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RESEARCH PAPER

Modification of photosynthesis and growth responses to elevated CO_2 by ozone in two cultivars of winter wheat with different years of release

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Abstract

The beneficial effects of elevated CO_2 on plants are expected to be compromised by the negative effects posed by other global changes. However, little is known about ozone (O_3)-induced modulation of elevated CO_2 response in plants with differential sensitivity to O_3. An old (*Triticum aestivum* cv. Beijing 6, O_3 tolerant) and a modern (*T. aestivum* cv. Zhongmai 9, O_3 sensitive) winter wheat cultivar were exposed to elevated CO_2 (714 ppm) and/or O_3 (72 ppb, for 7 h d^{-1}) in open-topped chambers for 21 d. Plant responses to treatments were assessed by visible leaf symptoms, simultaneous measurements of gas exchange and chlorophyll *a* fluorescence, *in vivo* biochemical properties, and growth. It was found that elevated CO_2 resulted in higher growth stimulation in the modern cultivar attributed to a higher energy capture and electron transport rate compared with the old cultivar. Exposure to O_3 caused a greater growth reduction in the modern cultivar due to higher O_3 uptake and a greater loss of photosystem II efficiency (mature leaf) and mesophyll cell activity (young leaf) than in the old cultivar. Elevated CO_2 completely protected both cultivars against the deleterious effects of O_3 under elevated CO_2 and O_3. The modern cultivar showed a greater relative loss of elevated CO_2-induced growth stimulation due to higher O_3 uptake and greater O_3-induced photoinhibition than the old cultivar at elevated CO_2 and O_3. Our findings suggest that the elevated CO_2-induced growth stimulation in the modern cultivar attributed to higher energy capture and electron transport rate can be compromised by its higher O_3 uptake and greater O_3-induced photoinhibition under elevated CO_2 and O_3 exposure.

Keywords: elevated CO_2, *in vivo* biochemical parameters, ozone, photosynthesis, relative growth rate, stomatal conductance, *Tritium aestivum* L., winter wheat.

Introduction

The atmospheric concentration of CO_2 is predicted to increase accompanied by a concurrent rise in background ozone (O_3) level in the 21st century (Prather *et al.*, 2001; IPCC, 2007). The projected rise in atmospheric CO_2 level is expected to increase the growth and yield of many agricultural crops (Long, 1991; Kimball *et al.*, 1995; Long *et al.*, 2006). The

Abbreviations: A, CO_2 assimilation rate; A_{sat}, area-based light-saturated net photosynthetic rate; CFA, charcoal-filtered air; C_i, intercellular CO_2 concentration; ETR, electron transport rate; F_0, minimum fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence; g_s, stomatal conductance; J_{max}, maximum electron transport rate for RuBP regeneration; *K*, allometric coefficient; NAR, net assimilation rate; NPQ, non-photochemical quenching; O_3, ozone; OTC, open-topped chamber; PPFD, photosynthetic photon flux density; PSII, photosystem II; Q, photon flux; q_P, photochemical quenching coefficient; RGR, relative growth rate; RGR_r, relative growth rate of root; RGR_s, relative growth rate of shoot; V_{cmax}, maximum rate of carboxylation by Rubisco; WUE_{int}, intrinsic water-use efficiency; Φ_{PSII}, quantum yield of PSII.

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positive effects of increased atmospheric CO_2 concentration on crop growth and yield may be compromised by the deleterious aspects of atmospheric O_3 on crop systems (Long, 1991; McKee *et al.*, 1995; McKee *et al.*, 2000; Long *et al.*, 2006; Ainsworth *et al.*, 2008*a*). However, little is known about the extent of O_3-induced modification of the beneficial effects of elevated CO_2 on crop plants that would have differential responses to atmospheric O_3.

Elevated CO_2 can cause an increase in biomass and yield of 30–40% in many crops including wheat (10–20%) (Kimball, 1983; Poorter, 1993; McKee and Woodward, 1994; Tuba *et al.*, 1994). The extent of the beneficial effect of elevated CO_2 depends largely on the sink strength of a plant (Stitt, 1991; Bowes, 1993; Sicher *et al.*, 2010). Wheat breeding in China, as elsewhere, has progressed over time with reduced plant height (less biomass production) and an increase in grain yield through higher flag leaf photosynthesis (current photosynthesis) and a higher harvest index (Manderscheid and Weigel, 1997; Jiang *et al.*, 2003; Biswas *et al.*, 2008*a*). Consequently, the sink strength as well as the extent of the CO_2 response in wheat cultivars is decreasing following years of cultivar release (Manderscheid and Weigel, 1997). This may incur a penalty on the potential beneficial effects of elevated CO_2 on agricultural production and food security using future high-yielding modern crop cultivars, as the plant CO_2 response will be modified further by other global changes including atmospheric O_3 (Ainsworth *et al.*, 2008*b*). It is therefore important to ensure that selection for improved responsiveness to elevated CO_2 is not at the expense of tolerance to other features of global climatic and atmospheric change, notably increased temperature, O_3, and drought to maximize the benefit of elevated CO_2 on the major food crops (Ainsworth *et al.*, 2008*b*).

The extent of downregulation of photosynthesis under elevated CO_2 depends on the duration of CO_2 exposure, the plant species, the plant developmental stage, the canopy leaf position, and leaf age (McKee *et al.*, 1995; Osborne *et al.*, 1998). The possible physiological mechanisms of downregulation of photosynthesis to elevated CO_2 include a decrease in the amounts and activity of Rubisco, and in the capacity for regeneration of the substrate ribulose-1,5-bisphosphate (RuBP) (Stitt, 1991; Bowes, 1993; Sage, 1994). In addition, the intrinsic limitation of photosynthesis under elevated CO_2 shifts from CO_2 fixation in carboxylation towards energy capture by the photochemical component of photosynthesis (Long and Drake, 1992). Therefore, it should be beneficial for plants to invest relatively more resources into energy capture and electron transport rate at the expense of reduced carboxylation capacity (Long and Drake, 1992; Medlyn, 1996). Whilst growth and yield responses of wheat to elevated CO_2 and their underlying mechanisms have been well studied (McKee *et al.*, 1995; Manderscheid and Weigel, 1997), little is known about the mechanistic physiological responses of wheat cultivars with different years of release (i.e., differential sink sizes) to elevated CO_2.

In contrast, both old and modern wheat cultivars have been well characterized for their differential responses to O_3 (Barnes *et al.*, 1990; Biswas *et al.*, 2008*a*, *b*, 2009; Biswas and Jiang, 2011). O_3-induced loss of photosynthesis and growth is higher in the recently released winter wheat cultivars due to higher stomatal conductance, a larger reduction in antioxidative activities, and lower levels of dark respiration leading to higher oxidative damage to proteins and integrity of the cellular membrane than in the older cultivars (Biswas *et al.*, 2008*a*). It has been reported that the decline in photosynthetic capacity induced by O_3 is caused primarily by a decrease in the maximum *in vivo* rate of Rubisco carboxylation due to a reduction in the activity and/or quantity of Rubisco (Pell *et al.*, 1992; Farage and Long, 1995, 1999; Long and Naidu, 2002; Biswas and Jiang, 2011). In contrast, the impacts of O_3 on light-harvesting processes and photosynthetic electron transport are believed to be of secondary importance (Nie *et al.*, 1993; Farage and Long, 1999).

