Modification of photosynthesis and growth responses to elevated CO2 by ozone in two cultivars of winter wheat with different years of release

D.K. Biswas1,2, H. Xu1, Y.G. Li1, B.L. Ma2 and G.M. Jiang1,3 *

1 State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, 20 Nanxincun, 100093, Beijing, PR China
2 Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, K1A0C6, Canada
3 State Key Laboratory of Crop Biology, Shandong Agricultural University, No. 61, Daizong Avenue, 271018, Tai’an, PR China

* To whom correspondence should be addressed. Email: jianggm@126.com

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Abstract

The beneficial effects of elevated CO2 on plants are expected to be compromised by the negative effects posed by other global changes. However, little is known about ozone (O3)-induced modulation of elevated CO2 response in plants with differential sensitivity to O3. An old (Triticum aestivum cv. Beijing 6, O3 tolerant) and a modern (T. aestivum cv. Zhongmai 9, O3 sensitive) winter wheat cultivar were exposed to elevated CO2 (714 ppm) and/or O3 (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 CO2 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 O3 caused a greater growth reduction in the modern cultivar due to higher O3 uptake and a greater loss of photosystem II efficiency (mature leaf) and mesophyll cell activity (young leaf) than in the old cultivar. Elevated CO2 completely protected both cultivars against the deleterious effects of O3 under elevated CO2 and O3. The modern cultivar showed a greater relative loss of elevated CO2-induced growth stimulation due to higher O3 uptake and greater O3-induced photoinhibition than the old cultivar at elevated CO2 and O3. Our findings suggest that the elevated CO2-induced growth stimulation in the modern cultivar attributed to higher energy capture and electron transport rate can be compromised by its higher O3 uptake and greater O3-induced photoinhibition under elevated CO2 and O3 exposure.

Keywords: elevated CO2, in vivo biochemical parameters, ozone, photosynthesis, relative growth rate, stomatal conductance, Triticum aestivum L., winter wheat.

Introduction

The atmospheric concentration of CO2 is predicted to increase accompanied by a concurrent rise in background ozone (O3) level in the 21st century (Prather et al., 2001; IPCC, 2007). The projected rise in atmospheric CO2 level is expected to increase the growth and yield of many agricultural crops (Long, 1991; Kimball et al., 1995; Long et al., 2006).
positive effects of increased atmospheric CO2 concentration on crop growth and yield may be compromised by the deleterious aspects of atmospheric O3 on crop systems (Long, 1991; McKee et al., 1995; McKee et al., 2000; Long et al., 2006; Ainsworth et al., 2008a). However, little is known about the extent of O3-induced modification of the beneficial effects of elevated CO2 on crop plants that would have differential responses to atmospheric O3.

Elevated CO2 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 CO2 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., 2008a). Consequently, the sink strength as well as the extent of the CO2 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 CO2 on agricultural production and food security using future high-yielding modern crop cultivars, as the plant CO2 response will be modified further by other global changes including atmospheric O3 (Ainsworth et al., 2008b). It is therefore important to ensure that selection for improved responsiveness to elevated CO2 is not at the expense of tolerance to other features of global climatic and atmospheric change, notably increased temperature, O3, and drought to maximize the benefit of elevated CO2 on the major food crops (Ainsworth et al., 2008b).

The extent of downregulation of photosynthesis under elevated CO2 depends on the duration of CO2 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 CO2 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 CO2 shifts from CO2 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 CO2 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 CO2.

In contrast, both old and modern wheat cultivars have been well characterized for their differential responses to O3 (Barnes et al., 1990; Biswas et al., 2008a, b, 2009; Biswas and Jiang, 2011). O3-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., 2008a). It has been reported that the decline in photosynthetic capacity induced by O3 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 O3 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 CO2 and O3 concentrations, the deleterious effect of O3 is often offset by the beneficial effect of elevated CO2 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 CO2 (Manderscheid and Weigel, 1997) but more sensitive to O3 compared with old cultivars (Barnes et al., 1990; Biswas et al., 2008a, 2009) in terms of growth and yield. It was therefore hypothesized that the beneficial effects of elevated CO2 on an old wheat cultivar could be attributed to its higher O3 tolerance under elevated CO2 and O3 conditions. As protection against O3 (i.e. the efficiency of metabolism of O3-induced reactive oxygen species) is an energy-dependent process (Tausz et al., 2007), it was also hypothesized that O3-induced loss of the beneficial effects of elevated CO2 on plants might be higher in a modern wheat cultivar than in an old cultivar under elevated CO2 and O3. An old and a modern cultivar of winter wheat were therefore utilized to test these hypotheses. Plant responses to elevated CO2 and/or O3 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 CO2 on crop cultivars and food security under changing climate conditions such as elevated CO2 and O3.

