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Effects of elevated CO_2 and water stress on population growth of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), on sweet pepper under environmentally controlled conditions

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A B S T R A C T

Weather events such as drought and elevated atmospheric CO_2 are likely to interact with plants in numerous ways with diverse mechanisms. As a consequence of changes in quality of plants, the performance parameters and population dynamics of herbivores are expected to be influenced. In this study, a split-plot design was used to evaluate the interaction of elevated CO_2 and irrigation regime on population growth of the two-spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae: Tetranychini), feeding on sweet pepper, *Capsicum annum* L. (Solanaceae), in environmentally controlled chambers. Results showed that exposure to elevated CO_2 significantly increased the C/N ratio in sweet pepper plants. Except in case of the adult stage, elevated CO_2 did not significantly increase the population density of other developmental stages or the overall population of *T. urticae*. However, water stress by itself and in combination with elevated CO_2 had significant effects on per capita population growth rate (r) and population density of mites. Maximum growth rate and population density of mites were observed at a combination of elevated CO_2 and intermediate water stress. Further studies, especially in field conditions, investigating the impact of elevated CO_2 and water stress on population size and growth of herbivores in other plant species may contribute to a greater understanding of the implications of global climate change on future crop productivity.

Introduction

Future climate changes such as increased atmospheric CO_2 and drought have the potential to change plant-herbivore dynamics (Casteel et al., 2012). Sweet pepper, *Capsicum annum* L. (Solanaceae), is a commercially important crop which is cultivated in many parts of Iran (Tabatabaei et al., 2014) and has economic value in the food, drug and spice industry (Rohami et al., 2010). *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), also known as the two-spotted spider mite, is a highly polyphytophagous species with a host range of over 1100 plant species worldwide (Grbić et al., 2011) and is a critical pest of greenhouse and field crops, including sweet pepper, in Iran (Khanjani, 2005; Beyzavi et al., 2013).

The sensitivity of plants to herbivorous feeding under drought conditions has been discussed by many authors (Mattson and Haack, 1987; Oi et al., 1989; Louda and Collinge, 1992; Huberty and Denno, 2004). According to them, water deficit causes physiological changes in plants, including a reduced synthesis of secondary metabolites or protective compounds (which have negative effects on feeding mites), increased content of soluble nitrogen (amino acids, amides) and free sugars, which improves the overall nutritional potential of plant tissues, and raised temperature of leaf surface, which stimulates the development of mites and contributes to a shorter duration of their individual stages, i.e., eggs, larvae, protonymphs, deutonymphs and adults. Other studies have also observed that water stressed plants may be a more suitable background for the development and fecundity of insects as many plant nutrients are more concentrated or better balanced in them, compared to well-irrigated plants (House, 1974; Haack & Slansky, 1987). Increasing the content of those nutrients and improving their balance should logically encourage pest reproduction. Nevertheless, the correlation between water stress and the growth and reproduction of herbivores such as spider mites depends on circumstances.

Studies have shown that water stress can result in an increase (Chandler et al., 1979; Hollingsworth and Berry, 1982; Youngman et al., 1988; Colijn and Lindquist, 1986; Nikolova et al., 2014; Shibuya et al., 2015) or decrease (Specht, 1965; Mellors et al., 1984; Oloumi-Sadeghi et al., 1988) in the population density of spider mites. However, in some cases no impact has been observed (Ferree and Hall,

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1980; Mellors and Propts, 1983; Sadras et al., 1998; Gillman et al., 1999).

Many studies have investigated the effects of elevated atmospheric CO_2 on plants and herbivores. In general, it is reported that increase of atmospheric CO_2 level leads to an increase in fixed-carbon rate, accumulation of total non-structural carbohydrates (TNC) in leaves and reduction of other leaf compounds, especially nitrogen and minerals (Hsiao and Jackson, 1999). These changes increase the C/N ratio, drastically reduce the quality of plant nutrients, and have a significant effect on herbivores (Ayres, 1993). A study by Boutaleb Joutei et al. (2000) showed that elevated CO_2 levels significantly stimulate the growth of common bean (*Phaseolus vulgaris* L.) and decrease the leaf nitrogen content, which is the result of an increase in TNC concentrations. Also, females of the two-spotted spider mite, *Tetranychus urticae*, feeding on plants grown in elevated CO_2 conditions showed a reduction in their progeny which could eventually lead to less damage to the host plants. In contrast, Heagle et al. (2002) studied the reproduction of *T. urticae* on white clover (*Trifolium repens* L.) under elevated levels of CO_2 and reported a significant increase in the mites' reproduction rate which could mean the increase of damage to some plant species in future elevated atmospheric CO_2 concentrations. Other effects including increasing their food intake, lengthened developmental times and reduction of growth and food transformation rates of herbivores feeding on plants under high concentration of atmospheric CO_2 have been reported (Lincoln, 1993; Roth and Lindroth, 1995; Watt et al., 1995). In addition to these, elevated CO_2 reduces plant stomatal conductance, which often increases water use efficiency, conserves soil moisture, and may ameliorate drought stress (Leakey et al., 2009).