In the combined presence of elevated CO_2 and O_3 concentrations, the deleterious effect of O_3 is often offset by the beneficial effect of elevated CO_2 on many crop plants including wheat, although results are variable depending on the crop cultivars, developmental stage, and other growth conditions (Polle and Pell, 1999; McKee *et al.*, 2000; Cardoso-Vilhena *et al.*, 2004). Previous studies have demonstrated that modern wheat cultivars are less responsive to elevated CO_2 (Manderscheid and Weigel, 1997) but more sensitive to O_3 compared with old cultivars (Barnes *et al.*, 1990; Biswas *et al.*, 2008*a*, 2009) in terms of growth and yield. It was therefore hypothesized that the beneficial effects of elevated CO_2 on an old wheat cultivar could be attributed to its higher O_3 tolerance under elevated CO_2 and O_3 conditions. As protection against O_3 (i.e. the efficiency of metabolism of O_3-induced reactive oxygen species) is an energy-dependent process (Tausz *et al.*, 2007), it was also hypothesized that O_3-induced loss of the beneficial effects of elevated CO_2 on plants might be higher in a modern wheat cultivar than in an old cultivar under elevated CO_2 and O_3. An old and a modern cultivar of winter wheat were therefore utilized to test these hypotheses. Plant responses to elevated CO_2 and/or O_3 were determined by simultaneous measurements of gas exchange and chlorophyll *a* fluorescence, *in vivo* biochemical parameters, and growth analysis. The results from this study may be valuable in understanding the extent of the beneficial effects of elevated CO_2 on crop cultivars and food security under changing climate conditions such as elevated CO_2 and O_3.

Materials and methods

Plant establishment and gas treatments

An old (*Triticum aestivum* cv. Beijing 6; released in 1961) and a modern (*T. aestivum* L. cv. Zhongmai 9; released in 1997) winter wheat cultivar were selected to assess photosynthetic acclimation and growth under elevated CO_2 and/or O_3. The study was carried out at the experimental station at the Institute of Botany of the Chinese Academy of Sciences. In a temperature-controlled double-glazed greenhouse, three germinated seeds were each sown in 60 plastic pots (6 cm diameter, 9 cm high) per cultivar for each of the

two runs, which were carried out continuously by adjusting planting dates. The pots were filled with local field top soil (clay loam) ideal for wheat growth. Organic C, total N, total P, and total K in the soil were determined as 1.24, 0.045, 0.296, and 14.7 g kg^{-1}, respectively. The seedlings were thinned to one per pot d 7 after planting. On d 8 after planting, 15 pots per cultivar were moved to each of four open-topped chambers (OTCs) placed in the same greenhouse. The plants were allowed to grow up to d 17 after planting to adapt to the chamber environments before starting O_3 and CO_2 treatments. During this adaptation period, all plants received charcoal-filtered air (<5 ppb O_3) and ambient CO_2. The chambers were illuminated by natural daylight supplemented with fluorescence light providing a photosynthetic photon flux density (PPFD) of ~220 µmol m^{-2} s^{-1} at canopy height during the 14 h photoperiod. An artificial light source was continuously used to extend the day length and to maximize light intensity in the OTCs. The average midday light level (PPFD) in the chambers was ~1230 µmol m^{-2} s^{-1}. The temperature in the OTCs fluctuated from 17 °C (night) to 27 °C (day), and relative humidity varied from 57 to 85% during the experiment runs. Plants were irrigated as required to avoid drought, and the hard soil crust formed after irrigation was broken to ensure better aeration in the soil.

Pure CO_2 was dispensed for 24 h a day through manual mass flow meters into blowers and then into the chambers to produce the elevated CO_2 treatment. The concentration of CO_2 in the OTCs was monitored during the day and night using an infrared gas analyser (GFS-3000; Walz, Germany). O_3 was generated by electrically discharging ambient oxygen (Balaguer *et al*., 1995) with an O_3 generator (CF-KG1; Beijing Sumsun Hi-Tech. Co., China) and then was bubbled through distilled water before entering the higher O_3 chambers. Water traps were used to remove harmful compounds other than O_3 (Balaguer *et al*., 1995). The flow of O_3-enriched air into the OTCs was regulated by manual mass flow controllers. O_3 concentrations in the OTCs were continuously monitored at ~10 cm above the plant canopy using an O_3 analyser (APOA-360; Horiba, Japan), which was cross-calibrated once before starting O_3 treatment with another O_3 monitor (ML 9810B; Eco-Tech, Canada). The concentrations of CO_2 and O_3 in the four OTCs was averaged over the entire experimental period: control [CO_2, 385 ± 4 ppm+carbon-filtered air (CFA), 4 ± 0.02 ppb O_3]; O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb for 7 h d^{-1}). To minimize the effects from the chambers and environmental heterogeneities, plants and O_3 treatments were switched among the chambers every other day and the location of the plants within the chambers was randomized each time.

Visible symptoms of O_3 damage

Visible symptoms were assessed on all leaves of the main stem of each plant after termination of gas treatments. The percentage of mottled or necrotic areas on the leaves was assessed for five plants per cultivar sampled from each of the four gas treatments.

Photosystem II (PSII) functionality

On d 19 of fumigation treatment, five plants per cultivar were sampled from each of the four treatments and taken into an adjacent laboratory for dark adaptation (40 min) to ensure maximal oxidization of the primary quinone acceptor. Modulated chlorophyll fluorescence measurements were made in the middle of two fully expanded leaves (i.e. mature: leaf 3, and recently developed: leaf 4) using a PAM-2000 (Heinz Walz, Germany). The room temperature was maintained at 25 °C during measurements. The minimum fluorescence, F_0, was determined with modulated light, which was sufficiently low (<1 µmol m^{-2} s^{-1}) so as not to induce any significant variable fluorescence. The maximum fluorescence, F_m, was determined using a 0.8 s saturating pulse at 8000 µmol m^{-2} s^{-1}. Data obtained after recording fluorescence key parameters included F_0, F_m, variable fluorescence, F_v (=F_m–F_0), and maximum photochemical efficiency in the dark-adapted state, F_v/F_m (Krause and Weis, 1991).

Simultaneous measurement of gas exchange and chlorophyll a fluorescence

Two fully expanded leaves (i.e. mature: leaf 3, and recently developed: leaf 4) of each of the sampled plants (four plants per cultivar per treatment) were used for simultaneous measurements of gas exchange and chlorophyll *a* fluorescence with a portable Gas Exchange Fluorescence System (GFS-3000; Heinz Walz). The system was connected to a PC with data acquisition software (GFS-Win; Heinz Walz) and calibrated to the zero point prior to measurements. The measurement was programmed for simultaneously measurement of gas exchange and chlorophyll *a* fluorescence (Biswas and Jiang, 2011). Relative humidity was maintained at 65% and leaf temperature was set at 25 °C in the leaf chamber. The flow rate was set at 400 µmol s^{-1} and a CO_2 concentration of 400 ppm was maintained in the leaf chamber. The leaf was illuminated with a PPFD of 1500 µmol m^{-2} s^{-1} of internal light source in the leaf chamber. Steady-state fluorescence and maximum and minimum fluorescence were recorded along with gas-exchange parameters. In addition, dark-adapted (at least 40 min) steady-state fluorescence and maximum and minimum fluorescence were also recorded in leaf 3 and leaf 4 of the sampled plants with the same environmental settings in the leaf chamber except for the light used for gas exchange and light-adapted fluorescence parameters using the Gas Exchange and Fluorescence System. Data obtained as part of the gas exchange measurements included the area-based light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i). Plant intrinsic water-use efficiency (WUE_{int}) at the instantaneous level was calculated as the ratio of A_{sat}/g_s (Guehl *et al*., 1995). After recording fluorescence key parameters in both dark- and light-adapted states, chlorophyll *a* fluorescence parameters were calculated as follows:

$$\text{Quantum yield of PSII, } \Phi_{PSII}=(F_m'-F_s)/F_m' \quad (1)$$

$$\text{Photochemical quenching coefficient, } q_P=(F_m'-F_s)/(F_m'-F_0') \quad (2)$$

$$\text{Non-photochemical quenching, NPQ}=(F_m-F_m')/F_m' \text{ (Bilger and Bjorkman, 1990)} \quad (3)$$

$$\text{Electron transport rate, ETR}=\text{yield}\times\text{PAR}\times 0.5\times 0.85 \text{ (Meyer } et\ al.\text{, 1997)} \quad (4)$$

where F_m', F_0' and F_s are the maximum, minimum, and steady-state fluorescence, respectively in the leaf adapted to 1500 µmol m^{-2} s^{-1} PPFD and F_m is the maximum fluorescence in the dark-adapted leaf.