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 CO2 and/or O3. 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 \(d\) 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 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) at canopy height during the 14th 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 \(\mu\)mol 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 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 analyzer (GFS-3000; Walz, Germany). O\(_3\) was generated by electrically discharging ambient oxygen (Balaguer \textit{et al.}, 1995) with an O\(_3\) generator (CF-KG; 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 \textit{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\(_3\) analyzer (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+charcoal-filtered air (CFA), 4 ± 0.02 ppb O\(_3\)]; O\(_3\) (ambient CO\(_2\), 385 ± 4 ppm+elevated O\(_3\), 72 ± 5 ppm 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 ppm 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 \(\mu\)mol 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 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). Data obtained after recording fluorescence key parameters included \(F_o\), \(F_m\), variable fluorescence, \(F_v=\frac{F_m-F_o}{\varphi_F}\), and maximum photochemical efficiency in the dark-adapted state, \(F_{v'/m}\) (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 \(\mu\)mol 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 \(\mu\)mol 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 \(A_{sat}/g_s\) (Guelh \textit{et al.}, 1995). After recording fluorescence key parameters in both dark- and light-adapted states, chlorophyll \(a\) fluorescence parameters were calculated as follows:

\[
\text{Quantum yield of PSII, } \varphi_F = \frac{(F_m'-F_o')}{F_m'} = \frac{(F_m'-F_o')}{F_m'} \quad (1)
\]

\[
\text{Photochemical quenching coefficient, } q_p \equiv \frac{(F_m'-F_o')}{(F_m'-F_i')} = \frac{(F_m'-F_o')}{(F_m'-F_i')} \quad (2)
\]

\[
\text{Non-photochemical quenching, } \text{NPOQ} = \frac{(F_m-F_o')}{F_m'} = \frac{(F_m-F_o')}{F_m'} \quad (\text{Bilger and Bjorkman, 1990}) \quad (3)
\]

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

where \(F_o', F_m'\) and \(F_i\) are the maximum, minimum, and steady-state fluorescence, respectively in the leaf adapted to 1500 \(\mu\)mol 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 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (\(A_{max}\)) 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,

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the CO2 concentration of 700 ppm in the leaf chamber was maintained to visualize photosynthetic acclimation (if any) to elevated CO2. 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/CI 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/CI 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 (Vcmax) and maximum electron transport rate for RuBP regeneration (Jmact). The program followed the model proposed by Farquhar et al. (1980). Data obtained as a part of the A/Q response curve included CO2 assimilation rate (A), g, and WUEint.

Determination of growth and resource allocation

Plants were sampled for growth analysis before O3 and elevated CO2 treatments (on d 17 after planting) and after 21 d of O3 and elevated CO2 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 (RGRs), relative growth rate of root (RGRr), 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 CO2 and two levels of O3) for leaf 3 and leaf 4 on the measurable variables. The data were also analysed for the overall effect of CO2, O3, 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 ≤ 0.05).

Results

Visible O3 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 O3 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 O3 injury were found in leaf 4 and leaf 5. Elevated O3 led to higher visible O3 injury both in leaf 2 and 3 in the modern cultivar than in the old one. Leaf 2 demonstrated a greater amount of visible O3 injury than leaf 3, irrespective of cultivars. There was no visible symptom of O3 injury in any leaf of the plants exposed to ambient CO2, elevated CO2, and elevated CO2 and O3.


dark-adapted chlorophyll a fluorescence

Overall, elevated CO2 significantly (P < 0.01) increased Fv/Fm both in mature (leaf 3) and young (leaf 4) leaves of wheat cultivars (data not shown). Elevated CO2 significantly (P < 0.05) increased Fm and Fv in the young leaf. Elevated O3 significantly decreased Fv/Fm in mature (P < 0.001) and young (P < 0.1) leaves. Exposure to O3 decreased Fm and Fv in the mature leaf, but increased Fv/Fm and Fv in the young leaf. The variety × CO2 interaction was non-significant for all dark-adapted fluorescence parameters. The old cultivar exhibited a higher Fv/Fm value in the mature leaf than the modern cultivar at elevated O3 (variety × O3, P < 0.05).