While exposure of plants to elevated atmospheric CO_2 decreases the nutritional quality of leaves by increasing C/N ratios (Ainsworth et al., 2002), drought often increases available nitrogen in plant tissues (Huberty and Denno, 2004). Thus, increased CO_2 concentrations and drought may alter plant interactions with herbivores. While the majority of ecological climate change studies examine effects of single climate change variables on biological systems, information on the interactive effect of elevated CO_2 and drought is not well understood. A study by Casteel et al. (2012) showed that soybean plants [*Glycine* max (L.) Merr.] exposed to elevated CO_2 were more vulnerable to Japanese beetle (*Popillia japonica* Newman) damage. However, a combination of elevated CO_2 and reduced soil water content neutralized the effect of elevated CO_2 and, as a result, susceptibility was not impacted.

This study aimed to investigate the combined effects of different levels of CO_2 concentration and watering regimes on (a) the population density and per capita growth rate (r) of *T. urticae*, per sweet pepper plant and (b) C/N ratio of sweet pepper plants, reared under environmentally controlled conditions.

Materials and methods

Experimental conditions

Research was conducted in a greenhouse designed for CO_2 studies where two transparent plastic-walled chambers were built within the greenhouse, each measuring 300 × 110 × 370 cm, maintained at 25 ± 2 °C, 65 ± 5 RH, and photoperiod of L14:D10. Based on the predictions that atmospheric CO_2 levels are expected to significantly increase during this century (Forster et al., 2007; Prior et al., 2011), two CO_2 levels, 400 ± 10 (the ambient level) and 700 ± 10 ppm (the elevated level), were used in this study. The appropriate levels of CO_2 were released into the chambers from a tank via digital multi-range timer (Shiva Amvaj, Isfahan, Iran). The level of CO_2 in the chambers was adjusted and monitored regularly by an infrared CO_2 analyzer (S157/S158, Qubit systems Inc. Kingston, ONT, Canada).

Host plant cultivation

Seedlings of sweet pepper (var. Dimaz, Anbari Agricultural Seeds Company, Mashhad, Iran), which is commonly grown in Iran for commercial production, were sown in May 2017 in plastic pots (15 cm diameter, 14.5 cm height) containing a 1:2:2:1 mixture of clay, sand, peat, and vermicompost, respectively, under greenhouse conditions with ambient CO_2 level. The plants were irrigated with tap water and received 100% of sweet pepper water requirement every three days (as calculated below).

Calculation of field capacity and sweet pepper water requirement

The field capacity (FC) of the soil used throughout the experiments was calculated based on the "weight method" (Mahmoodnia et al., 2013) by weighing a plastic pot (15 cm diameter, 14.5 cm height) filled with 1:2:2:1 ratios of dry clay, sand, peat and vermicompost, then watering the pot until saturated and covering the pot with aluminum foil, and leaving it in the rearing conditions for 48 h. When the excess water drained away, the pot was weighed again. The difference between the initial weight (pot with dried soil) and second weight (pot with saturated soil) revealed the FC. The FC was estimated base on three replicates. According to Sadreghaen et al. (2011) the optimum soil moisture for growth of sweet pepper plant under environmentally controlled conditions is 60–70% of FC which was determined to be 200 cc for each potted plant in each time of irrigation. The appropriate time for irrigation of sweet pepper is when the average soil moisture in root developmental zone of the plant decreases to 25.2% of the field capacity of the soil (Tabatabaei et al., 2014). Under experimental conditions, soil moisture of pots was measured on consecutive days with a soil moisture probe (2900F Quick Draw Probe, Soil Moisture Equipment Corp., Santa Barbara, CA) and the proper interval for irrigation in the experimental condition was determined to be every three days.

