Modification of photosynthesis and growth responses to elevated CO\(_2\) by ozone in two cultivars of winter wheat with different years of release

D.K. Biswas\(^1,2\), H. Xu\(^1\), Y.G. Li\(^1\), B.L. Ma\(^2\) and G.M. Jiang\(^1,3\)*

\(^1\) 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

Received 3 September 2012; Revised 12 December 2012; Accepted 2 January 2013

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 (\textit{Triticum aestivum} cv. Beijing 6, O\(_3\) tolerant) and a modern (\textit{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 \textit{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, \textit{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\(_2\), \textit{in vivo} biochemical parameters, ozone, photosynthesis, relative growth rate, stomatal conductance, \textit{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 \textit{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 \textit{et al.}, 1995; Long \textit{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, b, 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 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 sow in 60 plastic pots (6cm diameter, 9 cm high) per cultivar for each of the
Response of an old and a modern wheat cultivar to elevated CO₂ and O₃

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 O₃ and CO₂ treatments. During this adaptation period, all plants received charcoal-filtered air (<5 ppb O₃) and ambient CO₂. 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 CO₂ was dispensed for 24 h a day through manual mass flow meters into blowers and then into the chambers to produce the elevated CO₂ treatment. The concentration of CO₂ in the OTCs was monitored during the day and night using an infrared gas analyser (GFS-3000; Walz, Germany). O₃ was generated by electrically discharging ambient oxygen (Balaguer et al., 1995) with an O₃ generator (CF-KG); Beijing Sumsun Hi-Tech, Co., China) and then was bubbled through distilled water before entering the higher O₃ chambers. Water traps were used to remove harmful compounds other than O₃ (Balaguer et al., 1995). The flow of O₃-enriched air into the OTCs was regulated by manual mass flow controllers. O₃ concentrations in the OTCs were continuously monitored at ~10 cm above the plant canopy using an O₃ analyser (APOA-360; Horiba, Japan), which was cross-calibrated once before starting O₃ treatment with another O₃ monitor (ML 9810B; Eco-Tech, Canada). The concentrations of CO₂ and O₃ in the four OTCs was averaged over the entire experimental period: control [CO₂, 385 ± 4 ppm+carbon-filtered air (CFA), ± 0.02 ppm O₃]; O₃ (ambient CO₂, 385 ± 4 ppm+elevated O₃, 72 ± 5 ppm O₃ for 7 h d⁻¹, 9.00–16.00 h); elevated CO₂ (CO₂, 714 ± 16 ppm+CFA, 4 ± 0.02 ppm O₃); and elevated CO₂+O₃ (elevated CO₂, 714 ± 16 ppm+elevated O₃, 72 ± 5 ppm for 7 h d⁻¹). To minimize the effects from the chambers and environmental heterogeneities, plants and O₃ 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 O₃ 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 oxidation 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, F₀, 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, Fₘ, was determined using a 0.8 s saturating pulse at 8000 µmol m⁻² s⁻¹. Data obtained after recording fluorescence key parameters included Fₐ, Fₘ, variable fluorescence, Fv = (Fₐ − F₀), and maximum photochemical efficiency in the dark-adapted state, Fv/Fₘ (Krause and Weiss, 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 CO₂ 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 (Aₛ), stomatal conductance (gₛ) and intercellular CO₂ concentration (Cᵢ). Plant intrinsic water-use efficiency (WUEᵢ) at the instantaneous level was calculated as the ratio of Aₛ/gₛ (Guelh et al., 1995). After recording fluorescence key parameters in both dark- and light-adapted states, chlorophyll a fluorescence parameters were calculated as follows:

\[
\text{Quantum yield of PSII, } \Phi_{\text{PSII}} = \frac{(F_{\text{m}}' - F_{\text{i}})}{(F_{\text{m}}' - F_{\text{0}})} \]

\[
\text{Photochemical quenching coefficient, } q_p = \frac{(F_{\text{m}}' - F_{\text{i}})}{(F_{\text{m}}' - F_{\text{0}})} \]

\[
\text{Non-photochemical quenching, } \text{NPQ} = \frac{(F_{\text{m}} - F_{\text{i}})}{F_{\text{m}}'} \quad (1)
\]

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

where F₀, Fₐ and Fₘ are the maximum, minimum, and steady-state fluorescence, respectively in the leaf adapted to 1500 µmol m⁻² s⁻¹ PPFD and Fₘ is the maximum fluorescence in the dark-adapted state.