Table 1. Development of visible symptoms of O3 damage in different leaves of an old (released in 1961) and a modern (released in 1997) winter wheat cultivar exposed to elevated CO2 and/or O3. Fully developed leaves of the main stem of each sampled plant were named from the oldest (leaf 1) to the youngest (leaf 5). Control (CO2, 385 ± 4 ppm+CFA, 4 ± 0.02 ppb O3); elevated CO2 (CO2, 714 ± 16 ppm+CFA, 4 ± 0.02 ppb O3); O3 (ambient CO2, 385 ± 4 ppm + elevated O3, 72 ± 5 ppb O3 for 7 h d−1, 9.00–16.00 h); and elevated CO2+O3 (elevated CO2, 714 ± 16 ppm+elevated O3, 72 ± 5 ppb 7 h d−1). Overall, the modern cultivar showed significantly (P < 0.01) higher level of visible symptoms of O3 injury than the old cultivar. Results are shown as means ± 1 standard error (n = 10).

| Treatment | Visible symptoms of O3 damage (%) |
|-----------|----------------------------------|
|           | Leaf 1 | Leaf 2 | Leaf 3 | Leaf 4 | Leaf 5 |
| (a) Beijing 6 (1961) |        |        |        |        |        |
| Control   | 0      | 0      | 0      | 0      | 0      |
| CO2       | 0      | 0      | 0      | 0      | 0      |
| O3        | 100±2  | 62±6   | 34±4   | 0      | 0      |
| CO2+O3    | 38±4   | 0      | 0      | 0      | 0      |
| (b) Zhongmai 9 (1997) |        |        |        |        |        |
| Control   | 0      | 0      | 0      | 0      | 0      |
| CO2       | 0      | 0      | 0      | 0      | 0      |
| O3        | 100±2  | 84±9   | 59±5   | 0      | 0      |
| CO2+O3    | 42±5   | 0      | 0      | 0      | 0      |
Overall, the old cultivar displayed lower values of photosynthetic capacity \( (A) \) and elevated CO2+O3 (elevated CO2, 714 ± 16 ppm+elevated O3, 72 ± 5 ppb O3 for 7 h d\(^{-1}\), 9.00–16.00 h) and elevated CO2+O3 (elevated CO2, 714 ± 16 ppm+elevated O3, 72 ± 5 ppb for 7 h d\(^{-1}\)). Overall, elevated CO2 significantly \( (P < 0.05) \) increased \( F_{m} \) and \( F_{v} \) in the young leaf. Elevated CO2 considerably \( (P < 0.01) \) increased \( F_{m}/F_{v} \) in both matured and young leaves. Exposure to O3 decreased \( F_{m} \) and \( F_{v} \) in the mature leaf, but increased \( F_{m}/F_{v} \) in the young leaf. High O3 decreased \( F_{m}/F_{v} \) in the mature (\( P < 0.001 \)) and young \( (P < 0.01) \) leaves of wheat cultivars. Results are shown as means±1 standard error (\( n=10 \)). Means with the same letter were not significantly different.

