

Elevated CO₂ Alleviates the Effects of Drought on Wheat - Aphid Interaction

Haicui Xie (✉ hczie2008@126.com)

Hebei Normal University of Science and Technology

Fengyu Shi

Hebei Normal University of Science and Technology

Jingshi Li

Hebei Normal University of Science and Technology

miaomai Yu

Hebei Normal University of Science and Technology

Jia Fan

IPPCAAS

Shengyong Wu

IPPCAAS

Haiyun Xu

IPPCAAS

Research Article

Keywords: Triticum aestivum, Sitobion avenae, elevated CO₂, drought, nutritional quality, resistance

Posted Date: July 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-526566/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Due to rising concentration of atmospheric CO₂, climate change is predicted to intensify episodes of drought, however, our understanding of how combined environmental conditions will influence crop-insect interactions is limited. The direct effects of elevated CO₂ and drought stress on wheat (*Triticum aestivum* L.) nutritional quality, insect resistance and their indirect effects on the grain aphid (*Sitobion avenae*) performance are reported here. Elevated CO₂ was able to alleviate low water content in wheat caused by drought stress. Both elevated CO₂ and drought promoted soluble sugar accumulation in wheat. However, elevated CO₂ decreased and drought increased the amino acid content in wheat. Elevated CO₂ induced the down-regulation of jasmonic acid (JA) -dependent defense, but up-regulated the salicylic acid-dependent defense. Drought enhanced abscisic acid accumulation that promoted the JA-dependent defense in wheat. Aphid-induced phytohormone resistance in wheat was not influenced by elevated CO₂ and drought. The negative effects of drought on the performance of the aphid population was offset by positive effect of elevated CO₂. In conclusion, elevated CO₂ can alleviate the effects of drought stress on wheat nutritional quality and resistance, which results in unchanged damage to wheat from aphid populations under future elevated CO₂ and drier conditions.

Introduction

Elevated CO₂ is considered to be one of the main driving forces of global climate change, and results in increasing air temperature and altered precipitation patterns, which affect soil-water content and plant growth (IPCC, 2014; Li *et al.*, 2014). In agroecosystems, the co-evolution of plants and insects is influenced by distinct environmental factors, study of the effects of multiple environmental factors on plant-insect interactions is necessary to clarify the damage and occurrence trends of insect pests on plants under complex climate change (Murray *et al.*, 2013; Scherber *et al.*, 2013; Zandalinas *et al.*, 2018; Hervé Jactel *et al.*, 2019).

Elevated CO₂ and drought stress significantly influence plant nutritional quality and resistance of plants to insects. Elevated CO₂ levels alter the photosynthetic rate of plants, which further changes their primary and secondary metabolism (Zavala *et al.*, 2017). This change specifically influences the carbon:nitrogen ratio, secondary phytohormone signaling pathways, and insect resistant metabolites in plants (Zavala *et al.*, 2013; Sun *et al.*, 2015; Sun *et al.*, 2016; Guo *et al.*, 2017). Plants also develop various physiological responses for survival under drought stress, such as closing stoma, increasing carbohydrate and amino acid accumulation, and activating phytohormone signaling pathways, etc. (McDowell *et al.*, 2008; Lee & Luan, 2012; Danquah *et al.*, 2014; Ullah *et al.*, 2018; Xie *et al.*, 2019). However, the current understanding of the combined effects of elevated CO₂ and drought on plant-insect interactions is limited. One relevant study indicated that elevated CO₂- and drought-mediated changes in phytochemistry reduced forest tent caterpillar growth and food processing efficiency, the patterns were in a host-species-specific manner (Roth *et al.*, 1997). Previous studies have shown that increases in photosynthetic parameters from elevated CO₂ are diminished by drought (Nackley *et al.*, 2018). However, elevated CO₂ can also

promote stomatal closure, root growth and prolong physiological activity, which may attenuate the negative impact of drought stress on dry biomass, water status and water use efficiency (Wall 2001; Roy *et al.*, 2016; Li *et al.*, 2017; Uddin *et al.*, 2018; Miranda-Apodaca *et al.*, 2018; Li *et al.*, 2019b). Thus, there may be opposing effects of elevated CO₂ and drought on plant growth and development. More work is need to explain the intrinsic mechanisms underlying changes in plant-insect interactions under elevated CO₂ and drought stress.

Aphids are key economic pests that cause extensive feeding damage and transmit viruses for many types of crops (Züst & Agrawal 2016, Blackman & Eastop, 2000). Both crop nutritional quality and defense responses mediated by phytohormones are important factors in aphid feeding, growth, and population size (Sun *et al.*, 2016). Aphids are the most important of the sap sucking insects, the soluble sugar and free amino acids contents found in plant phloem are the main sources of carbon and nitrogen nutrients for aphids (Oehme *et al.*, 2013). Change in phytohormone content is an important index for plant resistance to aphids, and the amount of expression is increased by aphid feeding induction. This induced resistance from phytohormone is common in plants, but the resistance mechanism varies with different aphid species or plant genotypes (Kempel *et al.*, 2011; Züst & Agrawal, 2016). Recent studies have revealed that aphid feeding alters the content of phytohormones (e.g. abscisic acid, ABA; Jasmonic acid, JA; salicylic acid, SA), as well as the expression of defense genes (*AOC*, *LOX*, *PAL*, *PR-1*) related to JA and SA signaling pathways in plant tissues (Guo *et al.*, 2012; Zhang *et al.*, 2017). These phytohormones' signaling pathways work together to regulate plant defense response to aphids.

In this study, we used wheat (*Triticum aestivum* L.) and its key pest aphid (*Sitobion avenae*) to determine whether wheat nutritional quality and resistance to aphid change when grown under combined elevated CO₂ and drought conditions; and whether these changes in the host plants affect aphids performance. The results obtained in this study are expected to be useful to improve the control measures of aphids in wheat production under future elevated CO₂ and drought conditions.

Materials And Methods

Plant preparation and treatment with elevated CO₂ and drought

Six closed-dynamic CO₂ chambers (CDCC, Chen & Ge, 2004) were used in this experiment. The chambers were maintained at 25±1°C, 60-70% relative humidity, and 14 h light /10 h dark photoperiods with 9000 lx fluorescent lamp active radiation levels in each chamber. Three chambers were maintained at ambient CO₂ (~380 ppm) and the other three chambers were maintained at elevated CO₂ (~750±15 ppm). The ambient CO₂ conditions were supplied from the surrounding air entering the environmental chamber facilities, and the elevated CO₂ conditions were supplied from gas tanks. Wheat seeds were planted in pots (7.5 cm diameter x 9.0 cm height) with a sterilized loamy field soil (organic carbon content ~75 g kg⁻¹). Each pot was planted with one seeding. wheat plants were exposed to the CO₂ and drought treatments after seedling emergence. The wheat seeds were irrigated with water, and pot weight was measured per

day to maintain the well-watered treatment (soil moisture was kept at the 80% of field capacity moisture) and drought stress treatment (40% of field capacity moisture), respectively. Forty pots were used for each water level in each chamber. Wheat plants from half of the pots were used for the testing indicators, and the remaining pots were used for aphid rearing. To minimize positional effects, plants were randomly repositioned within each chamber daily. The experiment was performed in three replicates. All chambers were used for each treatment in different replicates.

For the phytohormone defense response analysis, the above wheat leaves with and without aphid infection were used as samples. Twenty-two wingless adults were transferred to each plant's leaves for 24 h at the two-leaf stage. After that, all adults were removed. The leaves were collected, frozen in liquid nitrogen immediately, and kept at -80°C until subsequent analysis (Zhang *et al.*, 2017).

Measurement of relative water content

Leaf relative water content (RWC) was measured with using the formula RWC (%) = (fresh weight-dry weight)*(turgor weight-dry weight) (Barrs & Weatherley, 1962). Fresh weight (FW) was measured and leaves were left to rehydrate in distilled water for 24 h at 15 °C in darkness to obtain the weight at full turgor (TW). Leaf dry weight (DW) was dried to constant weight.

The wheat leaves were placed in distilled water and maintained them at 4°C in darkness (to minimize respiration loss) until they reached a constant weight to obtain the turgid weight. Dry weight was obtained after placing the samples in an oven at 70°C for 48 h.

Measurement of soluble sugar, amino acid, and phytohormones

To quantify soluble sugar and amino acid concentrations in phloem, phloem exudates were obtained using the EDTA exudation technique described by Tetyuk *et al.* (2013).

