Does elevated CO_2 protect photosynthesis from damage by high temperature *via* modifying leaf water status in maize seedlings?

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Abstract

We hypothesized that decreased stomatal conductance (g_s) at elevated CO_2 might decrease transpiration (E), increase leaf water potential (Ψ_W), and thereby protect net photosynthesis rate (P_N) from heat damage in maize (*Zea mays* L) seedlings. To separate long-term effects of elevated CO_2, plants grew at either ambient CO_2 or elevated CO_2. During high-temperature treatment (HT) at 45°C for 15 min, leaves were exposed either to ambient CO_2 (380 µmol mol^{-1}) or to elevated CO_2 (560 µmol mol^{-1}). HT reduced P_N by 25 to 38% across four CO_2 combinations. However, the g_s and E did not differ among all CO_2 treatments during HT. After returning the leaf temperature to 35°C within 30 min, g_s and E were the same or higher than the initial values. Leaf water potential (Ψ_W) was slightly lower at ambient CO_2, but not at elevated CO_2. This study highlighted that elevated CO_2 failed in protecting P_N from 45°C *via* decreasing g_s and Ψ_W.

Additional key words: heat stress, leaf water potential; net photosynthesis; stomatal conductance; vapor pressure deficit.

Introduction

High air temperatures are a serious threat to crop production worldwide (Mearns *et al.* 1984). Heat stress affects photosynthetic and metabolic processes that ultimately influence the production of biomass, fruits, and grains (Hay and Walker 1989, Orbovic and Poff 2007). Rising atmospheric concentrations of CO_2 are expected to increase the frequency of extreme high-temperature events (Groisman and Knight 2008). Nevertheless, research on plant heat tolerance is not focused on the effects of elevated CO_2.

In a few C_3 species, elevated CO_2 improved the tolerance of photosynthesis to high temperatures (Taub *et al.* 2000, Hamilton *et al.* 2008, Gutiérrez *et al.* 2009). Hamilton *et al.* (2008) exposed whole plants of maize and other species to high-temperature stress at two growth CO_2 concentrations. They found that elevated CO_2 did not always protect P_N from damage. Moreover, Wang *et al.* (2008) reported that elevated CO_2 even decreased the tolerance to high temperatures in some cases. In C_4 species, P_N is usually near or at saturation for CO_2 at the current atmospheric concentration, thus, no positive effect of elevated CO_2 on P_N should be anticipated at high temperatures (Kim *et al.* 2007) as it is in C_3 species (Taub *et al.* 2000). However, indirect effects of CO_2 concentration could influence the tolerance of high temperatures even in C_4 species because of changes in g_s, E, and Ψ_W at elevated CO_2.

In the field, high-temperature stress is usually accompanied by high evaporative demand for water vapor. Thus, leaf desiccation can be involved in the damage caused by high temperatures. If desiccation is involved, then partial stomata closure caused by elevated CO_2 could mitigate the damage by high temperatures. Therefore, we hypothesized that decreased g_s at elevated CO_2 might protect P_N in C_4 species from damage caused by high-temperature stress, reducing E, and increasing leaf Ψ_W.

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Abbreviations: AC – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; CT – control; E – transpiration rate; EC – elevated CO_2 concentration; g_s – stomatal conductance; HT – high-temperature treatment; P_N – net photosynthetic rate; R_I – relative injury; T_{leaf} – leaf temperature; VPD – water vapor pressure deficit; WJC – water-jacketed cuvette; WUE – water-use efficiency; Ψ_W – water potential.

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Materials and methods

Growth: Maize (*Zea mays L.* cv. Silver Queen) plants were grown in two environment-controlled chambers under either ambient CO_2 (AC, 380 µmol mol^{-1}) or elevated CO_2 (EC, 560 µmol mol^{-1}) concentrations. Plants were grown in vermiculite and irrigated daily with a complete nutrient solution containing 14.5 mM total N. Day/night air temperatures were 29/17ºC, while soil temperature averaged 25.7 ± 0.33ºC/14.8 ± 0.41ºC. Light was provided by a mixture of high-pressure sodium and metal halide lamps (*Shanghai Yahong Electrical Lighting Co., Ltd*, China) at a photosynthetic photon flux density (PPFD) of 1,000 µmol m^{-2} s^{-1}, with a photoperiod of 12 h. Air humidity was 60% during the daytime. When the 4th leaf numbered from the bottom was fully expanded (14 d after emergence), a single leaf was exposed to a high-temperature treatment described below.