**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 CO2 and/or O3 for 21 d in OTCs. Control (CO2, 385 ± 4 ppm+CF, 4 ± 0.02 ppb O3); elevated CO2 (CO2, 714 ± 16 ppm+CF, 4 ± 0.02 ppb O3); O3 (ambient CO2, 385 ± 4 ppm+elevated O3, 72 ± 5 ppb O3 for 7 h d\(^{-1}\), 9.00–16.00 h) and elevated CO2+O3 (elevated CO2, 714 ± 16 ppm+elevated O3, 72 ± 5 ppb for 7 h d\(^{-1}\)). Overall, elevated CO2 significantly \( (P < 0.05) \) increased \( F_{m} \) and \( F_{v} \) in the young leaf. Elevated CO2 considerably \( (P < 0.01) \) increased \( F_{m}/F_{v} \) in both matured and young leaves. Exposure to O3 decreased \( F_{m} \) and \( F_{v} \) in the mature leaf, but increased \( F_{m}/F_{v} \) in the young leaf. High O3 decreased \( F_{m}/F_{v} \) in the mature (\( P < 0.001 \)) and young \( (P < 0.01) \) leaves of wheat cultivars. Results are shown as means±1 standard error (\( n=10 \)). Means with the same letter were not significantly different.

To O3 significantly decreased \( A_{sat} \) \( (P < 0.001) \) but increased \( C_{i} \) \( (P < 0.01) \) in the mature leaf. Elevated O3 did not alter \( A_{sat} \) and \( C_{i} \) but lowered \( g_{s} \) and increased WUEint 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 WUEint in the young leaf than the modern variety. The variety×CO2 interaction was non-significant for all gas exchange parameters in both leaves. The modern cultivar displayed higher \( C_{i} \) and a lower WUEint in the young leaf than the old cultivar at elevated O3 (variety×O3, \( P < 0.01 \)). Elevated CO2 ameliorated the O3-induced reduction in mesophyll cell activity (\( C_{i} \)) and photosynthetic capacity (\( A_{sat} \)) in both leaves of wheat cultivars under elevated CO2 and O3 (CO2×O3, \( P < 0.01 \); Table 3).

The old cultivar showed a higher WUEint in the mature leaf than the modern variety under elevated CO2 and O3 (variety×CO2×O3, \( P < 0.001 \)). Overall, elevated CO2 significantly \( (P < 0.01) \) increased \( \Phi_{PSII} \), \( q_{P} \), and ETR in the young leaf of wheat. Elevated CO2 also considerably increased \( q_{P} \) but decreased NPQ in the mature leaf. Exposure to O3 significantly \( (P < 0.01) \) increased NPQ in the mature leaf of wheat cultivars. Elevated O3 did not alter any light-adapted fluorescence parameter in the young leaf. The modern cultivar showed higher \( \Phi_{PSII} \), \( q_{P} \) and ETR in the mature leaf than the old cultivar under elevated CO2 (variety×CO2, \( P < 0.05 \)). The modern cultivar also showed considerably greater \( q_{P} \) in the young leaf than the old cultivar at elevated CO2 (variety×CO2, \( P < 0.01 \)). The variety×O3 interaction was non-significant for all light-adapted fluorescence parameters. The modern cultivar showed higher decreases in \( \Phi_{PSII} \) and \( q_{P} \) in the mature leaf than the old cultivar with combined gas treatment compared with elevated CO2 (variety×CO2×O3, \( P < 0.05 \); Table 4). The modern cultivar also a displayed higher NPQ in the young leaf than the old cultivar under elevated CO2 and O3 (variety×CO2×O3, \( P < 0.1 \)).

**In vivo biochemical parameters**

Elevated CO2 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 O3 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 CO2 (variety×CO2, \( P < 0.05 \)). The variety×CO2×O3 interaction was non-significant for all in vivo biochemical parameters.