For soluble sugar analysis, we followed the sample preparation method from Xie *et al.* (2019). Samples of 20-µL aliquots from each treatment were injected into the HPLC-MS/MS system. Sugars were separated with a Waters BEH Amide column (4.6 mm × 250 mm, 5 µm; Waters Corporation) using 75% acetonitrile as the mobile phase with isocratic elution at a flow rate of 1.0 mL/min. The column was maintained at 30°C.

For amino acid analysis, phloem exudates and 60 mL of pure water were transferred into 100 mL volumetric flasks. After ultrasonic extraction for 30 min, the volume was fixed to the scale mark with water. Then, 10 µL aliquots of each sample were injected into the HPLC-MS/MS system. Amino acids were separated using an ACQUITY HSS T3 column (100 mm × 2.1 mm, 1.8 µm; Waters Corporation) under gradient conditions with 5 mM ammonium acetate (A) and acetonitrile (B) as the mobile phases at a 0.3 mL/min flow rate. The gradient program is shown in Table S1. The column was maintained at 30°C.

For phytohormone (ABA, JA and SA) analysis, the sample preparation method and analysis conditions in HPLC-MS/MS system followed the protocol from Xie *et al.* (2019).

Gene expression by quantitative RT-qPCR

Before and after aphid infestation, the relative expression of wheat genes involved in the JA and SA defense signaling pathways was detected using RT-qPCR. *Lipoxygenase (LOX)* and *allene oxide synthase (AOS)* were the target genes for the JA-responsive pathway. They are involved in JA biosynthesis (Liu *et al.*, 2011). The SA synthesis enzymes *phenylalanine ammonia lyase (PAL)* and the induced SA marker protein *pathogenesis-related protein 1 (PR-1)* were the target genes for the SA-responsive pathway (Chen *et al.*, 2009). Actin was used as the internal control and was amplified using the primer sequences described in Liu *et al.* (2011). Primers of these genes were designed using Primer Premier 5.0, all primer sequences are listed in Table S2 (Zhang *et al.*, 2017). RT-qPCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The PCRs were performed in 20- μ L reaction volumes that contained 1 μ L of cDNA, 0.5 μ L each of 10 μ mol L⁻¹ forward and reverse primers, 10 μ L of 2 \times SybrGreen qPCR Mastermix, and 8.0 μ L of ddH₂O under the following thermal cycling conditions: 2 min at 95°C followed by 40 cycles of 10 s at 95°C and 30 s at 60°C.

Insect rearing and life table parameter measurements

The *S. avenae* population was originally collected from wheat plants in Hebei Province and were reared in the laboratory for 5 years in an indoor environment at 22 ± 1°C, 60±10% RH, and a 16 h light /8 h dark photoperiod. To avoid overcrowding, aphids were continuously transferred to new plants until the start of the experiment.

Wheat plants under the different treatments mentioned above were used to rear aphids in corresponding environmental chambers. Using a camel hair brush, a single newly emerged nymph (< 6 h old) was placed on each plant's leaves. To prevent the aphids from escaping, the plants were confined in transparent plastic column cages covered with double-deck gauze on top. Each nymph was examined daily to record their survival and the number of daily offspring until all parental aphids died. The offspring were removed after each count. Twenty aphids were reared in each treatment, with three replicates per treatment (total 60 individually caged aphids per treatment). Age-specific reproduction was used to construct a life table and the life table parameters (net reproductive rate R_0 , intrinsic rate of increase r_m , generation time T , and finite rate of increase λ) were calculated as reported by Birch (1948) and Maia (2000).

Statistical analyses

The effects of elevated CO₂ and drought stress on relative water content, soluble sugar content, and free amino acids content as well as aphid life table parameters were tested by two-way ANOVAs. The effects of elevated CO₂, drought and aphid infestation on phytohormone (ABA, JA, and SA) content as well as JA- and SA-related gene expression in wheat were tested by three-way ANOVAs. Least significant different

(LSD) tests were used to determine if treatment means significantly differed when ANOVAs indicated a factor was significant. For all analyses, $P < 0.05$ was considered the threshold for statistical significance.

Results

Relative water content in wheat

The relative water content of wheat showed different changes in response to elevated CO₂ and drought stress (Fig. 1, Table S3). Compared to the ambient CO₂ treatment, the relative water content in wheat increased by 6.63% under elevated CO₂ conditions (Fig. 1). However, compared to the well-watered treatment, the relative water content in wheat decreased by 10.9% under drought stress (Fig. 1). The interactions between elevated CO₂ and drought stress on relative water content in wheat were not significant (Table S3).

Soluble sugar and amino acid content in wheat

Elevated CO₂ and drought stress significantly promoted soluble sugar accumulation in wheat (Fig. 2, Table S4). Relative to the ambient CO₂ treatment, elevated CO₂ increased glucose and total soluble sugar content in wheat by 10.9% and 8.4%, respectively (Fig. 2B, D). Relative to the well-watered treatment, drought stress increased fructose, glucose, sucrose, and total soluble sugar content in wheat by 26.5%, 17.1%, 48.0%, and 18.1%, respectively (Fig. 2A-D). The interaction effects between elevated CO₂ and drought stress on soluble sugars in wheat were not significant (Table S4).

There were contrasting effects of elevated CO₂ and drought stress on amino acid accumulation in wheat (Fig. 3, Table S5). Elevated CO₂ significantly decreased the methionine, glycine, lysine, tryptophan, threonine, aspartic acid, histidine, and total amino acid content, compared to ambient CO₂ treatment (Table S5). Drought stress significantly increased the phenylalanine, glutamate, tyrosine, proline, tryptophan, aspartic acid, asparagine, and total amino acid content, compared to the well-watered treatment (Table S5). Furthermore, the negative interaction effects between elevated CO₂ and drought stress on alanine, arginine, and tryptophan were also significant (Table S5).

Phytohormone-dependent defense against aphids

Elevated CO₂, drought stress and aphid infestation significantly influenced the wheat phytohormone content (Fig. 4, Table S6). Relative to the ambient CO₂ treatment, elevated CO₂ decreased JA, but increased SA content in wheat (Fig. 4B, C). Relative to the well-watered treatment, drought stress increased ABA and JA content in wheat (Fig. 4A, B). Aphid infestation significantly increased ABA, JA, and SA content in wheat compared with the uninfested treatment (Fig. 4A-C). Moreover, the interaction effects among elevated CO₂, drought stress, and infestation on the wheat phytohormones were not significant (Table S6).

Elevated CO₂, drought stress and aphid infestation significantly influenced the relative expression of genes JA and SA defense signaling pathway genes (Fig. 5, Table S7). Relative to the ambient CO₂ treatment, the relative of expression of *LOX* was downregulated, and the relative of expression of *PR-1* and *PAL* were upregulated in wheat grown under elevated CO₂ (Fig. 5B-D). Relative to the well-watered treatment, the relative of expression of *AOS* and *LOX* were upregulated in wheat grown under drought stress (Fig. 5A, B). Aphid infestation upregulated the relative expression of *AOS*, *LOX*, *PAL*, and *PR-1* in wheat, compared to the uninfested treatment (Fig. 5A-D). Furthermore, the positive interaction effect between drought stress and infestation on the relative expression of *LOX* was significant (Table S7).

Changes in performance of aphid populations on wheat

There are contrasting effects of elevated CO₂ and drought stress on the life table parameters of aphids that feed on wheat (Fig. 6, Table S8). The R_0 , r , and λ values of aphid populations increased by 10.6%, 13.1%, and 2.5%, respectively, under elevated CO₂, compared to ambient CO₂ (Fig. 6A, C and D). The R_0 values of aphid populations decreased by 12.4% under drought stress compared to the well-watered treatment (Fig. 6A). The interaction effects between elevated CO₂ and drought stress on the life history table parameters of aphids were not significant (Table S8).

Discussion

Certain aspects of plant-aphid interactions have been well-studied over the past decade (Smith & Clement; 2012; Jaouannet *et al.*, 2014; Foyer *et al.*, 2016). The water content and nutritional quality (soluble sugar, amino acid, etc) and the secondary metabolic defense pathways in host plants are important limiting factors for aphids' growth and development (Douglas 1993; Bezemer & Jones, 1998; Mewis *et al.*, 2012; Züst & Agrawal, 2016). However, little is known about how combined abiotic stressors influence these limiting factors and further change plant-aphid interactions.

Relative water content changes in wheat under elevated CO₂ and drought stress

The effects of elevated CO₂ and drought stress on relative water content in this study were distinct. Previous studies indicated that elevated CO₂ promotes wheat root growth, which may help facilitate access of wheat to the sub-soil water in this study (Li *et al.*, 2017; Uddin 2018). Thus, elevated CO₂ may help alleviate lower water content caused by drought stress, which increases plant water availability and aphid feeding efficiency in wheat (Sun *et al.*, 2015; Guo *et al.* 2016).