Experimental design

When plants were 10–15 cm tall, they either were transferred to growth chambers with ambient CO_2 concentration or elevated CO_2 concentration, and left for three weeks to acclimate to the experimental conditions before infesting with test mites.

Within each CO_2 chamber, 15 test plants were divided into three groups of five (i.e., five replicates per CO_2 × irrigation combination), and the following watering regimes were conducted every three days during the experimental period: (Ainsworth et al., 2002) well watered (i.e., no water stress) plants which received 100% of plant water requirement; (Allen et al., 1994) intermediate water stressed plants which received 75% of plant water requirement; (Ayres, 1993) full water stressed plants which received 50% of plant water requirement. In this study, only one chamber was used per CO_2 treatment, therefore pseudoreplication (Hurlbert, 1984) was inevitable, however, pots in each chamber were moved randomly within the chamber (chamber swapping) every three days to minimize potential false effects caused by pseudoreplication (Quirk et al., 2013; Ryalls et al., 2013; Sherwin et al., 2013).

After three weeks, each test plant was infested with five newly emerged adult females of *T. urticae.* Mites were supplied from a stock culture which was established from individual females collected from an infested rose plant (*Rosa* sp. L.) in a greenhouse in the previous year, and maintained on sweet pepper. These sweet pepper plants were kept in a rearing room under controlled conditions (25 ± 2 °C, 60 ± 5% RH, and a photoperiod of L14:D10), and received 100% of sweet pepper water requirement every three days.

Infested test plants in each growth chamber were kept at the appropriate watering treatments and mite populations were allowed to develop for four weeks. After this period, the above ground parts of each plant were removed from the pots, and the number of mite eggs, immatures (larvae and nymphs), and adults on each plant were counted with the aid of a dissecting microscope. On each potted pepper plant, the number of each developmental stage per plant was considered as the population density of that specific stage, and subsequently, the total number of mites per plant was considered as the overall population density.

Calculation of the C/N ratio of potted plants

The C/N ratios were calculated by measuring the organic carbon and nitrogen content of potted plants in all six combinations of CO_2 × watering regime, using the Walkley Black method (Walkley, 1946) and Kjeldahl method (Kjeldahl, 1883), respectively.

Following the Walkley-Black method, a wet oxidation technique used for determining organic carbon in soil, plant, etc., organic matter was oxidized by heating plant material with a mixture of potassium dichromate and sulphuric acid. The excess of potassium dichromate was titrated with ferrous ammonium sulphate. Organic carbon was oxidized to carbon dioxide and the amount of organic carbon was determined by measuring the amount of dichromate used for its oxidation. Using the Kjeldal method for determining nitrogen content, dried plant samples were firstly ground and then digested using sulphuric acid and a catalyst, which decompose the organic substance by oxidation, and liberate the reduced nitrogen as ammonium sulphate. Afterwards, sodium hydroxide and boric acid were added and the digested sample was distilled, converting the ammonium to ammonia. In the final step, titration was performed using sulphuric acid to determine the amount of ammonia, and thus the amount of nitrogen present in the sample. The determined percentages of organic carbon and nitrogen were used to calculate the C/N ratio.

Statistical analysis

This experiment was designed as a 2 × 3 split-plot (as suggested by Hurlbert and White, 1993 to minimize the unintended effects of pseudoreplication) including two levels of CO_2 (ambient level and elevated level) assigned to main plots, and three levels of irrigation (100%, 75% and 50% of sweet pepper water requirement) allocated to sub-plots. Data were log transformed where needed to provide normality. C/N ratio of pepper plants as well as mite population data including number of eggs, immatures (larvae and nymphs), adults and total population were statistically analyzed using a general linear model (GLM) in Minitab Version 17 (Minitab, 2014) and means were compared using Fisher LSD test ($P < .05$). In this study five replicates were performed for each CO_2 × irrigation combination.

Population growth rate of mites over four weeks for each CO_2 × irrigation combination was estimated using the equation: $r = \mathrm{Ln}(N_{x+1}/N_x)/t$, where N_x is the population at time x, N_{x+1} the population at time x + 1 and t the difference in days between time x + 1 and x (Sadeghi-Namaghi and Amiri-Jami, 2018). The initial mite inoculum (five adult females) represented the beginning (N_x), and after four weeks the mite populations were counted again (N_{x+1}). Results were analyzed using a one-way ANOVA and means were compared using Fisher's LSD method ($P < .05$).