**Gas exchange parameters at elevated CO2 (700 ppm) under varying PPFDs**

Both cultivars, regardless of treatment, had increased \( A \), \( g_{s} \) and WUEint with increasing PPFDs at the CO2 concentration of 700 ppm in the leaf chamber (Fig. 2). None of the wheat cultivars showed photosynthetic acclimation to elevated CO2. Elevated CO2 resulted in higher \( A \) in the modern cultivar than in old one at high PPFDs. Exposure to O3 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 CO2 at higher PPFDs. Elevated CO2 exhibited a higher relative increase in \( g_{s} \) in the old cultivar compared with the modern one under different PPFDs. Exposure to O3 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 CO2 over different PPFDs. Elevated CO2 increased WUEint in both cultivars at higher PPFDs. Elevated O3 increased WUEint in the old cultivar but decreased WUEint 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 ($A_{\text{sat}}$), stomatal conductance ($g_s$), intercellular CO$_2$ concentration (C) and intrinsic water-use efficiency ($WUE_{\text{int}}$) 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 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 $A_{\text{sat}}$ ($P<0.01$) and $g_s$ ($P<0.01$) in both matured and young leaves, but decreased $WUE_{\text{int}}$ in the young leaf. Exposure to O$_3$ significantly decreased $A_{\text{sat}}$ ($P<0.01$), but increased C$_i$ ($P<0.1$) in the matured leaf. Elevated O$_3$ did not alter $A_{\text{sat}}$ but decreased $g_s$ ($P<0.05$) and increased $WUE_{\text{int}}$ ($P<0.01$) in the young leaf. Results are shown as means±1 standard error ($n=8$). Means with the same letter were not significantly different.

![Table 3](https://academic.oup.com/jxb/article-abstract/64/6/1485/585553)

Table 4. Yield ($F'_{v}/F'_{m}$), quantum yield ($\Phi_{PSII}$), 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 ($P<0.01$) $\Phi_{PSII}$, $q_p$, 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.

![Table 4](https://academic.oup.com/jxb/article-abstract/64/6/1485/585553)

WUE$_{\text{int}}$ 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$_c$, and RGR$_r$, but did not alter $K$ in wheat cultivars (data not shown). Elevated O$_3$ significantly ($P<0.05$) decreased RGR, RGR$_c$, RGR$_r$, and $K$ in wheat cultivars. Overall, the modern cultivar showed considerably higher ($P<0.001$) RGR, RGR$_c$, and RGR$_r$ values than the old one. The modern cultivar displayed higher RGR, RGR$_c$, and RGR$_r$ values than old one at elevated CO$_2$ (variety$\times$CO$_2$, $P<0.05$). The old cultivar showed considerably higher RGR, RGR$_c$, and RGR$_r$ values than the modern one at high O$_3$ (variety$\times$O$_3$, $P<0.005$). Elevated CO$_2$ significantly ameliorated the negative effects of O$_3$ on RGR, RGR$_c$, and RGR$_r$ under elevated CO$_2$ and O$_3$ (CO$_2$$\times$O$_3$, $P<0.05$). The
The 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 CO₂ (variety×CO₂×O₃, P <0.05; Table 5).

**Discussion**

**Visible symptoms of O₃ damage in the two cultivars of winter wheat as affected by elevated CO₂**

Scoring of visible symptoms in two wheat cultivars exposed to four treatment combinations of O₃ and CO₂ for 21 d revealed that only elevated O₃ 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 O₃-induced oxidative stress was higher in old and mature leaves, despite their lower O₃ uptake compared with recently developed leaves at the upper canopy (Noormets et al., 2010). Significant varietal difference was noted in the visible symptoms of O₃ 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 CO₂, elevated CO₂+O₃, and CFA, as found in an O₃-sensitive spring wheat cultivar exposed to elevated CO₂ and/or O₃ (Cardoso-Vilhena et al., 2004).
Elevated CO\textsubscript{2} is expected to increase the productivity of C\textsubscript{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 \textit{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\textsubscript{2}. Overall, elevated CO\textsubscript{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\textsubscript{2} also produced a larger increase in $A_{\text{sat}}$ in the mature leaf (41%) than in the young leaf (10%). Exposure to elevated CO\textsubscript{2} decreased WUE\textsubscript{int} in the young leaf due to a higher relative increase in $g_s$ (26%) at the CO\textsubscript{2} concentration of 400 ppm in the leaf chamber. However, elevated CO\textsubscript{2} increased both $g_s$ and WUE\textsubscript{int} in the young leaf at the CO\textsubscript{2} concentration of 700 ppm in the leaf chamber. This indicated that elevated CO\textsubscript{2} increased WUE\textsubscript{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\textsubscript{2}, as found in this study, is consistent with previous reports (Norby and O’Neil, 1991; Pettersson and McDonald, 1992; Wang \textit{et al}., 2000). Elevated CO\textsubscript{2} significantly increased $\Phi_{\text{PSII}}$, ETR, and $q_F$ 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 \textit{et al}. (2010), which demonstrated an increase in ETR in soybean at elevated CO\textsubscript{2}. Overall, elevated CO\textsubscript{2} significantly decreased NPQ in the mature leaf but did not alter NPQ in the young leaf. We found that a 10% increase
in \( A_{\text{sat}} \) in the young leaf was attributed to an increase in \( V_{\text{max}} \) and \( J_{\text{max}} \) by 27 and 29%, respectively, under elevated \( \text{CO}_2 \). These results indicated that mature and young leaves show differential strategies in energy acquisition and carbon assimilation. Our findings of higher levels of \( V_{\text{max}} \) and \( J_{\text{max}} \) in winter wheat under elevated \( \text{CO}_2 \) 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 \( \text{CO}_2 \) was reflected on growth, as elevated \( \text{CO}_2 \) significantly increased RGR, RGRs, RGRr, 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 \( \text{CO}_2 \).