Changes in nutrient quality in wheat under elevated CO₂ and drought stress

It has long been recognized that elevated CO₂ increased photosynthetic rate of C₃ plants, which increases carbohydrate accumulation (Barbehenn *et al.*, 2004; Chen *et al.*, 2005). Carbohydrate accumulation is also coordinated with the activation of specific physiological and molecular responses in plants grown under certain abiotic stresses, including drought, which mitigate the damaging effects of those stresses

on the plant (Zandalinas *et al.*, 2018). Previous studies have shown that individual elevated CO₂ or drought enhanced the carbohydrate accumulation in wheat (Sun *et al.*, 2009; Xie *et al.*, 2019). In this study, both elevated CO₂ and drought significantly promoted soluble sugar accumulation in wheat, there was no interaction between the two factors. However, aphid responses are typically species-specific to the increasing soluble sugar content in their host plants. Some reports have suggested that the increasing soluble sugar content in host plants are beneficial to aphid feeding. However, others studies have shown that increased soluble sugar content is not necessarily conducive to aphid growth (Slosser *et al.*, 2004; Alkhedir *et al.*, 2013; Li *et al.*, 2019a).

Many studies have shown that lower nitrogen concentration is found in plants grown in elevated CO₂ conditions, compared to plants grown in ambient CO₂ conditions. However, the interpretations for lower nitrogen concentration are inconsistent across the literature. One such interpretation is the presence of a dilution effect on nitrogen due to enhanced carbohydrates or biomass accumulation in plants grown under elevated CO₂ conditions (Nie *et al.*, 1995; Smart *et al.*, 2010; Novriyanti *et al.*, 2012). In this study, elevated CO₂ decreased the methionine, glycine, lysine, tryptophan, threonine, aspartic acid, histidine and total amino acid content in wheat, which may be due to a dilution effect of the soluble sugar accumulation in wheat grown under elevated CO₂. Aphids utilize a food source rich in carbohydrates, but relatively low in nitrogen concentration. Typically, they must obtain some essential amino acids from their host. Thus, the nitrogen concentration is often a limiting nutritional factor, as reflected in the strong correlations found between this variable and individual performance of aphids on their hosts (Sandstrom & Pettersson, 1994; Hansen & Moran, 2011). Sun *et al.* (2009) indicated that a decline in amino acid content could enhance the aphid ingestion efficiency in order to meet their nitrogen requirements. Therefore, the reduced the amino acid content in wheat grown under elevated CO₂ may enhance aphid feeding activity and lead to heavier ingestion in wheat plants.

Drought stress increased the amino acid content in wheat in this study. Previous studies have shown that the accumulation of amino acids enhances plant drought stress tolerance by regulating the activation of specific physiological and molecular responses in plants (Sugiyama *et al.*, 2014). In this study, the accumulated amino acids (tryptophan, tyrosine, and phenylalanine) are located downstream of the shikimic acid pathway, which adjusts metabolism towards secondary metabolite production and contributes to hormone metabolism in wheat (Tzin & Galili, 2010). Our study also shows that the opposing effects of elevated CO₂ and drought on amino acid accumulation in wheat, i.e., drought stress alleviates the reduced amino acid concentration caused by elevated CO₂. Thus, the total amino acid content was similar between the control and the combined elevated CO₂ and drought stress conditions. It is speculated that the combined elevated CO₂ and drought stress conditions may not influence aphid nitrogen absorption from wheat.

Phytohormone-dependent defense against aphids of wheat under elevated CO₂ and drought stress

Abiotic and biotic stress can activate phytohormone signal pathways. However, the method of regulation from combined stressors is currently complicated and unclear (Gupta *et al.*, 2017). Plant response to abiotic and biotic stress induced ABA, JA or SA defense signaling pathways (Adie *et al.*, 2007; Danquah *et al.*, 2014; Ahammed & Yu, 2016; Zandalinas *et al.*, 2016). The ABA signaling pathway activity up-regulated the JA signaling pathway, but suppressed the SA signaling pathway when plants were grown under abiotic stress, this also enhanced the effective resistance (JA signaling pathway) of host plants to herbivorous insects (Casteel *et al.*, 2008; Zavala *et al.*, 2008; Ahammed *et al.*, 2016; Guo *et al.*, 2016). In this study, elevated CO₂ reduced JA accumulation and the expression levels of the JA defense-related gene *LOX*, but enhanced SA accumulation, and the expression levels of the SA defense-related genes *PR-1* and *PAL*, i.e., elevated CO₂ induces the downregulation of JA-dependent defense, but up-regulates SA-dependent defense in wheat. Similar studies have also indicated that elevated CO₂ reduces the effective defense-JA signaling pathway and enhances the ineffective defense-SA signaling pathway against aphids (Sun *et al.* 2013, Guo 2017 Zavala *et al.* 2013, 2017, Haworth *et al.* 2015). Therefore, elevated CO₂ reduced the effective resistance of wheat to aphids by changing the phytohormone signal pathways (Guo *et al.*, 2012, 2016; Sun *et al.*, 2013).

Drought stress increased ABA and JA accumulation and increased the expression levels of the JA defense-related genes *LOX* and *AOS* in wheat, i.e., drought stress enhanced ABA accumulation that promotes the JA signaling pathway activity in wheat, and enhanced its resistance to aphids (Guo *et al.*, 2012, 2016; Sun *et al.*, 2013). Thus, the wheat resistance response to aphids was different between elevated CO₂ and drought stress conditions. Regardless of elevated CO₂ and drought conditions, aphid infestation up-regulated the JA and SA signaling pathways by increasing JA and SA accumulation and their defense-related genes (*LOX*, *AOS*, *PR-1* and *PAL*) in this experiment (Table S6. 7). Thus, aphid induced resistance in wheat was not influenced by abiotic factors (elevated CO₂ and drought stress).

Changes in wheat - aphid interaction under heat and drought stress

Both elevated CO₂ and drought significantly influenced wheat nutritional quality and phytohormone-dependent defense. This indirectly influenced the performance of aphids in this study (Clissold & Simpson, 2015; Züst & Agrawal, 2016). The R_0 , r , and λ values of aphid populations increased when feeding on wheat grown under elevated CO₂. Elevated CO₂ promoted soluble sugar accumulation and decreased total amino acid content in wheat in this study. However, aphids need to ingest more food to increase their feeding effectiveness in order to meet nitrogen demand. This was confirmed when aphids excreted larger amounts of honeydew (Sun *et al.* 2009). In the present study, the increased relative water content in wheat also enhanced aphid feeding efficiency under elevated CO₂ conditions. And elevated CO₂ also weakened the effective JA-dependent defense for aphid (Sun *et al.* 2013). Thus, elevated CO₂ improved the occurrence of aphid populations in wheat. However, the R_0 value of aphid populations decreased when they fed on wheat grown under drought stress. The decreased fecundity may be relative to the lower water content reducing aphid feeding efficiency and higher JA-dependent defense in wheat grown under drought stress, although drought increased the soluble sugar and amino acid accumulation

in wheat. The above results and the similar aphids' performance between control and combined elevated CO₂ and drought treatments indicated that the positive effect of elevated CO₂ on the performance of aphid populations can be offset by the negative effects of drought.

Conclusions

Elevated CO₂ can alleviate the direct effects of drought on wheat nutritional quality and resistance and the indirect effects on aphid performance. Thus, it is speculated that the wheat may suffer invariable damage from aphid populations in the future under combined elevated CO₂ and drier conditions. Moving forward, it will be necessary to study the plant-insect interactions from the perspective of multiple environmental factors and species specificity, which will also further analysis of the relationship between pest occurrence and damage in plants.

Declarations

Acknowledgements

The authors thank the technical team of wheat pest (CAAS, Beijing, China) for providing the insect material and data analysis. This work was supported by the Science and Technology Research Project for Colleges and Universities in Hebei Province (BJ2020049) and the National Natural Science Foundation of China (31871966).