Results

Effect of elevated CO_2 on organic carbon, nitrogen and C/N ratio of sweet pepper

The organic carbon content of sweet pepper plants was not significantly influenced by CO_2 concentration or irrigation regime, however, their interaction caused significant changes (Table 1). Decreasing irrigation from no stress to intermediate stress in elevated CO_2 increased the carbon content by 16% (from 46.02 to 53.27). Also, in no stress irrigation regime, the carbon content for plants in elevated CO_2 was significantly lower by 16% compared to ambient CO_2 (46.02 and 54.6, respectively) (Table 3, Fig. 1E).

On the other hand, exposure to elevated CO_2 significantly decreased the nitrogen content and increased the C/N ratio in sweet pepper plants but irrigation regime and the interaction between CO_2 level and irrigation regime did not have significant effects on nitrogen or the C/N ratios (Tables 1, 2). Compared to ambient CO_2, elevated CO_2 concentration caused nitrogen content to decrease by 34% (from 2.71 to 1.79) and C/N ratio to increase by 44% (from 20.96 to 30.17) (Table 2).

Effect of CO_2 and irrigation regime on population density of T. urticae

CO_2 and irrigation regime significantly influenced population density of *T. urticae* on sweet pepper (Table 1). Compared to ambient CO_2, the population density of adults per plant was 41% higher under elevated CO_2 (1.31 and 1.85, respectively) (Table 3). However, elevated CO_2 did not significantly increase the population density of other developmental stages or the overall population (Table 1). Water stress significantly increased the population density of all developmental stages, as well as the overall population (Table 1(. Intermediate water stress (75% irrigation) increased population density of eggs by 21% (from 1.95 to 2.35), immatures by 25% (from 1.73 to 2.17), adults by 26% (from 1.34 to 1.69) and total population by 17% (from 2.24 to 2.63), compared to the well watered treatment (100% irrigation) (Table 3). Also, applying full water stress (50% irrigation) increased population density of eggs by 22% (from 1.95 to 2.37), immatures by 31% (from 1.73 to 2.27), adults by 28% (from 1.34 to 1.72) and total population by 21% (from 2.24 to 2.70) compared to the well watered treatment (Table 3).

In addition, the interaction between CO_2 and irrigation regime was statistically significant for all developmental stages of *T. urticae* including eggs, immatures (larvae and nymphs) and adults, as well the total population (Table 1). Depiction of graphs based on comparison of mean values for *T. urticae* population density of different life stages and the total population are shown in Fig. 1. Mite population, for all developmental stages, reached a peak at a combination of elevated CO_2 × intermediate water stress which was also the observed pattern for total population (Fig. 1). Population increase at the elevated CO_2 × intermediate water stress compared with the ambient CO_2 × intermediate water stress was 47% (from 1.90 to 2.80) for eggs, 55% (from 1.70 to 2.64) for immatures, 86% (from 1.18 to 2.19) for adults and 42% (from 2.17 to 3.09) for overall population (Table 3; Fig. 1).

In addition, the adult stage showed a significant increase by 51% (from 1.37 to 2.07) at elevated CO_2 × full water stress, compared to ambient CO_2 × full water stress (Table 3; Fig. 1C), but such pattern was not observed for other stages or the overall population (Table 3; Fig. 1A,B,D).

Furthermore, for all stages and total population of *T. urticae* in ambient CO_2, decreasing the irrigation from 100% to 75% does not cause any significant population change, however, decreasing the irrigation from 75% to 50% causes significant population increase by 30% (from 1.70 to 2.21) for immatures and 22% (from 2.17 to 2.64) for total population, but no significant increase was observed for eggs or adults (Table 3; Fig. 1). On the other hand, decreasing the irrigation from 100% to 75% in elevated CO_2 resulted in significant population increase by 59% (from 1.76 to 2.80) for eggs, 81% (from 1.46 to 2.64) for immatures, 70% (from 1.29 to 2.19) for adults and 51% (from 2.05 to 3.09) for total population (Table 3; Fig. 1), however, decreasing the irrigation from 75% to 50% resulted in significantly lower number of eggs by 16% (from 2.80 to 2.35) (Table 3; Fig. 1A) but had no significant effect on other stages or the total population (Table 3; Fig. 1B,C,D).