The modern cultivar demonstrated higher levels of ETR, \( \Phi_{\text{PSII}} \), and \( F_{\text{v}}/F_{\text{m}} \) in the mature leaf but showed higher \( q_{\text{P}} \) in the young leaf than the old one at high \( \text{CO}_2 \). This suggested that the modern cultivar had a higher level of energy capture and electron transport rate compared with the old one at elevated \( \text{CO}_2 \). In addition, the modern wheat also displayed higher electron-use efficiency for RuBP regeneration, as documented by a lower value of \( J_{\text{max}}/V_{\text{max}} \) compared with the old one at elevated \( \text{CO}_2 \) (variety×\( \text{CO}_2 \), \( P < 0.05 \)). The intrinsic limitation of photosynthesis under elevated \( \text{CO}_2 \) shifts from \( \text{CO}_2 \) 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 \( \text{CO}_2 \) (Long and Drake, 1992; Medlyn, 1996). In agreement with the abovementioned idea, we found that the modern cultivar showed higher relative increases in RGR, RGRs, and RGRr, than old one at elevated \( \text{CO}_2 \).

### Table 5. Relative growth rate of whole plant (RGR), relative growth rate of shoot (RGRs), relative growth rate of root (RGRr), allometric coefficient (\( K=\text{RGR}/\text{RGR}_s \)), 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 \( \text{CO}_2 \) and/or \( \text{O}_3 \) for 21 d in OTCs. Control (\( \text{CO}_2 \), 385±4 ppm+\( \text{CFA} \), 4±0.02 ppb \( \text{O}_3 \)); elevated \( \text{CO}_2 \) (\( \text{CO}_2 \), 714±16 ppm+\( \text{CFA} \), 4±0.02 ppb \( \text{O}_3 \)); \( \text{O}_3 \) (ambient \( \text{CO}_2 \), 385±4 ppm+elevated \( \text{O}_3 \), 72±5 ppb \( \text{O}_3 \) for 7 h d−1, 9.00–16.00 h); and elevated \( \text{CO}_2+\text{O}_3 \) (elevated \( \text{CO}_2 \), 714±16 ppm+elevated \( \text{O}_3 \), 72±5 ppb 7 h d−1). Overall, elevated \( \text{CO}_2 \) significantly (\( P < 0.001 \)) increased RGR, RGRs, RGRr, and NAR, but did not alter \( K \). Exposure to \( \text{O}_3 \) significantly (\( P < 0.05 \)) decreased RGR, RGRs, RGRr, 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) | RGRs (g g−1 d−1) | RGRr (g g−1 d−1) | \( K \) | SLA (cm2 g−1) | NAR (g m−2 d−1) |
|-------------------|-----------------|-----------------|-----------------|------|--------------|-----------------|
| (a) Beijing 6 (1961) |                 |                 |                 |      |              |                 |
| Control           | 0.065±0.003a    | 0.068±0.003a    | 0.056±0.003a    | 0.83±0.05b | 603±34ab     | 2.34±0.13       |
| \( \text{CO}_2 \) | 0.067±0.002b    | 0.070±0.003a    | 0.059±0.003a    | 0.84±0.05b | 548±32ab     | 2.54±0.13       |
| \( \text{O}_3 \)  | 0.062±0.002c    | 0.066±0.003c    | 0.050±0.003c    | 0.76±0.04c | 658±30a      | 2.17±0.12       |
| \( \text{CO}_2+\text{O}_3 \) | 0.066±0.002c    | 0.069±0.003c    | 0.056±0.003c    | 0.82±0.04c | 571±30b      | 2.37±0.12       |
| (b) Zhongmai 9 (1997) |                 |                 |                 |      |              |                 |
| Control           | 0.075±0.002a    | 0.077±0.003a    | 0.064±0.003a    | 0.84±0.04a | 592±30b      | 2.43±0.12       |
| \( \text{CO}_2 \) | 0.081±0.002a    | 0.084±0.003c    | 0.071±0.003a    | 0.84±0.05a | 527±32ab     | 2.76±0.13       |
| \( \text{O}_3 \)  | 0.054±0.002c    | 0.058±0.003c    | 0.040±0.003c    | 0.71±0.04c | 665±30a      | 1.98±0.12       |
| \( \text{CO}_2+\text{O}_3 \) | 0.076±0.002c    | 0.083±0.003c    | 0.065±0.003b    | 0.78±0.04c | 492±30c      | 2.48±0.12       |