High-temperature treatment (HT): P_N, g_s, and intercellular CO_2 concentration (C_i) of nonstressed leaves (control, CT) were measured over a range of CO_2 concentrations and temperatures with a portable photosynthesis system (*CIRAS-2*, *PP-Systems*, Amesbury, MA, USA) equipped with a LED light source, at the growth PPFD of 1,000 µmol m^{-2} s^{-1}. In a preliminary test, P_N were significantly reduced after exposure to 45ºC for 15 min, but recovered to almost the initial rates after 24 h. Thus, HT of 45ºC for 15 min was chosen for this study, because it produced significant, but slowly reversible damage to photosynthesis.

For each measurement, single, intact 4th leaf attached to the plants, numbered from the bottom, with leaf area of *ca*. 40 cm^2 was placed inside a water-jacketed cuvette (WJC) with an internal fan (Bunce 2006) in the following arrangement:

Combination	Growth	During HT
AA	AC	AC
AE	AC	EC
EA	EC	AC
EE	EC	EC

Leaf temperature was measured using a miniature thermister (*NTC 5k, ET Enterprises Ltd.*, UK) pressed against the lower leaf surface. These leaf sections were then exposed to gradually increasing leaf temperatures (T_{leaf}) from 35 to 45ºC, held at 45ºC for 15 min, and then T_{leaf} was gradually decreased again to 35ºC (Fig. 1). The whole HT cycle was completed within 1 h. During this cycle, light intensities were maintained at 1,000 µmol m^{-2} s^{-1} PPFD, and the water vapor content of air in the WJC was held constant. A *Ciras-1* portable photosynthesis system (*PP-Systems*, Amesbury, MA) programmed to use an external air supply and leaf chamber was used to record the CO_2 concentration, P_N, g_s, C_i, T_{leaf}, and water vapour pressure deficit (VPD) for the intact leaf inside the WJC throughout the temperature cycle (Bunce 2006). The reduction in photosynthesis due to the HT was quantified as: (*1*) the differences between P_N before and after HT for each temperature, and (*2*) the percentage decrease in P_N after HT for each temperature calculated as: $[(P_{NHT} - P_{NC})/P_{NC}] \times 100$, where P_{NHT} is P_N after HT treatments and P_{NC} is P_N before HT treatments for each temperature, as in Ameye *et al.* (2012). Water-use efficiency (WUE) was calculated as a ratio of P_N to *E*. Nine leaves from 9 different plants were subjected to HT for each of the four combinations of growth and measurement CO_2 concentration. For P_N measurements, 9 leaves per treatment were sampled.

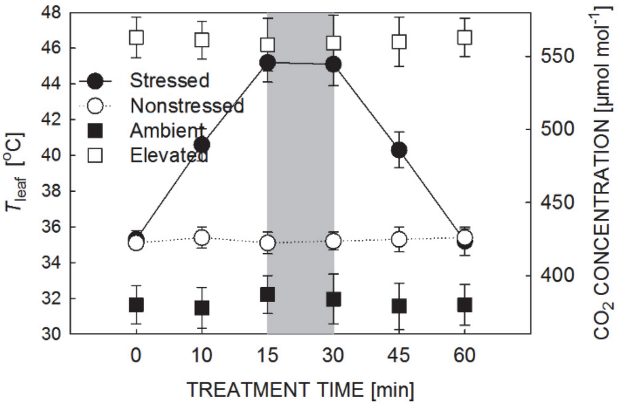

Fig. 1. Time courses of leaf temperatures (T_{leaf}) and CO_2 concentrations during the heat stress cycles. During the whole temperature cycle, light was maintained at PPFD of 1,000 µmol m^{-2} s^{-1}. *Vertical bars* represent SE.

Water potential (Ψ_W) and electrolyte leakage: Leaf samples were collected from the control and stressed, 4th leaves after the HT. For the measurement of leaf Ψ_W, leaf disc of a 6-mm diameter was removed from the leaf section in the WJC and immediately sealed in a sample chamber for determination of Ψ_W using a *Wescor HR-33* dew point hygrometer (*Wescor, Inc.*, Logan, UT), as previously described by Sicher and Barnaby (2012). Electrolyte leakage was expressed as relative injury (R_I) according to the procedure of Warren *et al.* (1996) with minor modification. Five leaf discs taken from the leaf inside the WJC, washed 3 times, and placed in glass tubes containing 30 mL of distilled water. The tubes were kept at room temperature (about 20ºC) for 16 h. The tubes were then gently shaken for 15 min and electrical conductivity was measured with an *Okaton 510* conductivity meter (*Eutech Instrumenta*, Singapore). The conductivity values were designated as T_1. The tubes were then boiled in a microwave oven for 3 min to release all electrolytes. After cooling to room temperature, the tubes were briefly shaken and the conductivity was measured as the total ion content (T_2). Average conductivity of distilled water served as the basic ion content (T_0). The R_I after HT was calculated by $[(T_1-T_0)/(T_2-T_0)] \times 100$. Because of the limited size of the leaf material inside the WJC, discs for water potential and electrolyte leakage measurements were taken from different plants. Before sampling, they were subjected to HT in a similar fashion as those for the P_N measurements.