Determination of A/C_i and A/Q response curves

A/C_i (where A is CO_2 assimilation rate) and A/Q (where Q is photon flux) response curves were recorded only in the recently developed leaf (leaf 4) of each plant using an automatic curve program with a portable Gas Exchange Fluorescence System (GFS-3000; Heinz Walz). Three plants per cultivar were selected randomly from each treatment for *in vivo* biochemical parameters. The system connected to a PC was calibrated to zero point prior to measurements. The leaf chamber environment conditions (temperature, flow rate, and relative humidity) were kept the same as described above. Firstly, A/C_i curve was recorded and then the A/Q response curve was started automatically. For A/C_i curves, the steady-state rate of net photosynthesis under a saturating irradiance of 1500 µmol m^{-2} s^{-1} (A_{sat}) was determined at external CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 600 and 800 ppm. For the A/Q response curves,

the CO_2 concentration of 700 ppm in the leaf chamber was maintained to visualize photosynthetic acclimation (if any) to elevated CO_2. Gas exchange parameters in response to PPFDs of 1800, 1500, 1000, 500, 300, 150, 80, 50, 20, 0 (μmol m^{-2} s^{-1}) at the leaf surface level were recorded. Each step of the A/C_i and A/Q curves lasted for 4 and 3 min, respectively, with data being recorded twice at the end of each step. The data obtained for the A/C_i curve of each plant were analysed using a curve-fitting program (Photosynthesis Assistant, version 1.1; Dundee Scientific, UK) to obtain the maximum rate of carboxylation by Rubisco (V_{cmax}) and maximum electron transport rate for RuBP regeneration (J_{max}). The program followed the model proposed by Farquhar *et al.* (1980). Data obtained as a part of the A/Q response curve included CO_2 assimilation rate (A), g_s and WUE_{int}.

Determination of growth and resource allocation

Plants were sampled for growth analysis before O_3 and elevated CO_2 treatments (on d 17 after planting) and after 21 d of O_3 and elevated CO_2 exposure (on d 38 after planting). Five plants per cultivar were harvested from each of the four treatment chambers and partitioned into shoot and root before being dried to a constant weight at 72 °C. The difference in dry weight between the pre-fumigation and final harvest was used to calculate the relative growth rate of whole plants and plant parts over 21 d. The mean plant relative growth rate (RGR), relative growth rate of shoot (RGR_s), relative growth rate of root (RGR_r), allometric coefficient (K), specific leaf area and net assimilation rate (NAR) were calculated as described by Hunt (1990).

Statistical analysis

The experiment consisted of two blocks (i.e. two runs) in which the four gas treatments were assigned to the chambers in a randomized complete block design. The results from two runs were checked for homogeneity of variance prior to analysis and were then combined for statistical analysis. Analyses of variance was performed for the eight treatment combinations (i.e. two cultivars, two levels of CO_2 and two levels of O_3) for leaf 3 and leaf 4 on the measurable variables. The data were also analysed for the overall effect of CO_2, O_3, and cultivar, and for all interactions. Statistical analysis of the data was performed using a general linear model within the SPSS package (PASW Statistics 18.0, Chicago, USA). A Tukey comparison of means was performed when the F-test showed significance ($P \leq 0.05$).

Results

Visible O_3 injury

Fully developed leaves of the main stem of each sampled plant were named from the oldest (leaf 1) to the youngest (leaf 5) to assess visible O_3 injury of wheat plants. Scoring of visible symptoms demonstrated that there was no difference in the extent of premature leaf senescence (leaf 1) between the cultivars. There was significant cultivar variation in development of visible injury appearing in leaf 2 and leaf 3 (Table 1). No visible symptoms of O_3 injury were found in leaf 4 and leaf 5. Elevated O_3 led to higher visible O_3 injury both in leaf 2 and 3 in the modern cultivar than in the old one. Leaf 2 demonstrated a greater amount of visible O_3 injury than leaf 3, irrespective of cultivars. There was no visible symptom of O_3 injury in any leaf of the plants exposed to ambient CO_2, elevated CO_2, and elevated CO_2 and O_3.

Dark-adapted chlorophyll a fluorescence

Overall, elevated CO_2 significantly ($P < 0.01$) increased F_v/F_m both in mature (leaf 3) and young (leaf 4) leaves of wheat cultivars (data not shown). Elevated CO_2 significantly ($P < 0.05$) increased F_m and F_v in the young leaf. Elevated O_3 significantly decreased F_v/F_m in mature ($P < 0.001$) and young ($P < 0.1$) leaves. Exposure to O_3 decreased F_m and F_v in the mature leaf, but increased F_o, F_m, and F_v in the young leaf. The variety×CO_2 interaction was non-significant for all dark-adapted fluorescence parameters. The old cultivar exhibited a higher F_v/F_m value in the mature leaf than the modern cultivar at elevated O_3 (variety×O_3, $P < 0.05$). Elevated CO_2 considerably ameliorated O_3-induced alterations in basic fluorescence parameters in the mature leaf of both cultivars under elevated CO_2 and O_3 (CO_2×O_3, $P < 0.05$). The modern cultivar displayed higher F_o, F_m, and F_v values, along with a lower value of F_v/F_m in the mature leaf, than the old cultivar under combined gas treatment (variety×CO_2×O_3, $P < 0.05$; Table 2).

Simultaneous measurements of gas exchange and chlorophyll a fluorescence at ambient CO_2 concentration (400 ppm)

Overall, elevated CO_2 significantly ($P < 0.05$) increased A_{sat} and g_s but decreased C_i in both mature and young leaves of wheat cultivars at the CO_2 concentration of 400 ppm in the leaf chamber (data not shown). Elevated CO_2 also decreased WUE_{int} in the young leaf but not in the mature leaf. Exposure

Table 1. Development of visible symptoms of O_3 damage in different leaves of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO_2 and/or O_3. Fully developed leaves of the main stem of each sampled plant were named from the oldest (leaf 1) to the youngest (leaf 5). Control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb 7 h d^{-1}). Overall, the modern cultivar showed significantly ($P < 0.01$) higher level of visible symptoms of O_3 injury than the old cultivar. Results are shown as means±1 standard error (n=10).

Treatment	Visible symptoms of O_3 damage (%)				
	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5
(a) Beijing 6 (1961)					
Control	0	0	0	0	0
CO_2	0	0	0	0	
O_3	100 ± 2	62 ± 6	34 ± 4	0	0
CO_2+O_3	38 ± 4	0	0	0	0
(b) Zhongmai 9 (1997)					
Control	0	0	0	0	0
CO_2	0	0	0	0	0
O_3	100 ± 2	84 ± 9	59 ± 5	0	0
CO_2+O_3	42 ± 5	0	0	0	0

Table 2. Minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), and maximum photochemical efficiency of PSII (F_v/F_m) in leaf 3 and leaf 4 of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO_2 and/or O_3 for 21 d in OTCs. Control (CO_2, 385±4 ppm+CFA, 4±0.02 ppb O_3); elevated CO_2 (CO_2, 714±16 ppm+CFA, 4±0.02 ppb O_3); O_3 (ambient CO_2, 385±4 ppm+elevated O_3, 72±5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h) and elevated CO_2+O_3 (elevated CO_2, 714±16 ppm+elevated O_3, 72±5 ppb for 7 h d^{-1}). Overall, elevated CO_2 significantly (P <0.05) increased F_m and F_v in the young leaf. Elevated CO_2 considerably (P <0.01) increased F_v/F_m in both matured and young leaves. Exposure to O_3 decreased F_m and F_v in the matured leaf, but increased F_0, F_m, and F_v in the young leaf. High O_3 decreased F_v/F_m in the mature (P <0.001) and young (P <0.1) leaves of wheat cultivars. Results are shown as means±1 standard error (n=10). Means with the same letter were not significantly different.