Author contributions

Haicui Xie and Haiyun Xu designed the experiment and wrote the manuscript. Fengyu Shi and Jingshi Li performed the experiment. Shengyong Wu, Miaomiao Yu and Jia Fan provided the insect, reagents and materials.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

References

1. Adie BA, Pérez-Pérez J, Pérez-Pérez MM, Godoy M, Sánchez-Serrano JJ, Schmelz EA, Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and

- the activation of defenses in *Arabidopsis*. *Plant Cell* 19: 1665-1681.
<https://doi.org/10.1105/tpc.106.048041>.
2. Ahammed GJ, Li X, Zhou J, Zhou YH, Yu JQ (2016) Role of hormones in plant adaptation to heat stress. In: Ahammed G, Yu JQ. (eds) *Plant Hormones under Challenging Environmental Factors*. Springer, Dordrecht.
 3. Alkhedir H, Karlovsky P, Vidal S (2013) Relationship between water soluble carbohydrate content, aphid endosymbionts and clonal performance of *Sitobion avenae* on cocksfoot cultivars. *PLoS One* 8(1): e54327. <https://doi.org/10.1371/journal.pone.0054327>.
 4. Barbehenn RV, Chen Z, Karowe DN, Spickard A (2004) C₃ grasses have higher nutritional quality than C₄ grasses under ambient and elevated atmospheric CO₂. *Global Change Biol* 10(9): 1565-1575. <https://doi.org/10.1111/j.1365-2486.2004.00833.x>.
 5. Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J Biol Sci* 15: 413-428. <https://doi.org/10.1071/bi9620413>.
 6. Bezemer TM, Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO₂: quantitative analyses and guild effects. *Oikos* 82: 212-222. <https://www.jstor.org/stable/3546961>
 7. Birch LC (1948) The intrinsic rate of natural increase of an insect population. *J Anim Ecol* 17: 15-26. <https://www.jstor.org/stable/1605>
 8. Blackman RL, Eastop VF (2000) *Aphids on the world's crops: an identification and information guide*. John Wiley & Sons Ltd.: New York. <https://doi.org/10.1080/00305316.2001.10417292>.
 9. Casteel CL, O'Neill BF, Zavala JA, Bilgin DD, Berenbaum MR, Delucia EH (2008) Transcriptional profiling reveals elevated CO₂ and elevated O₃ alter resistance of soybean (*Glycine max*) to Japanese beetles (*Popillia japonica*). *Plant Cell Environ* 31: 419-434. <https://doi.org/10.1111/j.1365-3040.2008.01782.x>.
 10. Chen FJ, Ge F (2004) An experimental instrument to study the effects of changes in CO₂ concentrations on the interactions between plants and insects-CDCC-1 chamber. *Entomol Knowledge* 41: 279-281. (in Chinese with English abstract)
 11. Chen FJ, Ge F, Parajulee MN (2005) Impact of elevated CO₂ on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environ Entomol* 34(1): 37-46. <https://doi.org/10.1603/0046-225X-34.1.37>.
 12. Chen Z, Zheng Z, Huang J, Lai Z, Fan B (2009) Biosynthesis of salicylic acid in plants. *Plant Signal Behav* 4, 493-496. <https://doi.org/10.4161/psb.4.6.8392>.
 13. Clissold FJ, Simpson SJ (2015) Temperature, food quality and life history traits of herbivorous insects. *Curr. Opin. Insect Sci* 11: 63-70. <https://doi.org/10.1016/j.cois.2015.10.011>.
 14. Danquah A, Zelicourt DA, Colcombet J, Hirt H (2014) The role of ABA and MAPK signaling pathways in plant abiotic stress responses. *Biotechnol Adv* 32: 40-52. <https://doi.org/10.1016/j.biotechadv.2013.09.006>.

15. Douglas AE (1993) The nutritional quality of phloem sap utilized by natural aphid populations. *Ecol Entomol* 18: 31-38. <https://doi.org/10.1111/j.1365-2311.1993.tb01076.x>.
16. Foyer CH, Rasool B, Davey JW, Hancock RD (2016) Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation, *J Exp Bot* 67(7): 2025-203. <https://doi.org/10.1093/jxb/erw079>.
17. Guo HJ, Peng XH, Gu LY, Wu JQ, Ge F, Sun YC (2017) Up-regulation of MPK4 increases the feeding efficiency of the green peach aphid under elevated CO₂ in *Nicotiana attenuata*. *J Exp Bot* 21-22: 5923-5935. <https://doi.org/10.1093/jxb/erx394>.
18. Guo HJ, Sun Y, Ren Q, Zhu S K, Kang L, Wang CZ, Li CY, Ge F (2012) Elevated CO₂ reduces the resistance and tolerance of tomato plants to *Helicoverpa armigera* by suppressing the JA signaling pathway. *PLoS One* 7(7): e41426. <https://doi.org/10.1371/journal.pone.0041426>.
19. Guo HJ, Sun YC, Peng XH, Wang QY, Harris M, Ge F (2016) Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *J Exp Bot* 3: 681-693. <https://doi.org/10.1093/jxb/erv481>.
20. Gupta A, Hisano H, Hojo Y, Matsuura T, Ikeda Y, Mori IZ, Senthil-Kumar M (2017) Global profiling of phytohormone dynamics during combined drought and pathogen stress in *Arabidopsis thaliana* reveals ABA and JA as major regulators. *SCI REP-UK* 7: 4017. <https://doi.org/10.1038/s41598-017-03907-2>.
21. Hansen A K, Moran NA (2011) Aphid genome expression reveals host–symbiont cooperation in the production of amino acids. *PNAS* 108 (7): 2849-2854. <https://doi.org/10.1073/pnas.1013465108>.
22. Haworth M, Killi D, Materassi A, Raschi A (2015) Coordination of stomatal physiological behavior and morphology with carbon dioxide determines stomatal control. *Am J Bot* 102: 677- 688. <https://doi.org/10.3732/ajb.1400508>.
23. Hervé J, Koricheva J, Castagneyrol B (2019) Responses of forest insect pests to climate change: not so simple. *Curr Opin Insect sci* 35: 103-108. <https://doi.org/10.1016/j.cois.2019.07.010>.
24. IPCC. (2014). "Impacts, adaptation and vulnerability," in Working Group II Contribution to the Fifth Assessment Report of the Intergovernmental Panel on climate Change, 1132, eds C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, et al. (Cambridge: Cambridge University Press).
25. Jaouannet M, Rodriguez PA, Thorpe P, Lenoir CJG, Macleod R, Escudero-Martinez C, Bos JIB (2014) Plant immunity in plant-aphid interactions. *Fron Plant Sci* 5: 663. <https://doi.org/10.3389/fpls.2014.00663>.
26. Kempel A, Schaedler M, Chrobock T, Fischer Markus Kleunen M van (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. *PNAS* 108(14):5685-5689. <https://doi.org/10.1073/pnas.1016508108>.
27. Lee SC, Luan S (2012) ABA signal transduction at the crossroad of biotic and abiotic stress responses. *Plant Cell Environ* 35: 53-60. <https://doi.org/10.1111/j.1365-3040.2011.02426.x>.

28. Li LK, Wang MF, Pokharel SS, Li CX, Parajulee MN, Chen FJ, Fang WP (2019a) Effects of elevated CO₂ on foliar soluble nutrients and functional components of tea, and population dynamics of tea aphid, *Toxoptera aurantii*. *Plant Physiol Bioch* 145: 84-94. <https://doi.org/10.1016/j.plaphy.2019.10.023>.
29. Li S, Li X, Wei Z, Liu F (2019b) ABA-mediated modulation of elevated CO₂ on stomatal response to drought. *Curr Opin Plant Biol* 13: 1-7. <https://doi.org/10.1016/j.pbi.2019.12.002>.
30. Li X, Yang YQ, Sun XD, Lin HM, Chen JH, Ren J, Hu XY (2014) Comparative physiological and proteomic analyses of poplar (*Populus yunnanensis*) plantlets exposed to high temperature and drought. *PLoS One* 9: e107605. <https://doi.org/10.1371/journal.pone.0107605>.
31. Li YF, Li X, Yu JJ, Liu FL (2017) Effect of the transgenerational exposure to elevated CO₂ on the drought response of winter wheat: Stomatal control and water use efficiency. *Environ Exp Bot* 136: 78-84. <https://doi.org/10.1016/j.envexpbot.2017.01.006>.
32. Liu X, Meng J, Starkey S, Smith CM (2011) Wheat gene expression is differentially affected by a virulent Russian wheat aphid biotype. *J Chem Ecol* 37, 472-482. <https://doi.org/10.1007/s10886-011-9949-9>.
33. Maia A, Luiz AJB, Campanhola C (2000) Statistical inference on associated fertility life table parameters using Jackknife technique: computational aspects. *J Econ Entom* 93: 511-518. <https://doi.org/10.1603/0022-0493-93.2.511>.
34. McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178(4): 719-739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>.
35. Mewis I, Khan MAM, Glawischnig E, Schreiner M, Ulrichs C (2012) Water stress and aphid feeding differentially influence metabolite composition in *Arabidopsis thaliana* (L.). *PLoS One* 7: e48661. <https://doi.org/10.1371/journal.pone.0048661>.
36. Miranda-Apodaca J, Pérez-López U, Lacuesta M, Mena-Petite A, Muñoz-Rueda A (2018) The interaction between drought and elevated CO₂ in water relations in two grassland species is species-specific. *J Plant Physiol* 220: 193-202. <https://doi.org/10.1016/j.jplph.2017.11.006>.
37. Murray TJ, Ellsworth DS, Tissue DT, Riegler M (2013) Interactive direct and plant-mediated effects of elevated atmospheric [CO₂] and temperature on a eucalypt feeding insect herbivore. *Global Change Biol* 19: 1407-1416. <https://doi.org/10.1111/gcb.12142>.
38. Nackley LL, Betzelberger A, Skowno A, West AG, Ripley BS, Bond WJ, Midgley G (2018) CO₂ enrichment does not entirely ameliorate *Vachellia karroo* drought inhibition: A missing mechanism explaining savanna bush encroachment. *Environ Exp Bot* 155: 98-106. <https://doi.org/10.1016/j.envexpbot.2018.06.018>.
39. Nie GY, Hendrix DL, Webber AN, Kimball BA, Long SP (1995) Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO₂ concentration in the field. *Plant Physiol* 108(3): 975-983. <https://doi.org/10.1104/pp.108.3.975>.