Statistical analysis: For the Ψ_W and electrolyte leakage analyses, $n = 4$. Two-way analysis of variance (*ANOVA*) was used to test the effects of growth CO_2, exposure CO_2, and their interaction for the leaf gas exchange data. Means were compared using *Fisher*'s protected LSD test. A program used for calculations is *StatView* (*SAS Institute Inc.*, USA).

Results

The HT reduced P_N in all combinations of growth and measurement CO_2 concentration (Table 1). P_N decreased after HT by 24–38% compared with the CT in all CO_2 combinations. The decrease in P_N was significantly lesser when the HT occurred at AC rather than at EC (Table 1). In the plants grown and exposed to AC, P_N was the highest at the beginning of HT and at the end of HT in all combinations of CO_2. It suggested that EC did not protect P_N from the damage caused by HT (Fig. 2). Both growth CO_2 and its interaction with exposure CO_2 had no significant effect on the decrease of P_N (Table 1). Exposure to AC rather than EC prior to the HT resulted in higher g_s and E at both 35ºC and 40ºC in plants from both growth CO_2 concentrations (Fig. 3*A*). During the HT, g_s, E, VPD, WUE, and Ψ_W increased in all CO_2 combinations, while P_N declined (Figs. 2,3,4). After returning the T_{leaf} to 40ºC and then to 35ºC within 30 min, g_s and C_i were the same or higher than the initial values at those temperatures, while P_N

Table 1. Effects of CO_2 concentration during growth and during exposure to high temperature and their effects on the decreases in P_N after a heat stress treatment, compared with values at each temperature measured before the heat stress. Within each temperature, values with the same *lowercase letters* are not significantly different using *Fisher*'s LSD test. ns – not significant; * – $P \le 0.05$; ** – $P \le 0.01$. G, E – growth and exposure to CO_2 concentrations, respectively; AC, EC – ambient and elevated CO_2, respectively. T_{leaf} – leaf temperature. Values are ± SE for $n = 9$.

CO_2 treatments	Decrease in P_N [µmol(CO_2) m^{-2} s^{-1}]			Decrease in P_N [%]		
	T_{leaf} [ºC]			T_{leaf} [ºC]		
	35	40	45	35	40	45
$G_{AC}E_{AC}$	9.6 ± 2.2^b	10.9 ± 2^b	7.5 ± 1.6^a	26.6 ± 2.8^b	31.2 ± 2.7^b	25.5 ± 2.5^b
$G_{AC}E_{EC}$	13.3 ± 2.0^a	15.5 ± 2.2^a	9.5 ± 2.0^a	35.0 ± 2.5^{ab}	41.6 ± 2.8^{ab}	35.1 ± 3.8^{ab}
$G_{EC}E_{AC}$	11.9 ± 1.5^a	12.3 ± 2.3^a	6.1 ± 1.9^b	34.0 ± 1.9^{ab}	36.9 ± 2.7^{ab}	24.8 ± 3.8^b
$G_{EC}E_{EC}$	14.3 ± 3.9^a	16.9 ± 3.4^a	9.8 ± 1.9^a	37.6 ± 4.9^a	44.7 ± 4.4^a	38.1 ± 4.6^a
G	ns	ns	ns	ns	ns	ns
E	*	**	*	ns	*	**
Interaction	ns	ns	ns	ns	ns	ns