Treatment	F_0		F_m		F_v		F_v/F_m	
	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4
(a) Beijing 6 (1961)								
Control	248±12^{c}	237±19^{bc}	1356±48^{c}	1299±88^{c}	1108±38^{c}	1062±71^{c}	0.82±0.00^{a}	0.82±0.01^{ab}
CO_2	246±13^{c}	216±21^{c}	1345±53^{c}	1236±87^{c}	1099±42^{c}	1020±73^{c}	0.82±0.00^{a}	0.82±0.01^{ab}
O_3	252±15^{c}	261±22^{bc}	1209±55^{c}	1303±97^{c}	957±46^{d}	1041±77^{c}	0.79±0.00^{c}	0.80±0.01^{c}
CO_2+O_3	242±14^{c}	277±21^{bc}	1246±50^{c}	1605±81^{b}	1004±44^{cd}	1328±74^{b}	0.81±0.00^{ab}	0.83±0.01^{a}
(b) Zhongmai 9 (1997)								
Control	308±15^{ab}	291±20^{ab}	1713±51^{a}	1635±82^{b}	1405±41^{a}	1344±75^{b}	0.82±0.00^{a}	0.82±0.01^{ab}
CO_2	273±13^{bc}	263±21^{bc}	1509±53^{b}	1459±87^{bc}	1236±42^{b}	1196±77^{bc}	0.82±0.00^{a}	0.82±0.01^{ab}
O_3	275±17^{bc}	271±24^{bc}	1216±57^{c}	1384±91^{bc}	941±47^{d}	1113±78^{bc}	0.77±0.00^{d}	0.80±0.01^{c}
CO_2+O_3	323±12^{a}	347±22^{a}	1586±53^{ab}	1956±84^{a}	1262±42^{b}	1608±76^{a}	0.80±0.00^{bc}	0.82±0.01^{ab}

to O_3 significantly decreased A_{sat} (P <0.001) but increased C_i (P <0.1) in the mature leaf. Elevated O_3 did not alter A_{sat} and C_i but lowered g_s and increased WUE_{int} in the young leaf. Overall, the old cultivar displayed lower values of A_{sat} and g_s in the mature leaf but showed a lower g_s and higher WUE_{int} in the young leaf than the modern variety. The variety×CO_2 interaction was non-significant for all gas exchange parameters in both leaves. The modern cultivar displayed higher C_i and a lower WUE_{int} in the young leaf than the old cultivar at elevated O_3 (variety×O_3, P <0.01). Elevated CO_2 ameliorated the O_3-induced reduction in mesophyll cell activity (C_i) and photosynthetic capacity (A_{sat}) in both leaves of wheat cultivars under elevated CO_2 and O_3 (CO_2×O_3, P <0.01; Table 3). The old cultivar showed a higher WUE_{int} in the mature leaf than the modern variety under elevated CO_2 and O_3 (variety×CO_2×O_3, P <0.001).

Overall, elevated CO_2 significantly (P <0.01) increased Φ_{PSII}, q_P, and ETR in the young leaf of wheat. Elevated CO_2 also considerably increased q_P but decreased NPQ in the mature leaf. Exposure to O_3 significantly (P <0.01) increased NPQ in the mature leaf of wheat cultivars. Elevated O_3 did not alter any light-adapted fluorescence parameter in the young leaf. The modern cultivar showed higher Φ_{PSII}, q_P and ETR in the mature leaf than the old cultivar under elevated CO_2 (variety×CO_2, P <0.05). The modern cultivar also showed considerably greater q_P in the young leaf than the old cultivar at elevated CO_2 (variety×CO_2, P <0.1). The variety×O_3 interaction was non-significant for all light-adapted fluorescence parameters. The modern cultivar showed higher decreases in Φ_{PSII} and q_P in the mature leaf than the old cultivar with combined gas treatment compared with elevated CO_2 (variety×CO_2×O_3, P <0.05; Table 4). The modern cultivar also a displayed higher NPQ in the young leaf than the old cultivar under elevated CO_2 and O_3 (variety×CO_2×O_3, P <0.1).

In vivo biochemical parameters

Elevated CO_2 significantly (P <0.05) increased V_{cmax}, J_{max}, and J_{max}/V_{cmax} in the young leaf of wheat cultivars (Fig. 1). Exposure to O_3 did not alter the *in vivo* biochemical parameters in the young leaf. Overall, the modern cultivar showed considerably (P <0.05) lower values of J_{max} and J_{max}/V_{cmax} than the old cultivar. The old cultivar displayed a higher J_{max}/V_{cmax} value than the modern one at elevated CO_2 (variety×CO_2, P <0.05). The variety×CO_2×O_3 interaction was non-significant for all *in vivo* biochemical parameters.

Gas exchange parameters at elevated CO_2 (700 ppm) under varying PPFDs

Both cultivars, regardless of treatment, had increased A, g_s and WUE_{int} with increasing PPFDs at the CO_2 concentration of 700 ppm in the leaf chamber (Fig. 2). None of the wheat cultivars showed photosynthetic acclimation to elevated CO_2. Elevated CO_2 resulted in higher A in the modern cultivar than in old one at high PPFDs. Exposure to O_3 showed a higher relative increase in A in the old cultivar than in the modern one under higher PPFDs. The combined gas treatment decreased A in the modern cultivar but increased A in the old one compared with elevated CO_2 at higher PPFDs. Elevated CO_2 exhibited a higher relative increase in g_s in the old cultivar compared with the modern one over different PPFDs. Exposure to O_3 decreased g_s in the old cultivar but increased g_s in the modern one under varying PPFDs. The combined gas treatment resulted in a decline in g_s in both cultivars compared with elevated CO_2 over different PPFDs. Elevated CO_2 increased WUE_{int} in both cultivars at higher PPFDs. Elevated O_3 increased WUE_{int} in the old cultivar but decreased WUE_{int} in the modern one at higher PPFDs. The combined gas treatment resulted in a greater increase in

Table 3. Light saturated rate of net assimilation (A_{sat}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and intrinsic water-use efficiency (WUE_{int}) at instantaneous level in leaf 3 and leaf 4 of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO_2 and/or O_3 for 21 d in OTCs. The leaf chamber CO_2 concentration was maintained at 400 ppm during gas exchange measurements: control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb for 7 h d^{-1}). Overall, elevated CO_2 significantly increased A_{sat} (P <0.01) and g_s (P <0.1) in both matured and young leaves, but decreased WUE_{int} in the young leaf. Exposure to O_3 significantly decreased A_{sat} (P <0.001), but increased C_i (P <0.1) in the matured leaf. Elevated O_3 did not alter A_{sat} but decreased g_s (P <0.05) and increased WUE_{int} (P <0.001) in the young leaf. Results are shown as means±1 standard error (n=8). Means with the same letter were not significantly different.