Table 1
Analysis of variance (ANOVA) results for the effect of CO_2 concentration and irrigation regime on: Organic carbon (C) content, nitrogen (N) content and C/N ratio of sweet pepper plants; Population density of various developmental stages and total population of *Tetranychus urticae*.[†]

	CO_2 Level			Irrigation regime		CO_2 Level × Irrigation regime		
Component	F	P	Error 1	F	P	F	P	Error 2
C	0.03 (1,8)	0.856	37.16	0.10 (2,16)	0.909	4.24 (2,16)	0.033*	29.51
N	9.93 (1,8)	0.014*	0.63	0.58 (2,16)	0.573	0.67 (2,16)	0.526	0.427
C/N	8.29 (1,8)	0.021*	76.83	0.11 (2,16)	0.897	0.13 (2,16)	0.879	82.716
Egg	1.74 (1,8)	0.224	0.109	4.91 (2,16)	0.022*	9.72 (2,16)	0.002*	0.114
Immatures	1.65 (1,8)	0.234	0.13	6.33 (2,16)	0.009*	10.38 (2,16)	0.001*	0.132
Adult	22.47 (1,8)	0.001*	0.0968	4.07 (2,16)	0.037*	7.65 (2,16)	0.005*	0.109
Total Population	3.88 (1,8)	0.084	0.0944	6.13 (2,16)	0.011*	10.55 (2,16)	0.001*	0.102

* $P < .05$.
† First and second number in parenthesis show factor and error degrees of freedom (d.f.), respectively. Error 1, Error term for CO_2 level; Error 2, Error term for irrigation regime, and CO_2 level × irrigation regime.