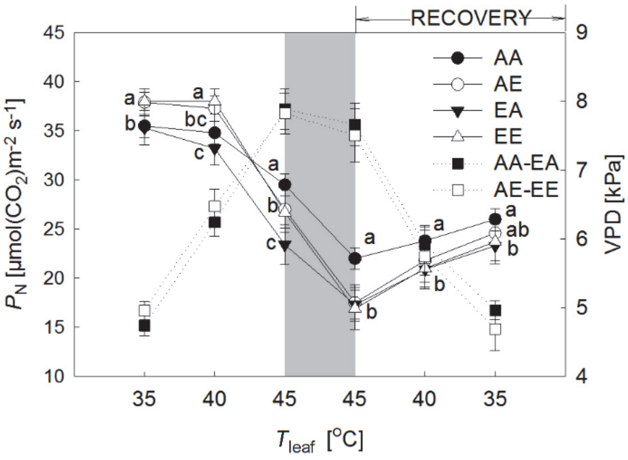

Fig. 2. Net photosynthetic rate (P_N) in response to the high-temperature treatment of 4 combinations of growth and exposure to CO_2 for maize seedlings, and the water vapor pressure deficit (VPD), to which leaves were exposed. P_N and VPD are depicted with *solid* and *dotted lines*, respectively. AA – growth and exposure at the ambient carbon dioxide concentration (AC, 380 µmol mol^{-1}), AE – growth at AC and exposure at the elevated concentration (EC, 560 µmol mol^{-1}), EE – growth and exposure at EC, and EA – growth at the EC and exposure at the AC. AA-EA – exposure to heat stress at AC across growth CO_2, AE-EE – exposure to heat stress at EC across growth CO_2. Within each temperature point, values among different [CO_2] treatments with same *lowercase letter* are not significantly different ($P>0.05$). Grey area represents the 15-min period of exposure to high temperature. *Vertical bars* represent SE for $n = 9$. T_{leaf} – 45ºC.

was lower than initial values (Figs. 2,3). Leaf Ψ_W was slightly lower after the HT at AC, but not at EC (Fig. 4*A*).

There were higher values of R_I from leaf tissue after the HT at both CO_2 concentrations, but the values of R_I were lower at AC than at EC (Fig. 4*C*).

Discussion

Our results underlined that the damage to P_N due to 45ºC was greater under EC, regardless of EC used for plant growth, compared to the plants grown and exposed to AC. Thus, EC failed in protecting P_N from 45ºC in this maize cultivar. Hamilton *et al.* (2008) showed that EC increased the damage to P_N caused by the HT in maize at 50ºC. In their study, maize, pigweed, lambs quarters, and pea were subjected to 3 different temperature treatments. Results showed that the negative EC effects occurred mainly at the highest temperature. In maize, CO_2 effects similar to those observed here occurred also at 45ºC (Wang *et al.* 2008). The lower P_N (Fig. 2), and higher g_s (Fig. 3*A*), and C_i (Fig. 3*B*) indicated that nonstomatal inhibition of P_N

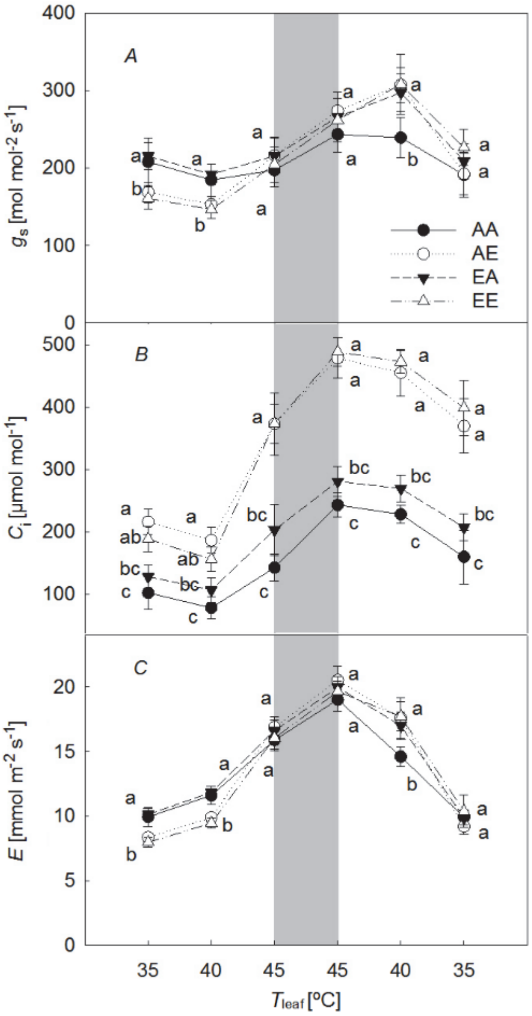

Fig. 3. Effects of a high-temperature treatment on *A*: stomatal conductance (g_s), *B*: intracellular CO_2 (C_i) and *C*: transpiration (*E*) under 4 combinations of growth and exposure CO_2 concentrations. AA – growth and exposure at the ambient carbon dioxide concentration (AC, 380 µmol mol^{-1}), AE – growth at AC and exposure at the elevated concentration (EC, 560 µmol mol^{-1}), EE – growth and exposure at EC, and EA – growth at EC and exposure at AC. Within each temperature point, values among different CO_2 treatments with same *lowercase letter* are not significantly different using *Fisher*'s LSD test ($P > 0.05$). The grey area represents the high-temperature treatment of 15 min. *Vertical bars* represent SE for $n = 9$.