Treatment	A_{sat} (μmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})		C_i (ppm)		WUE_{int} (μmol mol^{-1})	
	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4
(a) Beijing 6 (1961)								
Control	8.68 ± 0.63^{a}	14.73 ± 0.85^{ab}	0.20 ± 0.02^{ab}	0.28 ± 0.01^{bc}	333 ± 8^{bc}	393 ± 6^{a}	43.30 ± 4.41^{ab}	52.89 ± 3.43^{bc}
CO_2	8.31 ± 0.60^{a}	14.38 ± 0.76^{ab}	0.19 ± 0.02^{ab}	0.28 ± 0.01^{bc}	331 ± 8^{bc}	348 ± 5^{cd}	44.05 ± 4.31^{ab}	52.89 ± 3.07^{bc}
O_3	3.75 ± 0.48^{b}	13.50 ± 0.98^{b}	0.13 ± 0.02^{b}	0.16 ± 0.01^{d}	360 ± 5^{a}	359 ± 7^{bcd}	31.11 ± 3.54^{b}	82.71 ± 3.97^{a}
CO_2+O_3	8.55 ± 0.52^{a}	16.50 ± 0.76^{a}	0.17 ± 0.02^{ab}	0.29 ± 0.01^{bc}	321 ± 5^{c}	360 ± 5^{bc}	51.24 ± 4.84^{a}	56.66 ± 3.07^{b}
(b) Zhongmai 9 (1997)								
Control	8.93 ± 0.75^{a}	15.55 ± 0.76^{ab}	0.21 ± 0.02^{ab}	0.32 ± 0.01^{ab}	332 ± 9^{bc}	388 ± 5^{a}	43.30 ± 4.35^{ab}	49.23 ± 3.31^{bc}
CO_2	10.08 ± 0.73^{a}	16.70 ± 0.74^{a}	0.25 ± 0.02^{a}	0.32 ± 0.01^{ab}	334 ± 8^{bc}	342 ± 5^{d}	40.68 ± 4.40^{ab}	51.90 ± 3.34^{bc}
O_3	4.87 ± 0.53^{b}	13.70 ± 0.71^{b}	0.16 ± 0.02^{ab}	0.24 ± 0.01^{c}	352 ± 8^{ab}	391 ± 5^{a}	36.20 ± 4.40^{ab}	59.47 ± 3.36^{b}
CO_2+O_3	10.08 ± 0.60^{a}	15.87 ± 0.77^{ab}	0.25 ± 0.02^{a}	0.37 ± 0.01^{a}	334 ± 9^{bc}	368 ± 5^{b}	40.72 ± 4.39^{ab}	42.64 ± 3.30^{c}

Table 4. Yield (F_v'/F_m'), quantum yield (Φ_{PSII}), photochemical quenching coefficient (q_P), non-photochemical quenching (NPQ), and electron transport rate (ETR) in leaf 3 and leaf 4 of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO_2 and/or O_3 for 21 days in OTCs. Chlorophyll *a* fluorescence parameters were recorded simultaneously with gas exchange measurement. Leaf chamber environment conditions (i.e. PPFD, temperature, relative humidity, flow rate, and CO_2 concentration) were the same as those used for gas exchange measurement: control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb for 7 h d^{-1}). Overall, elevated CO_2 significantly increased (P <0.01) Φ_{PSII}, q_P, and ETR in the young leaf but decreased NPQ in the matured leaf. Elevated O_3 did not alter any light-adapted fluorescence parameter in the young leaf but considerably increased NPQ (P <0.01) in the mature leaf. Results are shown as means±1 standard error (n=8). Means with the same letter were not significantly different.

Treatment	F_v'/F_m'		Φ_{PSII}		q_P		NPQ		ETR	
	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4
(a) Beijing 6 (1961)										
Control	0.60 ± 0.04	0.50 ± 0.01^{a}	0.098 ± 0.01^{a}	0.091 ± 0.00^{ab}	0.13 ± 0.01^{b}	0.18 ± 0.01^{bcd}	2.48 ± 0.02^{bc}	1.78 ± 0.01^{b}	46 ± 4^{ab}	57 ± 3^{ab}
CO_2	0.62 ± 0.04	0.48 ± 0.01^{ab}	0.079 ± 0.01^{ab}	0.095 ± 0.00^{ab}	0.13 ± 0.01^{b}	0.20 ± 0.01^{abc}	2.12 ± 0.02^{bc}	2.01 ± 0.01^{ab}	43 ± 4^{ab}	60 ± 3^{ab}
O_3	0.54 ± 0.03	0.50 ± 0.01^{a}	0.072 ± 0.01^{ab}	0.084 ± 0.01^{b}	0.13 ± 0.01^{b}	0.17 ± 0.01^{d}	4.87 ± 0.02^{a}	1.93 ± 0.01^{ab}	46 ± 3^{ab}	53 ± 3^{b}
CO_2+O_3	0.57 ± 0.03	0.51 ± 0.02^{a}	0.070 ± 0.01^{ab}	0.092 ± 0.00^{ab}	0.12 ± 0.01^{b}	0.18 ± 0.01^{bcd}	2.43 ± 0.02^{bc}	1.84 ± 0.01^{ab}	42 ± 3^{b}	58 ± 3^{ab}
(b) Zhongmai 9 (1997)										
Control	0.51 ± 0.04	0.51 ± 0.01^{a}	0.052 ± 0.01^{b}	0.086 ± 0.00^{ab}	0.10 ± 0.01^{b}	0.17 ± 0.01^{cd}	2.41 ± 0.02^{bc}	1.67 ± 0.01^{b}	37 ± 5^{b}	54 ± 3^{ab}
CO_2	0.59 ± 0.04	0.46 ± 0.01^{b}	0.103 ± 0.01^{a}	0.094 ± 0.00^{ab}	0.18 ± 0.01^{a}	0.21 ± 0.01^{ab}	1.93 ± 0.02^{c}	1.55 ± 0.01^{b}	57 ± 4^{a}	59 ± 3^{ab}
O_3	0.56 ± 0.03	0.51 ± 0.01^{a}	0.077 ± 0.01^{ab}	0.082 ± 0.00^{b}	0.14 ± 0.01^{b}	0.16 ± 0.01^{d}	4.54 ± 0.02^{ab}	1.56 ± 0.01^{b}	45 ± 4^{ab}	51 ± 3^{b}
CO_2+O_3	0.49 ± 0.02	0.46 ± 0.01^{b}	0.066 ± 0.01^{ab}	0.098 ± 0.00^{a}	0.13 ± 0.01^{b}	0.21 ± 0.01^{a}	3.16 ± 0.02^{abc}	2.73 ± 0.01^{a}	48 ± 5^{ab}	62 ± 3^{a}

WUE_{int} in the old cultivar than in the modern one relative to elevated CO_2 at higher PPFDs.

Plant growth and resource allocation

Overall, elevated CO_2 significantly (P <0.001) increased RGR, RGR_s, and RGR_r, but did not alter K in wheat cultivars (data not shown). Elevated O_3 significantly (P <0.05) decreased RGR, RGR_s, RGR_r, and K in wheat cultivars. Overall, the modern cultivar showed considerably higher (P <0.001) RGR, RGR_s, and RGR_r values than the old one. The modern cultivar displayed higher RGR, RGR_s, and RGR_r values than old one at elevated CO_2 (variety×CO_2, P <0.05). The old cultivar showed considerably higher RGR, RGR_s, and RGR_r values than the modern one at high O_3 (variety×O_3, P <0.05). Elevated CO_2 significantly ameliorated the negative effects of O_3 on RGR, RGR_s, and RGR_r under elevated CO_2 and O_3 (CO_2×O_3, P <0.05). The