40. Novriyanti E, Watanabe M, Kitao M, Utsugi H, Uemura A, Koike T (2012) High nitrogen and elevated [CO₂] effects on the growth, defense and photosynthetic performance of two eucalypt species. Environ Pollut 170: 124-130. <https://doi.org/10.1016/j.envpol.2012.06.011>.
41. Oehme V, Petra H, Claus PW, Zebitz AF (2013) Effects of elevated atmospheric CO₂ concentrations on phloem sap composition of spring crops and aphid performance. J Plant Interact8: 7484. <https://doi.org/10.1080/17429145.2012.736200>.
42. Roth S, McDonald EP, Lindroth RL (1997) Atmospheric CO₂ and soil water availability: consequences for tree-insect interactions. Can J Forest Res 27(8): 1281-1290. <https://doi.org/10.1139/x97-031>.
43. Roy J, Picon-Cochard C, Augusti A, Benot Marie-Lise Thiery L, Darosnville O, Landais D, Piel C, Defossez M, Devidal S, Escape C, Ravel O, Fromin N, Volaire F, Milcu A, Bahn M, Soussana Jean-Francois (2016) Elevated CO₂ maintains grassland net carbon uptake under a future heat and drought extreme. PNAS 113(22): 6224-6229. <https://doi.org/10.1073/pnas.1524527113>.
44. Sandström J, Pettersson J (1994) Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. J Insect Physiol40: 947-955. [https://doi.org/10.1016/0022-1910\(94\)90133-3](https://doi.org/10.1016/0022-1910(94)90133-3).
45. Scherber C, Gladbach DJ, Stevnbak K, Karsten RJ, Schmidt IK, Michelsen A, Albert KR, Larsen KS (2013) Multi-factor climate change effects on insect herbivore performance. Ecol Evol3: 1449-1460 <https://doi.org/10.1002/ece3.564>.
46. Slosser JE, Parajulee MN, Hendrix DL, Henneberry TJ, Pinchak WE (2004) Cotton aphid (Homoptera: Aphididae) abundance in relation to cotton leaf sugars. Environ Entomol 33(3), 690-699. <https://doi.org/10.16.3/0046-225X-33.3.690>.
47. Smart DR, Chatterton NJ, Bugbee B (2010) The influence of elevated CO₂ on non-structural carbohydrate distribution and fructan accumulation in wheat canopies. Plant Cell Environ17(4): 435-442. <https://doi.org/10.1111/j.1365-3040.1994.tb>.
48. Smith CM, Clement SL (2012) Molecular bases of plant resistance to arthropods. Annu Rev Entomol 57: 309-328. <https://doi.org/10.1146/annrev-ento-120710-100642>.
49. Suguiyama VF, Silva EA, Meirelles ST, Centeno DC, Braga MR (2014) Leaf metabolite profile of the Brazilian resurrection plant *Barbacenia purpurea* Hook. (Velloziaceae) shows two time-dependent responses during desiccation and recovering. Front Plant Sci5: 96. <https://doi.org/10.3389/fpls.2014.00096>.
50. Sun Y, Guo H, Ge F (2016) Plant-aphid interactions under elevated CO₂: some cues from aphid feeding behavior. Front Plant Sci 7: 502. <https://doi.org/10.3389/fpls.2016.00502>.
51. Sun Y, Guo H, Yuan L, Wei JN, Zhang WH, Ge F (2015) Plant stomatal closure improves aphid feeding under elevated CO₂. Global Change Biol 21(7): 2739-2748. <https://doi.org/10.1111/gcb.12858>.
52. Sun Y, Guo H, Zhu-Salzman K, Ge F (2013) Elevated CO₂ increases the abundance of the peach aphid on *Arabidopsis* by reducing jasmonic acid defenses. Plant Sci 210: 128-140. <https://doi.org/j.plantsci.2013.05.014>.

53. Sun YC, Guo HJ, Yuan L, Wei JN, Zhang WH, Ge F (2015) Plant stomatal closure improves aphid feeding under elevated CO₂. *Global Change Biol* 21: 2739-2748. <https://doi.org/10.1111/gcb.12858>.
54. Sun YC, Jing BB, Ge F (2009) Response of amino acid changes in *Aphis gossypii* (Glover) to elevated CO₂ levels. *J Appl Entomol* 133, 189-197. <https://doi.org/10.1111/j.1439-0418.2008.01341.x>.
55. Tetyuk O, Benning UF, Hoffmann-Benning S (2013) Collection and analysis of *Arabidopsis* phloem exudates using the EDTA-facilitated method. *J Vis Exp* 80: e51111. <https://doi.org/10.3791/51111>.
56. Tzin V, Galili G,(2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Mol Plant* 3: 956-972. <https://doi.org/10.1093/mp/ssq048>.
57. Uddin S, Löw M, Parvin S, Fitzgerald GJ, Tausz-Posch S, Armstrong R, O'leary Garry, Tausz M (2018) Elevated [CO₂] mitigates the effect of surface drought by stimulating root growth to access sub-soil water. *PloS One* 13(6): e0198928. <https://doi.org/10.1371/journal.pone.0198928>.
58. Ullah A, Manghwar H, Shaban M, Khan AH, Akbar A, Ali U, Ali E, Fahad S (2018) Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environ Sci Pollut R* 25: 33103-33118. <https://doi.org/10.1007/s11356-018-3364-5>.
59. Wall GW (2001) Elevated atmospheric CO₂ alleviates drought stress in wheat. *Agr Ecosyst Environ* 87: 261-271. [https://doi.org/10.1016/S0167-8809\(01\)00170-0](https://doi.org/10.1016/S0167-8809(01)00170-0).
60. Xie HC, Shi JQ, Shi FY, Xu HY, He KL, Wang ZY (2019) Aphid fecundity and aphid defenses in wheat exposed to a combination of heat and drought stresses. *J Exp Bot* 71(9): 2713-2722. <https://doi.org/10.1093/jxb/eraa017>.
61. Zandalinas SI, Balfagon D, Arbona V, Gomez-Cadenas A, Inupakutika MA, Mittler R (2016) ABA is required for the accumulation of APX1 and MBF1c during a combination of water deficit and heat stress. *J Exp Bot* 67: 5381-5390. <https://doi.org/10.1093/jxb/erw299>.
62. Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. *Physiol Plantarum* 162: 2-12. <https://doi.org/10.1111/ppl.12540>.
63. Zavala JA, Gog L, Giacometti R (2017) Anthropogenic increase in carbon dioxide modifies plant-insect interactions. *Ann Appl Biol* 170: 68-77. <https://doi.org/10.1111/aab.12319>.
64. Zavala JA, Casteel CL, DeLucia EH, Berenbaum MR (2008) Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *PNAS* 105: 10631-10631. <https://doi.org/10.1073/pnas.0800568105>.
65. Zavala JA, Nabity PD, DeLucia EH (2013) An emerging understanding of mechanisms governing insect herbivory under elevated CO₂. *Annu Rev Entomol* 58: 79-97. <https://doi.org/10.1146/annurev-ento-120811-153544>.
66. Zhang Y, Fan J, Francis F, Chen JL (2017) Watery saliva secreted by the grain aphid *Sitobion avenae* stimulates aphid resistance in wheat. *J Agr Food Chem* 65: 8798-8805. <https://doi.org/10.1021/acs.jafc.7b03141>.