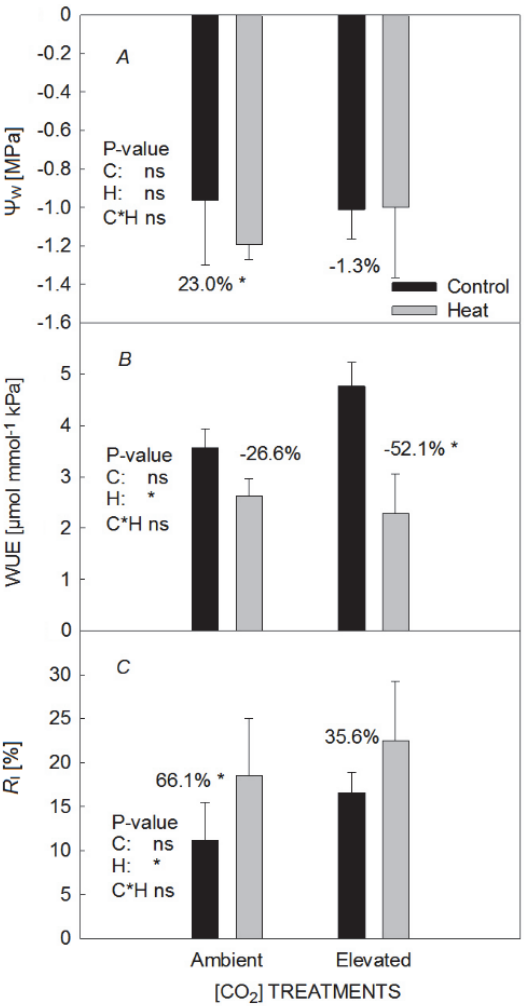

Fig. 4. High-temperature treatment induced changes in *A*: water potential (Ψ_W), *B*: water-use efficiency (WUE), and *C*: leaf relative injury (R_I) in leaves grown and measured at ambient and elevated CO_2. CO_2 effect (C), heat stress effect (H), and their interaction effect were inserted into each panel. ns – not significant; * – $P < 0.05$. *Vertical bars* represent SE, with $n = 4$ for Ψ_W and R_I and $n = 9$ for WUE.

occurred after the HT. This was also found after HT stress in grapes (Luo *et al.* 2011) and in birch (Ranney and Peet 1994).

With high g_s and VPD during the HT, the values of E were 16 to 21 mmol m^{-2} s^{-1}, approximately twice of those at 35ºC (Fig. 4). Thus, it was remarkable that the HT did not lower Ψ_W substantially. The lowest Ψ_W observed (−1.2 MPa) was too high to cause inhibition of P_N in this cultivar (Barnaby and Sicher 2012). Thus, the HT did not produce damaging leaf Ψ_W at two CO_2 concentrations despite of high VPD and E. The lack of stomata response to CO_2 concentration and the lack of significant leaf water deficits during the HT stress explainned why our hypothesis was contradicted by the experimental data. Our results indicated that damage to P_N occurred at 45ºC without the involvement of low leaf Ψ_W.

The premise of our hypothesis was that part of the damage to P_N caused by HT in combination with high VPD would be caused by low leaf Ψ_W resulting from high E. In such situation, we expected EC to decrease g_s and reduce the drop in leaf Ψ_W, thus preventing the damage. This scenario did not occur for two reasons in this maize cultivar. First, T_{leaf} of 45ºC was high enough to cause damage to P_N in this species. Fig. 3 clearly indicated that during HT no differences in g_s occurred between the AC and EC. This was even true comparing CO_2 concentrations of 200 and 1,000 μmol mol^{-1} (data not shown). In addition, because the HT occurred at high VPD, it could be either HT or high VPD that eliminated the stomata response to CO_2. Reduced CO_2 effect on g_s at high VPD has been reported in other species, *e.g.*, potato and sorghum (Bunce 2003), *Bromus japonicas* (Maherali et al. 2003), and wheat and barley (Bunce 2000b). An increase in g_s with temperature at constant water VPD occurs in many species including soybean, sunflower, tomato, turnip, winter wheat, barley (Bunce 2000a,b), and castor bean (Dai *et al.* 1992). Current study did not find a major weakening of membrane integrity by the heat stress. At this point, Xu *et al.* (2011) also found no ion leakage in maize after heat stress.

Conclusion: EC did not protect photosynthesis from inhibition caused by high temperature by modifying the leaf water status at high leaf temperature in maize. Actually, EC increased the inhibition of photosynthesis during the high temperature treatment.

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