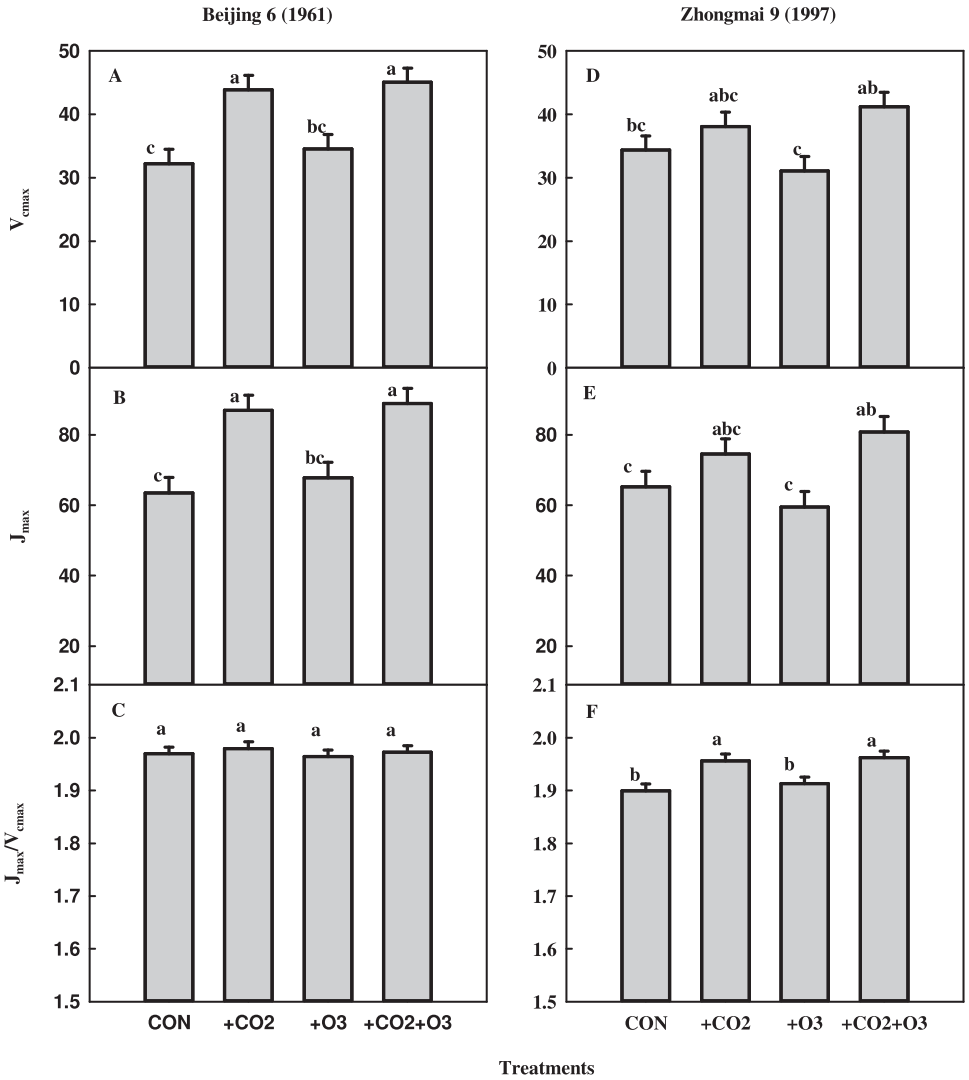

Fig. 1. Maximum *in vivo* rate of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate for RUBP regeneration (J_{max}) and J_{max}/V_{cmax} in the young leaf (leaf 4) of an old (released in 1961) and a modern (released in 1997) wheat cultivar exposed to elevated CO_2 and/or O_3 for 21 d in OTCs. Control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h) and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb for 7 h d^{-1}). Overall, elevated CO_2 significantly (P <0.05) increased V_{cmax}, J_{max}, and J_{max}/V_{cmax} in the young leaf. Exposure to O_3 did not alter *in vivo* biochemical parameters in the young leaf. Results are shown as means±1 standard error (n=6).

combined gas treatment resulted in a greater reduction in RGR, RGR_r, and K in the modern cultivar than in the old one relative to elevated CO_2 (variety×CO_2×O_3, P <0.05; Table 5).

Discussion

Visible symptoms of O_3 damage in the two cultivars of winter wheat as affected by elevated CO_2

Scoring of visible symptoms in two wheat cultivars exposed to four treatment combinations of O_3 and CO_2 for 21 d revealed that only elevated O_3 showed a differential degree of visible symptoms, varying with leaf age and wheat cultivar. The extent of visible symptoms increased with leaf age, regardless of cultivar. Visible symptoms varied from 62 to 84% and from 34 to 59% in leaf 2 and leaf 3, respectively. This suggested that O_3-induced oxidative stress was higher in old and mature leaves, despite their lower O_3 uptake compared with recently developed leaves at the upper canopy (Noormets *et al.*, 2010). Significant varietal difference was noted in the visible symptoms of O_3 damage that developed on leaf 2 and leaf 3. The modern cultivar showed a higher level of visible symptoms than the old cultivar, irrespective of leaf levels. No visible symptom was found on leaves of the two winter wheat cultivars exposed to elevated CO_2, elevated CO_2 and O_3, and CFA, as found in an O_3-sensitive spring wheat cultivar exposed to elevated CO_2 and/or O_3 (Cardoso-Vilhena *et al.*, 2004).

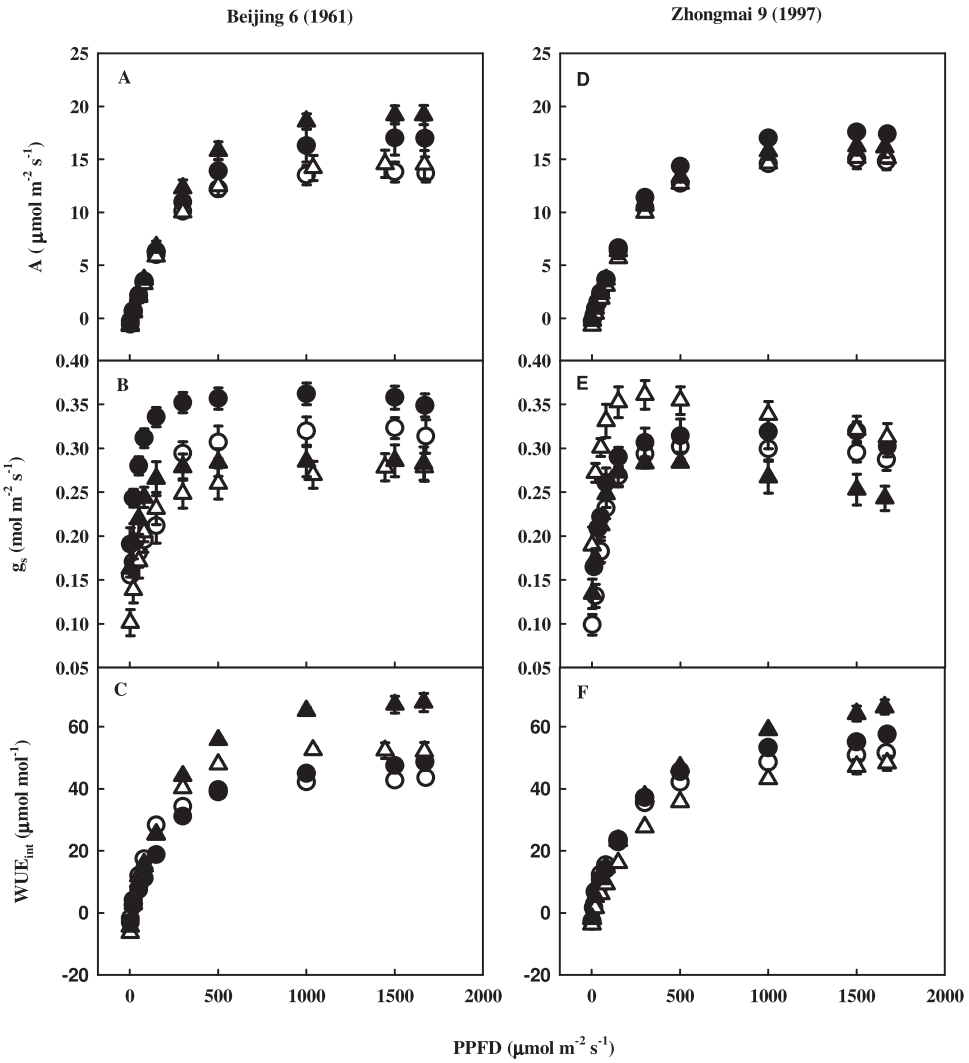

Fig. 2. Assimilation rate (*A*), stomatal conductance (g_s), and intrinsic water-use efficiency (WUE_{int}) at instantaneous level in the young leaf (leaf 4) of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar under varying levels of PPFD after they were exposed to elevated CO_2 and/or O_3 for 21 d in OTCs. Leaf chamber CO_2 concentration was maintained at 700 ppm. Control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3; open circles); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3; filled circles); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h; open triangles); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb for 7 h d^{-1}; filled triangles). Results are shown as means±1 standard error (*n*=6).