Figures

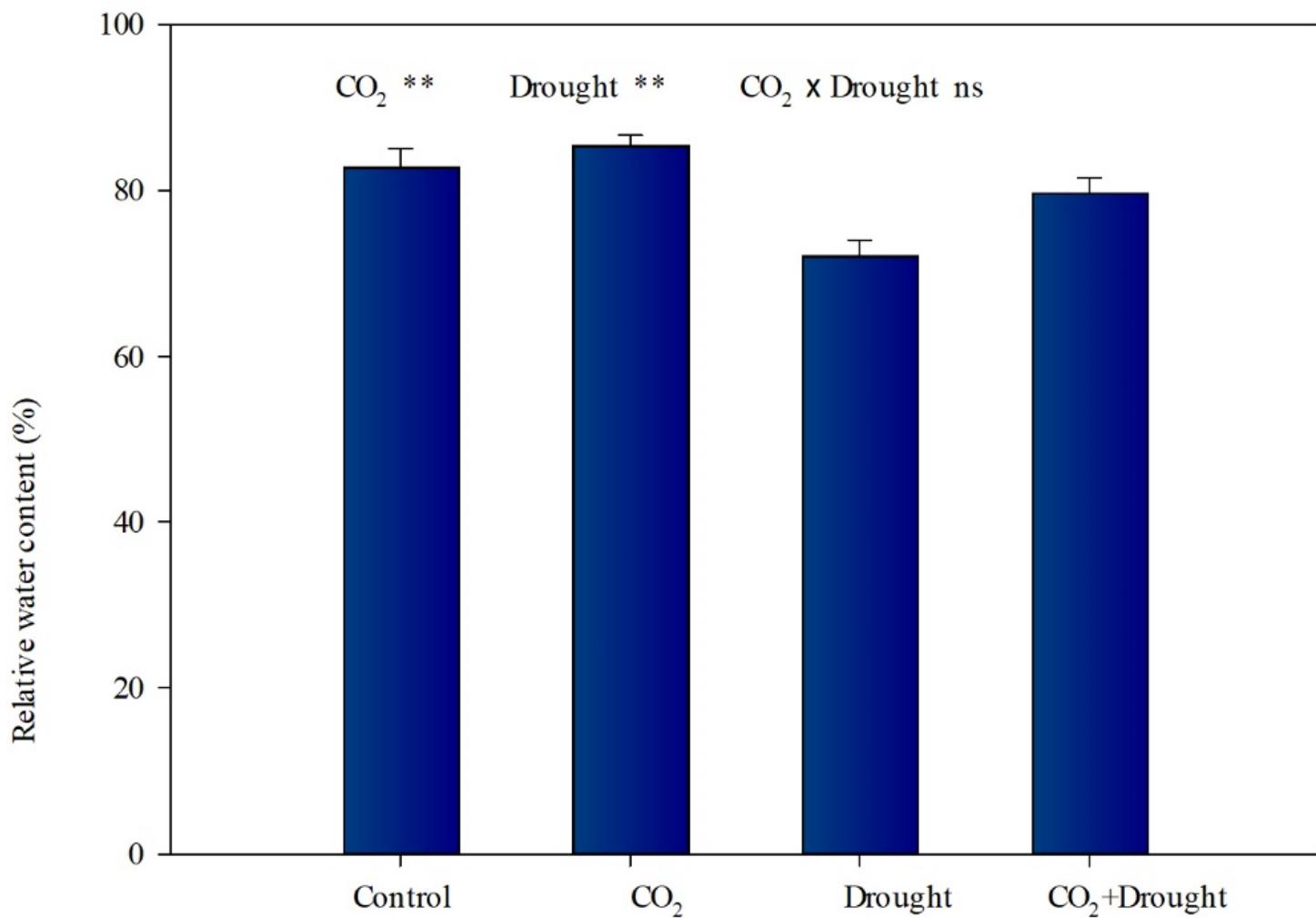


Figure 1

Relative water content of *Triticum aestivum* grown under elevated CO₂ and drought conditions. Each value shown represents the mean (\pm SE) of three replicates. P values are provided for two-way ANOVA on the effects of elevated CO₂ and drought treatments on relative water content. Significant differences: * , P < 0.05; ** , P < 0.01; ns, P > 0.05.

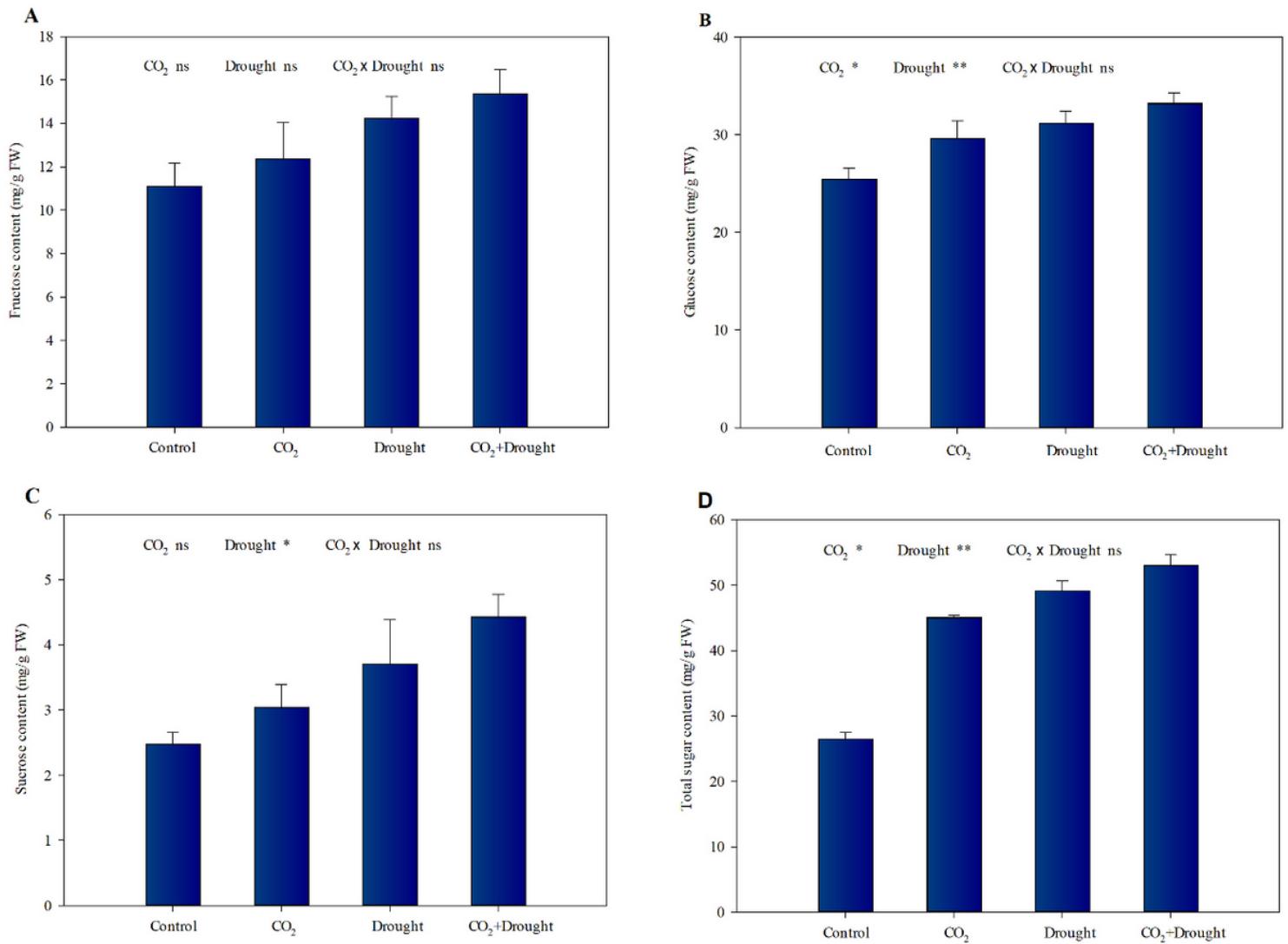


Figure 2

Soluble sugars content of *Triticum aestivum* grown under elevated CO₂ and drought conditions. A, fructose; B, glucose; C, sucrose; D, total soluble sugars. Each value shown represents the mean (\pm SE) of three replicates. P values are provided for two-way ANOVA on the effects of elevated CO₂ and drought treatments on soluble sugar content. Significant differences: *, P < 0.05; **, P < 0.01; ns, P > 0.05.