**Photosynthetic and growth responses of an old and a modern wheat cultivar to elevated \( \text{O}_3 \)**

Exposure to \( \text{O}_3 \) significantly reduced the maximum photochemical efficiency of PSII (\( F_{\text{v}}/F_{\text{m}} \)) in wheat cultivars, but a higher reduction in \( F_{\text{v}}/F_{\text{m}} \) 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 \( \text{O}_3 \)-induced decrease in \( F_{\text{m}} \) in the mature leaf indicated the occurrence of damage to PSII reaction centres, whilst an \( \text{O}_3 \)-induced increase in both \( F_0 \) and \( F_{\text{m}} \) in the young leaf suggested the occurrence of photoinhibition due to an increase in non-radiative thermal deactivation (Butler, 1978). \( \text{O}_3 \)-induced damage to PSII in the mature leaf resulted in a significant reduction in \( A_{\text{sat}} \) accompanied by a greater increase in \( C_1 \). In contrast, \( \text{O}_3 \)-induced non-radiative thermal deactivation of PSII in the young leaf resulted in a non-significant reduction in \( A_{\text{sat}} \) with a significant decrease in \( g_s \). As a result, \( \text{O}_3 \) increased \( \text{WUE}_{\text{m}} \) 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 \( \text{O}_3 \) significantly increased NPQ in the mature leaf but not in the young leaf. These results also suggested that \( \text{O}_3 \)-induced loss of \( A_{\text{sat}} \) in the mature leaf might be due to both stomatal and non-stomatal limitations, as evidenced by the \( \text{O}_3 \)-induced reduction in \( g_s \) and increase in \( C_1 \) (Farage et al., 1991; Farage and Long, 1995; Biswas et al., 2008a; Biswas and Jiang, 2011). Greater negative effects of \( \text{O}_3 \) 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 \( \text{O}_3 \) is considered to constitute the primary cause of the \( \text{O}_3 \)-induced decline in \( \text{CO}_2 \) assimilation (Farage et al., 1991; Farage and Long, 1995). It has also been documented that the maximal effect of \( \text{O}_3 \) on Rubisco coincided with the period when Rubisco concentration reached its peak (Dann et al., 1995).
and Pell, 1989; Pell et al., 1992). We found that O₃ had no effect on Vcmax, Jmax, and Jₕ/Vcmax in the young leaf. This might be the cause underlying the non-significant reduction in Aₘ in the young leaf of wheat cultivars at elevated O₃, as found in the newly expanded leaf of soybean plants exposed to O₃ (Bernacchi et al., 2009). Nevertheless, the O₃-induced negative effect on photosynthesis resulted in a marked reduction in RGR, RGRₕ, RGRₘ, and K in wheat cultivars. Root growth was more negatively affected by O₃ than shoot growth, regardless of cultivar, as reported elsewhere (Davison and Barnes, 1998; Biswas et al., 2008a, b).