Photosynthetic and growth responses of an old and modern winter wheat cultivar to elevated CO_2

Elevated CO_2 is expected to increase the productivity of C_3 plants and enhance water-use efficiency at the leaf level through a simultaneous increase in photosynthesis and a decline in stomatal conductance (Cure and Acock, 1986; Eamus, 1991; Drake *et al.*, 1997). We found differential photosynthetic responses of the mature (leaf 3) and recently developed young (leaf 4) leaves of wheat cultivars to elevated CO_2. Overall, elevated CO_2 significantly increased F_v/F_m in both mature and young leaves with a larger increase in F_v/F_m in the former than in the latter. Elevated CO_2 also produced a larger increase in A_{sat} in the mature leaf (41%) than in the young leaf (10%). Exposure to elevated CO_2 decreased WUE_{int} in the young leaf due to a higher relative increase in g_s (26%) at the CO_2 concentration of 400 ppm in the leaf chamber. However, elevated CO_2 increased both g_s and WUE_{int} in the young leaf at the CO_2 concentration of 700 ppm in the leaf chamber. This indicated that elevated CO_2 increased WUE_{int} in the young leaf without high C_i-induced partial stomatal closure. A significant increase in g_s in wheat cultivars at elevated CO_2, as found in this study, is consistent with previous reports (Norby and O'Neil, 1991; Pettersson and McDonald, 1992; Wang *et al.*, 2000). Elevated CO_2 significantly increased Φ_{PSII}, ETR, and q_P in the young leaf but not in the mature leaf when chlorophyll *a* fluorescence was recorded simultaneously with gas exchange. The results are consistent with the report of Rascher *et al.* (2010), which demonstrated an increase in ETR in soybean at elevated CO_2. Overall, elevated CO_2 significantly decreased NPQ in the mature leaf but did not alter NPQ in the young leaf. We found that a 10% increase

Table 5. Relative growth rate of whole plant (RGR), relative growth rate of shoot (RGR_s), relative growth rate of root (RGR_r), allometric coefficient (K=RGR_r/RGR_s), specific leaf area (SLA), and net assimilation rate (NAR) of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO_2 and/or O_3 for 21 d in OTCs. Control (CO_2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O_3); elevated CO_2 (CO_2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O_3); O_3 (ambient CO_2, 385 ± 4 ppm+elevated O_3, 72 ± 5 ppb O_3 for 7 h d^{-1}, 9.00–16.00 h); and elevated CO_2+O_3 (elevated CO_2, 714 ± 16 ppm+elevated O_3, 72 ± 5 ppb 7 h d^{-1}). Overall, elevated CO_2 significantly (P <0.001) increased RGR, RGR_s, and RGR_r but did not alter K. Exposure to O_3 significantly (P <0.05) decreased RGR, RGR_s, RGR_r, and K in wheat cultivars. Results are shown as means±1 standard error (n=10). Means with the same letter were not significantly different.

Treatment	RGR (g g^{-1} d^{-1})	RGR_s (g g^{-1} d^{-1})	RGR_r (g g^{-1} d^{-1})	K	SLA (cm^2 g^{-1})	NAR (g m^{-2} d^{-1})
(a) Beijing 6 (1961)						
Control	0.065 ± 0.003^{c}	0.068 ± 0.003^{c}	0.056 ± 0.003^{d}	0.83 ± 0.05^{a}	603 ± 34^{ab}	2.34 ± 0.13
CO_2	0.067 ± 0.002^{c}	0.070 ± 0.003^{c}	0.059 ± 0.003^{d}	0.84 ± 0.05^{a}	548 ± 32^{ab}	2.54 ± 0.13
O_3	0.062 ± 0.002^{c}	0.066 ± 0.003^{c}	0.050 ± 0.003^{e}	0.76 ± 0.04^{b}	658 ± 30^{a}	2.17 ± 0.12
CO_2+O_3	0.066 ± 0.002^{c}	0.069 ± 0.003^{c}	0.056 ± 0.003^{d}	0.82 ± 0.04^{a}	571 ± 30^{ab}	2.37 ± 0.12
(b) Zhongmai 9 (1997)						
Control	0.075 ± 0.002^{b}	0.077 ± 0.003^{b}	0.064 ± 0.003^{b}	0.84 ± 0.04^{a}	592 ± 30^{ab}	2.43 ± 0.12
CO_2	0.081 ± 0.002^{a}	0.084 ± 0.003^{a}	0.071 ± 0.003^{a}	0.84 ± 0.05^{a}	527 ± 32^{ab}	2.76 ± 0.13
O_3	0.054 ± 0.002^{d}	0.058 ± 0.003^{d}	0.040 ± 0.003^{c}	0.71 ± 0.04^{c}	655 ± 30^{a}	1.98 ± 0.12
CO_2+O_3	0.076 ± 0.002^{b}	0.083 ± 0.003^{a}	0.065 ± 0.003^{b}	0.78 ± 0.04^{b}	492 ± 30^{b}	2.48 ± 0.12

in A_{sat} in the young leaf was attributed to an increase in V_{cmax} and J_{max} by 27 and 29%, respectively, under elevated CO_2. These results indicated that mature and young leaves show differential strategies in energy acquisition and carbon assimilation. Our findings of higher levels of V_{cmax} and J_{max} in winter wheat under elevated CO_2 are consistent with the fact that the short-term response can be attributed largely to stimulation of Rubisco at the vegetative stage of plants when sink strength is less limited (Sharkey, 1988; Long, 1991). However, the stimulation of photosynthesis by elevated CO_2 was reflected on growth, as elevated CO_2 significantly increased RGR, RGR_s, RGR_r, and NAR in the wheat cultivars. The results are consistent with the findings of Cardoso-Vilhena *et al.* (2004), which demonstrate an increased relative growth rate in a spring wheat cultivar under elevated CO_2.

The modern cultivar demonstrated higher levels of ETR, q_P, and Φ_{PSII} in the mature leaf but showed higher q_P in the young leaf than the old one at high CO_2. This suggested that the modern cultivar had a higher level of energy capture and electron transport rate compared with the old one at elevated CO_2. In addition, the modern wheat also displayed higher electron-use efficiency for RuBP regeneration, as documented by a lower value of J_{max}/V_{cmax} compared with the old one at elevated CO_2 (variety×CO_2, P <0.05). The intrinsic limitation of photosynthesis under elevated CO_2 shifts from CO_2 fixation in carboxylation towards energy capture by the photochemical components of photosynthesis (Long and Drake, 1992). It is therefore believed that an investment of relatively more resources into the components of light harvesting and electron transport at the expense of reduced carboxylation capacity is beneficial to a plant under elevated CO_2 (Long and Drake, 1992; Medlyn, 1996). In agreement with the above-mentioned idea, we found that the modern cultivar showed higher relative increases in RGR, RGR_s, and RGR_r than old one at elevated CO_2.

