (C2 = 40 °C and 1200 µmol CO_2 mol^{-1}; C4 = 40 °C and 400 µmol CO_2 mol^{-1}). CO_2 enrichment increased flower and fruit production per plant. However, high temperature delayed flower phenology, increased flower abortion and inhibited fruiting. Elevated CO_2 counteracted the harmful effects of high temperature, but not to an extent sufficient to avoid flower abortion and detrimental morphological features of fruit caused by a temperature of 40 °C. Due to the vulnerability faced in response to climate change, it is essential to further investigate plant responses to future climate change scenarios in order to understand plasticity and requirements, especially in crops cultivated in tropical regions where greater damage is expected for these kinds of crops.

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