The modern cultivar demonstrated a higher loss of PSII efficiency in the mature leaf than the old cultivar at elevated O₃ (variety × O₃, P < 0.01). This suggested that the old cultivar was relatively less sensitive to O₃ compared with the modern one, as has been found elsewhere (Barnes et al., 1990, 2008b). In a previous study, the extent of O₃ sensitivity of a large number of modern winter wheat cultivars in terms of growth and antioxidative activities was positively associated with O₃ uptake and loss of mesophyll cell activity (Biswas et al., 2008a). We found that the modern cultivar showed a greater loss of mesophyll cell activity, as documented by higher Cₖ in the young leaf than the old wheat cultivar at high O₃ (variety × O₃, P < 0.01). This can be explained by higher O₃ uptake, as evidenced by higher gₛ in both leaves of modern cultivars compared with the old cultivar at high O₃ (Biswas et al., 2008a, b). Consequently, the old cultivar demonstrated higher WUEₑ in the young leaf than the modern one at high O₃. However, higher O₃-induced physiological impairment resulted in greater reductions in RGR, RGRₕ, and RGRₘ in the modern cultivar compared with the old one. These results are consistent with our earlier reports that demonstrate higher O₃ sensitivity of the newly released winter wheat cultivars compared with older ones in terms of growth and grain yield (Biswas et al., 2008a, b; Biswas and Jiang, 2011).

**Differential responses of winter wheat cultivars to the combination of elevated CO₂ and O₃**

The deleterious aspects of atmospheric O₃ on crop systems may partly be offset by the beneficial effects of increased atmospheric CO₂ concentration on crop plants (Ainsworth et al., 2008a). In our study, elevated CO₂ fully protected both old and modern cultivars against the negative effects of O₃ under elevated CO₂ and O₃. However, the beneficial effects of elevated CO₂ on plants varied significantly between the two cultivars under elevated CO₂ and O₃. We found that the combined gas treatment resulted in higher O₃-induced photoinhibition due to non-radiative thermal deactivation of PSII, as evidenced by greater increases in F₀ and Fₘ in the mature leaf of the modern cultivar than that of the old one relative to elevated CO₂. High O₃-induced photoinhibition in the modern cultivar was associated with higher O₃ uptake, as documented by higher gₛ compared with the old cultivar at elevated CO₂ and O₃. Consequently, the combined gas treatment showed larger decreases in Fₚ and qₑ in the mature leaf of the modern cultivar than in that of the old one compared with elevated CO₂. In addition, the modern wheat displayed a greater increase in NPQ in the young leaf than the old one under elevated CO₂ and O₃ relative to elevated CO₂. Higher levels of photoinhibition and NPQ in the modern cultivar compared with the old cultivar at elevated CO₂ and O₃ might be due to a greater reduction in total antioxidant capacity in the modern cultivar at elevated CO₂ (Gillespie et al., 2011). Our results also indicated that the old cultivar had a higher WUEₑ in the young leaf than the modern one under elevated CO₂ and O₃. Although the modern cultivar displayed a higher energy capture and electron transport rate compared with the old one at elevated CO₂, the positive effect of elevated CO₂ on plants was largely diminished in the modern cultivar under combined elevated CO₂ and O₃ exposure. For instance, the modern cultivar showed greater reductions in RGR, RGRₕ, RGRₘ, and K than old one in combined elevated CO₂ and O₃ exposure relative to elevated CO₂. These results are in agreement with the notion that the beneficial effects of elevated CO₂ on plants may be compromised by nutrient limitation and other environmental stresses (Ainsworth et al., 2008b).

In conclusion, elevated CO₂ 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, O₃ induced a greater reduction in growth due to higher O₃ 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 O₃ 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 CO₂ fully protected both cultivars against the deleterious effects of O₃ under elevated CO₂ and O₃. The modern cultivar showed a greater relative loss of elevated CO₂-induced growth stimulation attributed to higher O₃ uptake and O₃-induced photoinhibition than the old one under combined elevated CO₂ and O₃ exposure. These results suggest that cultivar selection with improved responsiveness to elevated CO₂ as well as tolerance to O₃ can maximize agricultural production under the anticipated elevation of CO₂ and O₃ levels in the future.

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