Photosynthetic and growth responses of an old and a modern wheat cultivar to elevated O_3

Exposure to O_3 significantly reduced the maximum photochemical efficiency of PSII (F_v/F_m) in wheat cultivars, but a higher reduction in F_v/F_m was noted in the mature leaf (leaf 3) than in the young leaf (leaf 4). The two leaves also showed different mechanisms of photoinhibition. An O_3-induced decrease in F_m in the mature leaf indicated the occurrence of damage to PSII reaction centres, whilst an O_3-induced increase in both F_0 and F_m in the young leaf suggested the occurrence of photoinhibition due to an increase in non-radiative thermal deactivation (Butler, 1978). O_3-induced damage to PSII in the mature leaf resulted in a significant reduction in A_{sat} accompanied by a greater increase in C_i. In contrast, O_3-induced non-radiative thermal deactivation of PSII in the young leaf resulted in a non-significant reduction in A_{sat} with a significant decrease in g_s. As a result, O_3 increased WUE_{int} in the young leaf but not in the mature leaf. Analysis of the quenching components of chlorophyll *a* fluorescence recorded simultaneously with gas exchange indicated that O_3 significantly increased NPQ in the mature leaf but not in the young leaf. These results also suggested that O_3-induced loss of A_{sat} in the mature leaf might be due to both stomatal and non-stomatal limitations, as evidenced by the O_3-induced reduction in g_s and increase in C_i (Farage *et al.*, 1991; Farage and Long, 1995; Biswas *et al.*, 2008*a*; Biswas and Jiang, 2011). Greater negative effects of O_3 on the mature leaf of winter wheat cultivars, as found in this study, are consistent with observations made previously on a cultivar of spring wheat (Cardoso-Vilhena *et al.*, 2004). Loss of Rubisco triggered by exposure to O_3 is considered to constitute the primary cause of the O_3-induced decline in CO_2 assimilation (Farage *et al.*, 1991; Farage and Long, 1995). It has also been documented that the maximal effect of O_3 on Rubisco coincided with the period when Rubisco concentration reached its peak (Dann

and Pell, 1989; Pell *et al.*, 1992). We found that O_3 had no effect on V_{cmax}, J_{max}, and J_{max}/V_{cmax} in the young leaf. This might be the cause underlying the non-significant reduction in A_{sat} in the young leaf of wheat cultivars at elevated O_3, as found in the newly expanded leaf of soybean plants exposed to O_3 (Bernacchi *et al.*, 2009). Nevertheless, the O_3-induced negative effect on photosynthesis resulted in a marked reduction in RGR, RGR_s, RGR_r, and K in wheat cultivars. Root growth was more negatively affected by O_3 than shoot growth, regardless of cultivar, as reported elsewhere (Davison and Barnes, 1998; Biswas *et al.*, 2008*a*, *b*).

The modern cultivar demonstrated a higher loss of PSII efficiency in the mature leaf than the old cultivar at elevated O_3 (variety×O_3, P <0.01). This suggested that the old cultivar was relatively less sensitive to O_3 compared with the modern one, as has been found elsewhere (Barnes *et al.*, 1990, 2008*b*). In a previous study, the extent of O_3 sensitivity of a large number of modern winter wheat cultivars in terms of growth and antioxidative activities was positively associated with O_3 uptake and loss of mesophyll cell activity (Biswas *et al.*, 2008*a*). We found that the modern cultivar showed a greater loss of mesophyll cell activity, as documented by higher C_i in the young leaf than the old wheat cultivar at high O_3 (variety×O_3, P <0.01). This can be explained by higher O_3 uptake, as evidenced by higher g_s in both leaves of modern cultivar compared with the old cultivar at high O_3 (Biswas *et al.*, 2008*a*, *b*). Consequently, the old cultivar demonstrated higher WUE_{int} in the young leaf than the modern one at high O_3. However, higher O_3-induced physiological impairment resulted in greater reductions in RGR, RGR_s, and RGR_r in the modern cultivar compared with the old one. These results are consistent with our earlier reports that demonstrate higher O_3 sensitivity of the newly released winter wheat cultivars compared with older ones in terms of growth and grain yield (Biswas *et al.*, 2008*a*, *b*; Biswas and Jiang, 2011).

Differential responses of winter wheat cultivars to the combination of elevated CO_2 and O_3

The deleterious aspects of atmospheric O_3 on crop systems may partly be offset by the beneficial effects of increased atmospheric CO_2 concentration on crop plants (Ainsworth *et al.*, 2008*a*). In our study, elevated CO_2 fully protected both old and modern cultivars against the negative effects of O_3 under elevated CO_2 and O_3. However, the beneficial effects of elevated CO_2 on plants varied significantly between the two cultivars under elevated CO_2 and O_3. We found that the combined gas treatment resulted in higher O_3-induced photoinhibition due to non-radiative thermal deactivation of PSII, as evidenced by greater increases in F_0 and F_m in the mature leaf of the modern cultivar than that of the old one relative to elevated CO_2. High O_3-induced photoinhibition in the modern cultivar was associated with higher O_3 uptake, as documented by higher g_s compared with the old cultivar at elevated CO_2 and O_3. Consequently, the combined gas treatment showed larger decreases in Φ_{PSII} and q_P in the mature leaf of the modern cultivar than in that of the old one compared with elevated CO_2. In addition, the modern wheat displayed a greater increase in NPQ in the young leaf than the old one under elevated CO_2 and O_3 relative to elevated CO_2. Higher levels of photoinhibition and NPQ in the modern cultivar compared with the old cultivar at elevated CO_2 and O_3 might be due to a greater reduction in total antioxidant capacity in the modern cultivar at elevated CO_2 (Gillespie *et al.*, 2011). Our results also indicated that the old cultivar had a higher WUE_{int} in the young leaf than the modern one under elevated CO_2 and O_3. Although the modern cultivar displayed a higher energy capture and electron transport rate compared with the old one at elevated CO_2, the positive effect of elevated CO_2 on plants was largely diminished in the modern cultivar under combined elevated CO_2 and O_3 exposure. For instance, the modern cultivar showed greater reductions in RGR, RGR_s, RGR_r, and K than old one in combined elevated CO_2 and O_3 exposure relative to elevated CO_2. These results are in agreement with the notion that the beneficial effects of elevated CO_2 on plants may be compromised by nutrient limitation and other environmental stresses (Ainsworth *et al.*, 2008*b*). Our results also suggested that the beneficial effects of elevated CO_2 on the old cultivar were sustained due to lower O_3 uptake and lower O_3-induced photoinhibition under elevated CO_2 and O_3. In addition, a greater O_3-induced loss of the positive effects of elevated CO_2 on the modern cultivar suggests that elevated CO_2-induced growth stimulation in the recently released wheat cultivar attributed to higher energy capture and electron transport rate could be compromised by its higher O_3 uptake and greater O_3-induced photoinhibition under elevated CO_2 and O_3 conditions.

In conclusion, elevated CO_2 resulted in higher growth stimulation in the modern cultivar attributed to a higher energy capture and electron transport rate compared with the old cultivar. In contrast, O_3 induced a greater reduction in growth due to higher O_3 uptake and greater loss of PSII efficiency (in the mature leaf) and mesophyll cell activity (in the young leaf) in the modern cultivar than in the old one. Exposure to O_3 resulted in greater photoinhibition in the mature leaf compared with the young leaf. The mature and young leaves showed photoinhibition due to the occurrence of damage to PSII reaction centres and an increase in non-radiative thermal deactivation, respectively. Elevated CO_2 fully protected both cultivars against the deleterious effects of O_3 under elevated CO_2 and O_3. The modern cultivar showed a greater relative loss of elevated CO_2-induced growth stimulation attributed to higher O_3 uptake and O_3-induced photoinhibition than the old one under combined elevated CO_2 and O_3 exposure. These results suggest that cultivar selection with improved responsiveness to elevated CO_2 as well as tolerance to O_3 can maximize agricultural production under the anticipated elevation of CO_2 and O_3 levels in the future.

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