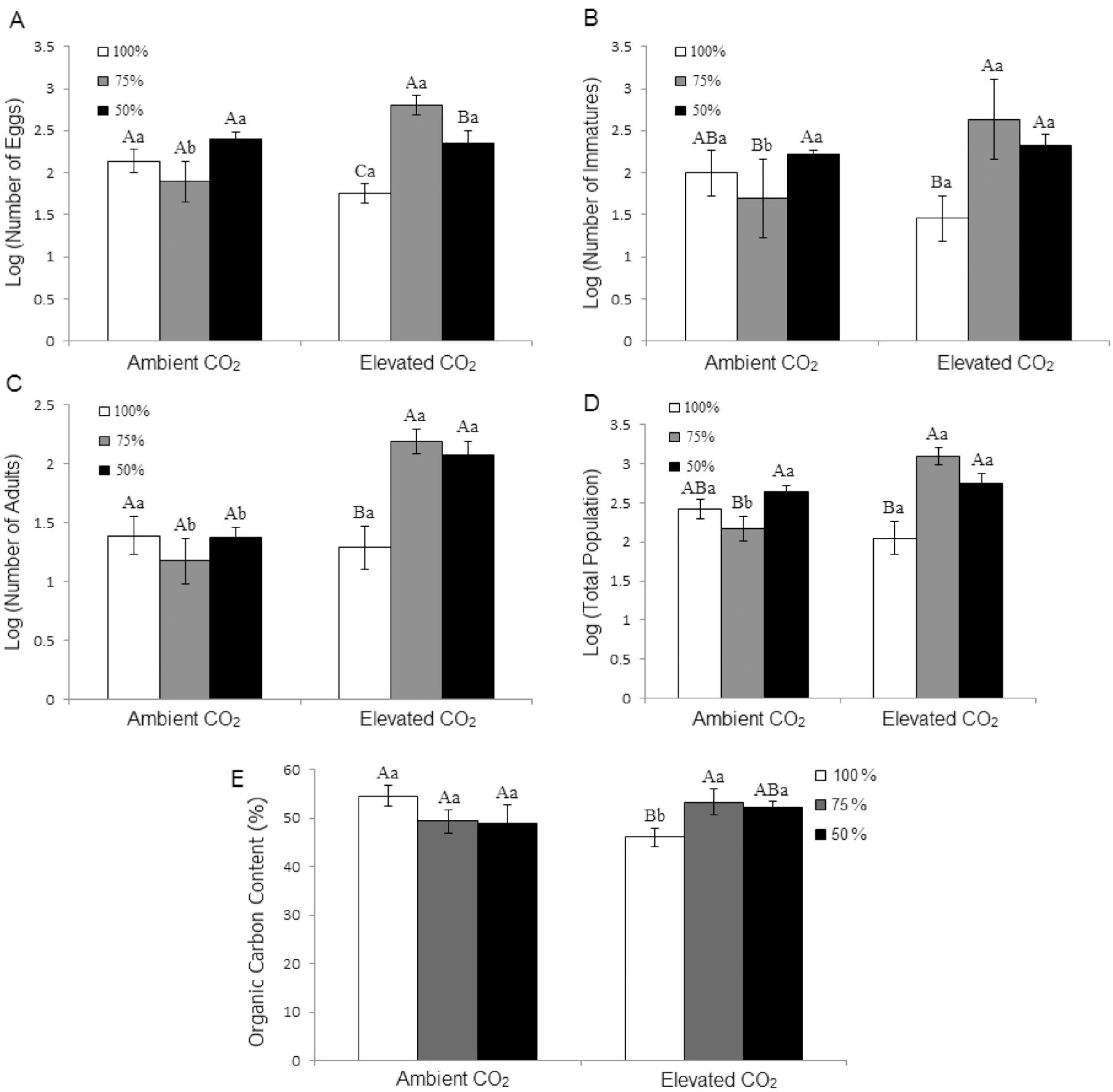

Fig. 1. Comparison of means for CO_2 and irrigation regime interaction on: Population density of different life stages and total population of *T.urticae* feeding on sweet pepper; Organic carbon content of sweet pepper. 100%, No water stress; 75%, Intermediate water stress; 50%, Full water stress (based on sweet pepper water requirement). Different uppercase letters show significant differences between three irrigation regimes within the same CO2 level, and different lowercase letters show significant differences between two CO2 levels within the same irrigation regime (LSD test, $P < .05$); Bars show mean standard error.

Table 2
Mean ± standard error for effect of CO_2 and irrigation regime on nitrogen (N) content and C/N ratio of sweet pepper.[†]

Variable	Ambient CO_2	Elevated CO_2	100%	75%	50%
N	2.71 ± 0.21A	1.79 ± 0.13B	2.28 ± 0.31a	2.39 ± 0.27a	2.08 ± 0.19a
C/N	20.96 ± 2.17A	30.17 ± 2.17B	25.18 ± 2.80a	24.88 ± 3.56a	26.65 ± 2.89a

Means with different upper-case letters show significant difference between levels of CO_2; Means with similar lower-case letters are not significantly different between levels of irrigation (LSD test, $P < .05$).
[†] 100%, No water stress; 75%, Intermediate water stress; 50%, Full water stress (based on sweet pepper water requirement).

Table 3
Two-way ANOVA results (mean ± SE) for effect of CO_2 concentration and irrigation regime on organic carbon content of sweet pepper plant, and population density per sweet pepper plant for different developmental stages and total population of *T. urticae* under environmentally controlled conditions.[†]

CO_2 level	Ambient CO_2			Elevated CO_2		
Irrigation regime	100%	75%	50%	100%	75%	50%
C	54.6 ± 2.10^{Aa}	49.37 ± 2.39^{Aa}	48.83 ± 3.97^{Aa}	46.02 ± 1.97^{Bb}	53.27 ± 2.67^{Aa}	52.26 ± 1.28^{ABa}
Egg	2.14 ± 0.14^{Aa}	1.90 ± 0.24^{Ab}	2.39 ± 0.10^{Aa}	1.76 ± 0.12^{Ca}	2.80 ± 0.12^{Aa}	2.35 ± 0.15^{Ba}
Immature	2.00 ± 0.11^{ABa}	1.70 ± 0.20^{Bb}	2.21 ± 0.04^{Aa}	1.46 ± 0.26^{Ba}	2.64 ± 0.12^{Aa}	2.33 ± 0.13^{Aa}
Adult	1.39 ± 0.16^{Aa}	1.18 ± 0.18^{Ab}	1.37 ± 0.08^{Ab}	1.29 ± 0.19^{Ba}	2.19 ± 0.10^{Aa}	2.07 ± 0.11^{Aa}
Total	2.42 ± 0.13^{ABa}	2.17 ± 0.21^{Bb}	2.64 ± 0.07^{Aa}	2.05 ± 0.15^{Ba}	3.09 ± 0.11^{Aa}	2.75 ± 0.12^{Aa}

Original data have been log transformed where needed to provide normality.
Means followed by different uppercase letters indicate significant difference between irrigation regimes within the same CO_2 level, while different lowercase letters indicate significant difference between CO_2 levels within the same irrigation regime (LSD test, $P < .05$).
[†] 100%, No water stress, 75%, Intermediate water stress; 50%, Full water stress (based on sweet pepper water requirement).