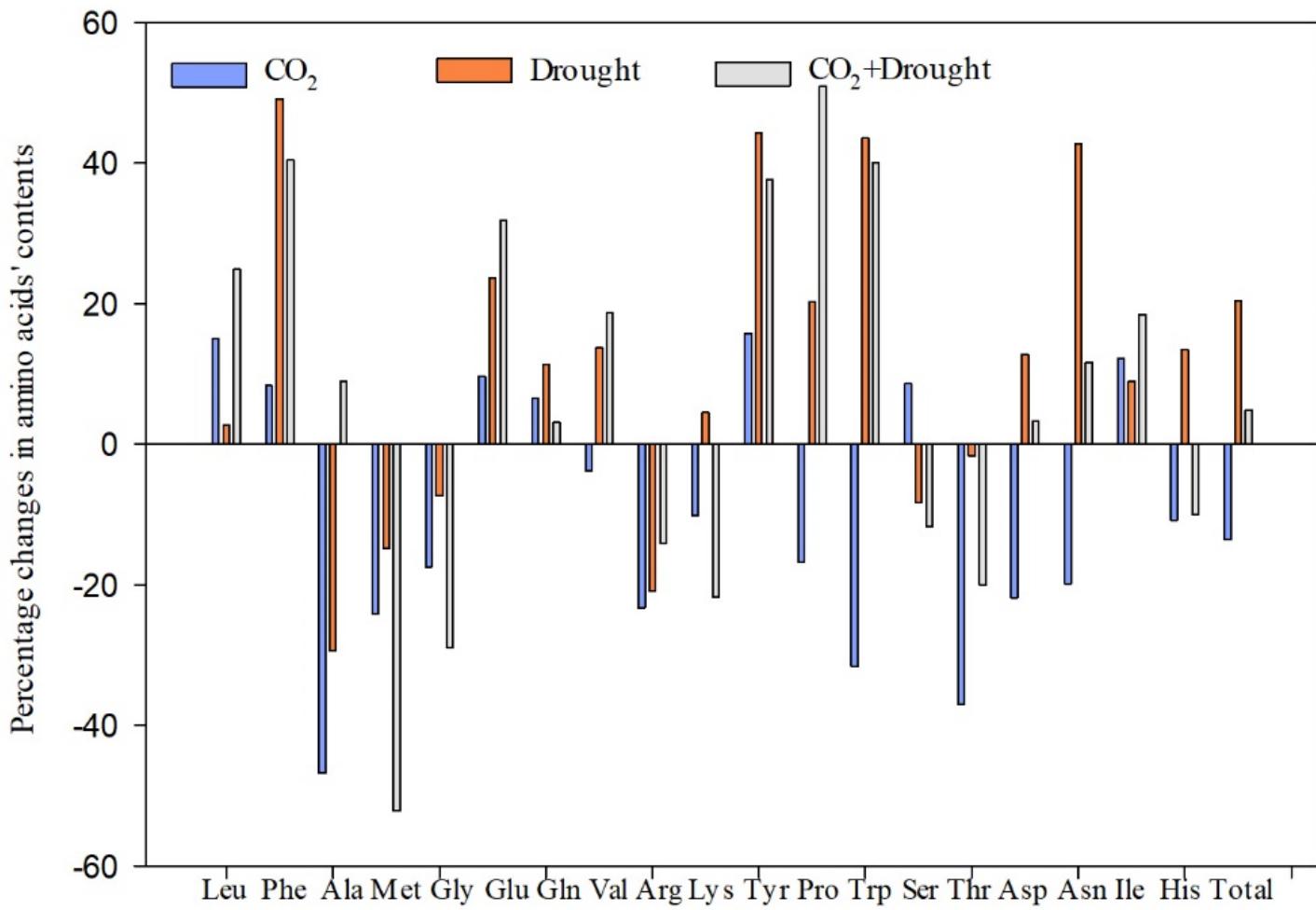


Figure 3

Percentage changes in amino acids content of *Triticum aestivum* grown under elevated CO₂ and drought conditions. Percentage change value (%) = (treatment- control)*100/control.

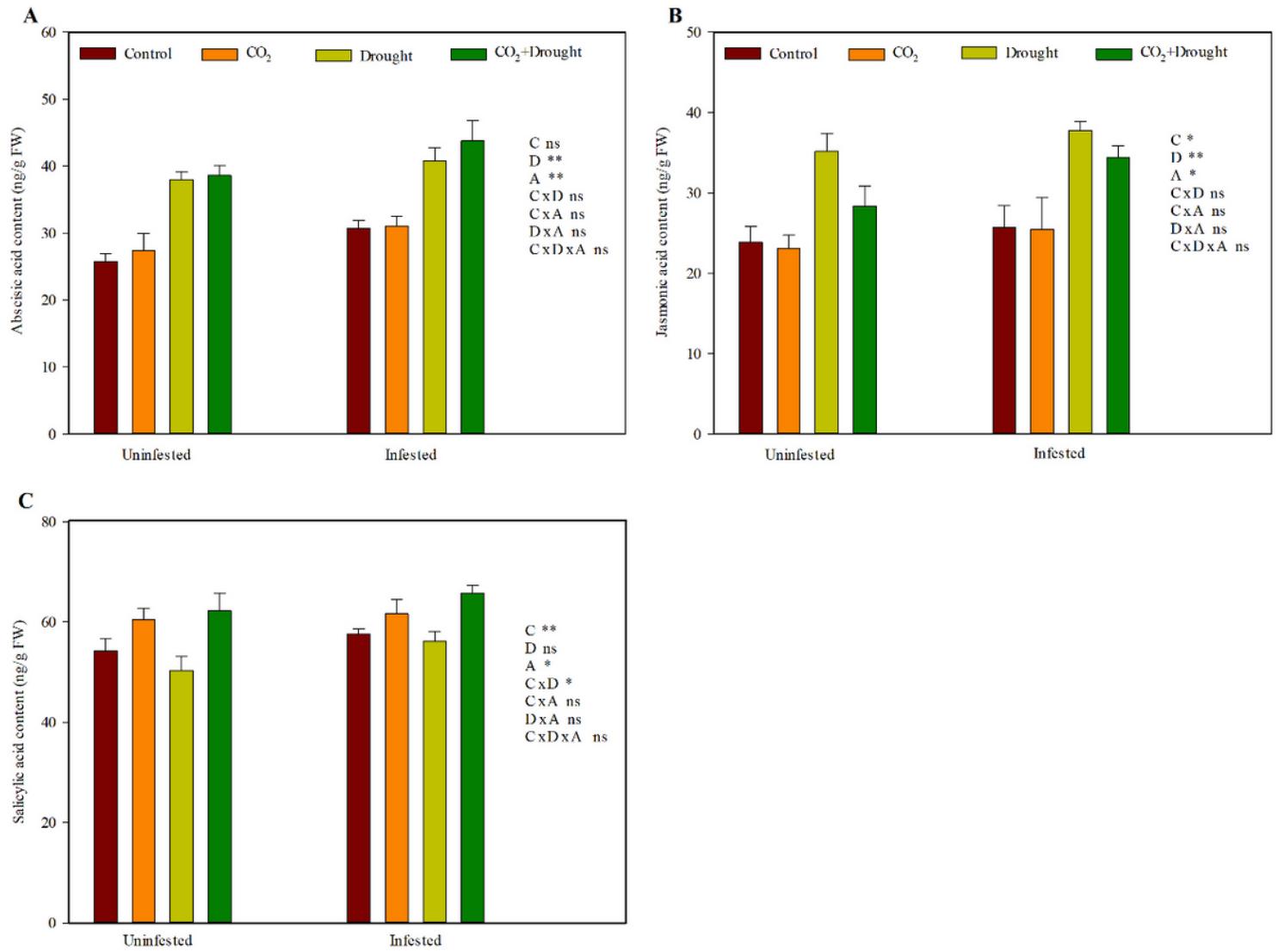


Figure 4

Phytohormone content of *Triticum aestivum* grown under elevated CO₂ and drought conditions with and without aphid infestation. A, abscisic acid (ABA); B, jasmonic acid (JA); C, salicylic acid (SA). Each value shown represents the mean (\pm SE) of three replicates. P values are provided for three-way ANOVA on the effects of elevated CO₂ (C), drought (D) and aphid (A) treatments on phytohormone content. Significant differences: *, P < 0.05; **, P < 0.01; ns, P > 0.05.