Effect of CO_2 and irrigation regime on per capita growth rate (r) of T. urticae

Mites feeding on plants reared under elevated $CO_2 \times$ intermediate water stress had the highest per capita population growth rate ($r = 0.197$). The lowest population growth rates of *T. urticae* were found in combinations of ambient $CO_2 \times$ intermediate water stress and elevated $CO_2 \times$ no water stress ($r = 0.12$ and 0.11, respectively). Decreasing irrigation from 75% to 50% in ambient CO_2, and from 100% to 75% in elevated CO_2 caused significant increase in per capita growth rate by 33% (from 0.12 to 0.16) and 79% (from 0.11 to 0.197), respectively (Table 4; Fig. 2).

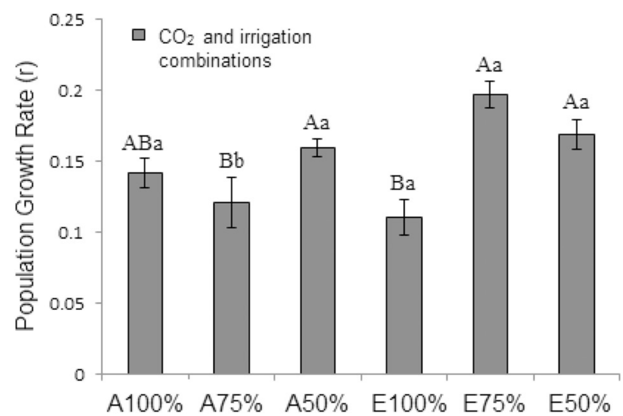

Fig. 2. Comparison of means for per capita growth rate (r) of *T. urticae* under different combinations of CO_2 and irrigation regime. A, ambient CO_2; E, elevated CO_2; 100%, No water stress; 75%, Intermediate water stress; 50%, Full water stress (based on sweet pepper water requirement). Different uppercase letters show significant differences between irrigation regimes within the same CO2 level, and different lowercase letters show significant differences between CO2 levels within the same irrigation regime. (LSD test, $P < .05$); Bars show mean standard error.

Discussion

Climatic variables such as atmospheric CO_2 concentration, temperature, rainfall, and drought interact with plants in numerous ways with diverse mechanisms. Increasing atmospheric CO_2 level will increase global temperature, and cause changes in rainfall patterns, which will result in many areas of the world becoming wetter and many other parts becoming drier. These set of changes will affect the plants as well as the interaction of plants and their herbivores (Villalpando et al., 2009).

In this study, exposure to elevated CO_2 decreased the nutritional quality of sweet pepper plants by increasing the C/N ratio. Also, water stress significantly increased the population density of all developmental stages, as well as the overall population of *T. urticae*. Except in case of adult stage population, which increased by 41%, elevated CO_2 did not significantly increase the population density of other developmental stages or the total population. However, the interactions of CO_2 with levels of water stress was found to influence the mites' population. Compared to ambient CO_2, intermediate water stress in elevated CO_2

Table 4
One-way ANOVA results (mean ± SE) for effect of different combinations of CO_2 concentration and irrigation regime on per capita growth rate (r) of *T. urticae* reared on sweet pepper under environmentally controlled conditions (d.f. = 5, 24; F = 7.58; $P < .000$).[†]

	Ambient CO_2			Elevated CO_2		
	100%	75%	50%	100%	75%	50%
r	0.14 ± 0.011^{ABa}	0.12 ± 0.018^{Bb}	0.16 ± 0.006^{Aa}	0.11 ± 0.013^{Ba}	0.197 ± 0.009^{Aa}	0.169 ± 0.010^{Aa}

Means with different uppercase letters are significantly different between three irrigation regimes within the same CO2 level, whereas means followed by different lowercase letters are significantly different between two CO2 levels within the same irrigation regime (LSD test, $P < .05$).
[†] 100%, No water stress; 75%, Intermediate water stress; 50%, Full water stress (based on sweet pepper water requirement).

caused the mite population to increase significantly and the highest population density was observed in the combination of elevated CO_2 × intermediate water stress. The least number of mites was observed in interactions of ambient CO_2 × intermediate water stress, as well as elevated CO_2 × no water stress combinations. It was also observed that in ambient CO_2, full water stress significantly increased the mites' population, in comparison to intermediate water stress, while in elevated CO_2, intermediate water stress drastically increased the mites' population, compared to no stress irrigation. In terms of per capita growth rate, mites feeding on plants reared under elevated CO_2 concentration × intermediate water stress had the highest per capita growth rate (r) compared to other treatments.