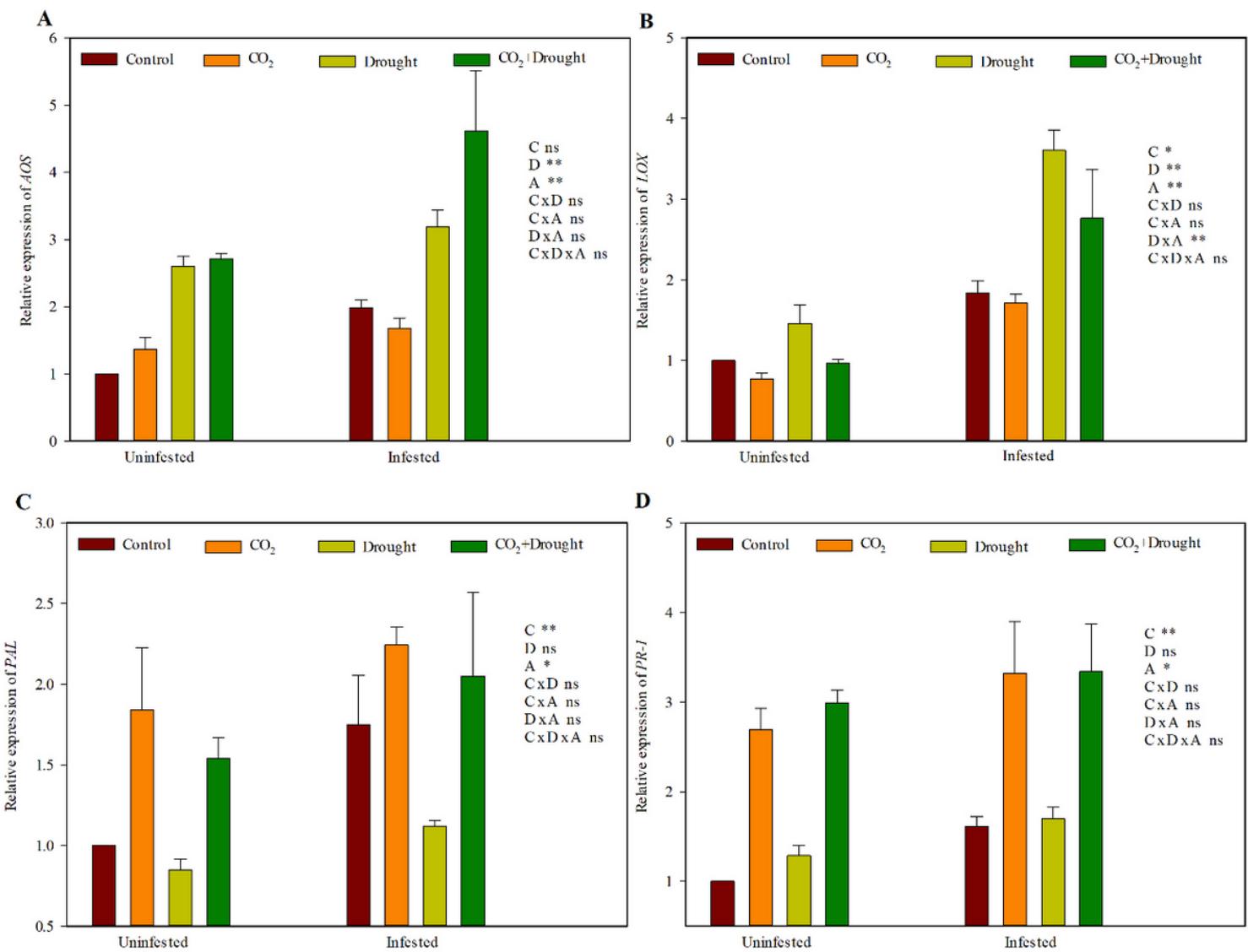


Figure 5

JA- and SA-related gene expression of *Triticum aestivum* grown under elevated CO₂ and drought conditions with and without aphid infestation. A, allene oxide synthase (AOS); B, Lipoxygenase (LOX) ; C, phenylalanine ammonia lyase (PAL), D, pathogenesis-related protein 1 (PR-1). Each value shown represents the mean (\pm SE) of three replicates. P values are provided for three-way ANOVA on the effects of elevated CO₂ (C), drought (D) and aphid (A) treatments on JA- and SA-related gene expression. Significant differences: *, P < 0.05; **, P < 0.01; ns, P > 0.05.

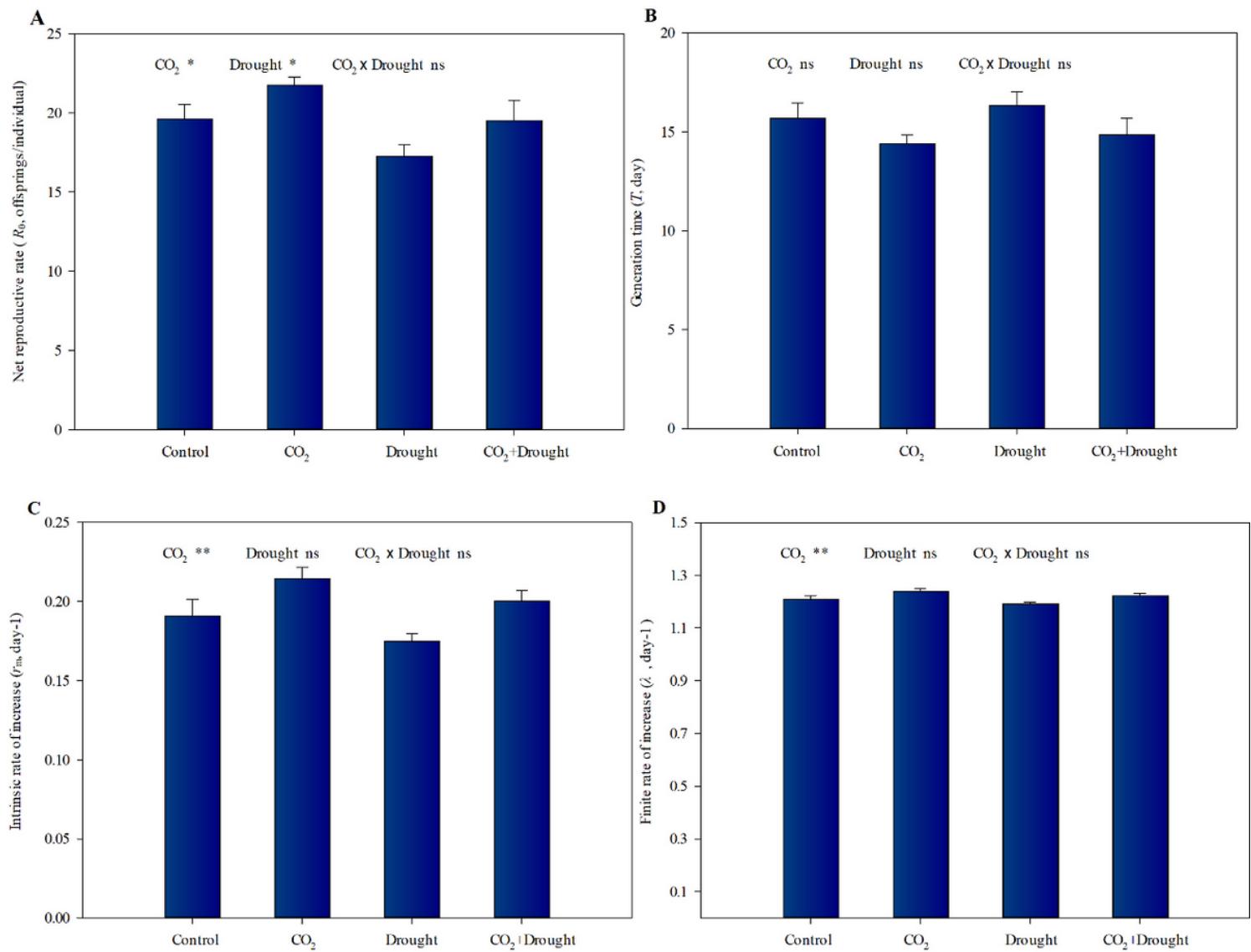


Figure 6

Life table parameters of aphid reared on *Triticum aestivum* grown under elevated CO_2 and drought conditions. A, net reproduction rate (R_0); B, generation time (T); C, intrinsic rate of increase (r_m); D, finite rate of increase (λ). Each value shown represents the mean ($\pm \text{SE}$) of three replicates. P values are provided for two-way ANOVA on the effects of elevated CO_2 and drought treatments on life table parameters. Significant differences: *, $P < 0.05$; **, $P < 0.01$; ns, $P > 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS18.docx](#)