Several studies (e.g., Allen et al., 1994; Dugas et al., 1997; Ainsworth et al., 2002; Dáder et al., 2016; Serret et al., 2018) have shown positive growth responses of plants to elevated CO_2 which results not only from increased uptake and assimilation of CO_2, but also from decreased transpiration by inducing the partial closure of stomatal guard cells of plants. According to Bazzaz (1990), reduction in transpiration, coupled with increased photosynthesis, can contribute to increased water use efficiency by plants and such responses of plants might partially improve the effects of drought. On the other hand, several authors (e.g., White, 1984; Mattson and Haack, 1987; Huberty and Denno, 2004) have reported that drought often increases nitrogen in plant tissues. As nitrogen is the primary component in plant foliage and a limiting nutrient which affects the growth of herbivores (Mattson Jr, 1980; Awmack and Leather, 2002), it is expected that increased CO_2 concentrations and water stress may alter plant-herbivore interactions. Several studies have investigated this subject and reported contradictory results. For example, Heagle et al. (2002) reported that elevated atmospheric CO_2 increased *T. urticae* population on white clover and correlations between mite population increases were significantly positive for foliar nonstructural carbohydrates and significantly negative for foliar nitrogen. Conversely, in the study by Boutaleb Joutei et al. (2000), elevated CO_2 increased C/N ratio in common bean but caused a reduction in *T. urticae* progeny. Yildrim and Akaroglu (2012) found that CO_2 enrichment did not affect population density of *Tetranychus cinnabarinus* (Boisduval) on Strawberry (*Fragaria* × *ananassa* Duchesne) directly.

In case of other herbivores, populations of six leaf miner species on oak decreased in elevated CO_2 (Stiling et al., 1999). Also, populations of a spittlebug (*Neophilaenus lineatus* L.) feeding on xylem of common rush (*Juncus squarrosus* L.) were decreased by elevated CO_2 (Brooks and Whittaker, 1999). But, populations of aphids such as *Myzus persicae* (Sulzer) on groundsel (*Senecio vulgaris* L.) and annual blue grass (*Poa annua* L.) often respond positively to elevated CO_2 (Bezemer et al., 1998). Similar results have been reported for *Aulacorthum solani* (Kalt.) on bean (*Vicia faba* L.) (Awmack et al., 1997) and *Sitobion avenae* (F.) on winter wheat (*Triticum aestivum* L.)(Awmack et al., 1996).

Physiological changes in plants caused by water deficit include reduction of secondary metabolites or protective compounds synthesis, better balance and concentration of plant nutrients, increased soluble nitrogen content, and raised temperature of leaf surface, which may lead to an increase in reproduction and population growth of mites, resulting in further damage to host plants (House, 1974; Haack and Slansky Jr, 1987; Mattson and Haack, 1987; Oi et al., 1989; Louda and Collinge, 1992; Huberty and Denno, 2004). Although most studies have shown that water stress leads to an increase in population density of spider mites (Chandler et al., 1979; Hollingsworth and Berry, 1982; Colijn and Lindquist, 1986; Youngman et al., 1988; Nikolova et al., 2014; Shibuya et al., 2015), the findings of Mellors et al. (1984) and those of Oloumi-Sadeghi et al. (1988) suggest that outbreaks of spider mites on soybean during periods of drought are not necessarily due only to physiological changes in plants caused by water stress. In both cases, water stress reduced the density of spider mite populations. Several factors such as high temperatures, lowered turgor pressure, and increased cell-sap viscosity, and the degree of imposed water stress have been proposed as explanations for the decline of aphid and mite populations on severely stressed host plants (Mattson and Haack, 1987). In the present study, the effects of these factors were not investigated.

Overall, our results indicate that in comparison to the ambient CO_2 concentration of the earth's atmosphere, future elevated CO_2 levels in combination with even intermediate levels of water stress are influential on *T. urticae* populations and have the potential to cause outbreaks and increase damage to sweet pepper and most probably other related host plants. However, similar studies with repeated experimental runs which would reduce the problems of pseudoreplication are much desired. Moreover, determining how the increased atmospheric CO_2 concentration in combination with water stress induce changes in plant quality, and consequently will impact the complex interactions with herbivorous insects and mites needs further studies. Such information are required to develop management strategies for the agricultural crops to adapt to future environmental conditions.

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Declarations of interest

None.

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