Determination of A/Cᵢ and A/Q response curves

A/Cᵢ (where A is CO₂ 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. First, A/Cᵢ curve was recorded and then the A/Q response curve was started automatically. For A/Cᵢ curves, the steady-state rate of net photosynthesis under a saturating irradiance of 1500 µmol m⁻² s⁻¹ (Aₛ) was determined at external CO₂ concentrations of 400, 300, 200, 100, 50, 400, 400, 600 and 800 ppm. For the A/Q response curves,
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/C 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 curve of each plant were analyzed 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 (Jmax). 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 were performed for the eight treatment combinations (i.e. two cultivars, then combined for statistical analysis. Analyses of variance was checked for homogeneity of variance prior to analysis and were randomized complete block design. The results from two runs were combined for statistical analysis. Analyses of variance 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 Fm/Fv 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 Fm/Fv in mature (P <0.001) and young (P <0.1) leaves. Exposure to O3 decreased Fm and Fv in the mature leaf, but increased Fm/Fv in the young leaf. The variety×CO2 interaction was non-significant for all dark-adapted fluorescence parameters. The old cultivar exhibited a higher Fm/Fv value in the mature leaf than the modern cultivar at elevated O3 (variety×O3, P <0.05). Elevated CO2 considerably ameliorated O3-induced alterations in basic fluorescence parameters in the mature leaf of both cultivars under elevated CO2 and O3 (CO2×O3, P <0.05). The modern cultivar displayed higher Fm, Fv, and Fv values, along with a lower value of Fm/Fv in the mature leaf, than the old cultivar under combined gas treatment (variety×CO2×O3, P <0.05; Table 2).

Simultaneous measurements of gas exchange and chlorophyll a fluorescence at ambient CO2 concentration (400 ppm)
Overall, elevated CO2 significantly (P <0.05) increased A max and g, but decreased Ci in both mature and young leaves of wheat cultivars at the CO2 concentration of 400 ppm in the leaf chamber (data not shown). Elevated CO2 also decreased WUEint in the young leaf but not in the mature leaf. Exposure

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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf 1</th>
<th>Leaf 2</th>
<th>Leaf 3</th>
<th>Leaf 4</th>
<th>Leaf 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Beijing 6 (1961)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O3</td>
<td>100±2</td>
<td>62±6</td>
<td>34±4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>38±4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(b) Zhongmai 9 (1997)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O3</td>
<td>100±2</td>
<td>84±9</td>
<td>59±5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>42±5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Overall, the old cultivar displayed lower values of O3 sat in both leaves of wheat cultivars. The variety×O3 interaction was non-significant for all gas exchange parameters in the young leaf. Elevated CO2 considerably increased Fv/Fm in the mature leaf but lowered \( A_{\text{sat}} \) in the young leaf than the old cultivar at high PPFDs. Exposure to O3 decreased Fv/Fm in the mature leaf, but increased \( F_{\text{v}}/F_{\text{m}} \) in the young leaf. High O3 decreased \( F_{\text{v}}/F_{\text{m}} \) in the mature leaf \((P<0.001)\) 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.

### Table 2. Minimum fluorescence \((F_{\text{0}})\), maximum fluorescence \((F_{\text{m}})\), variable fluorescence \((F_{\text{v}})\), and maximum photochemical efficiency of PSII \((F_{\text{v}}/F_{\text{m}})\) 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), elevated CO2 (CO2, 714±16 ppm+CF), (CO2, 4±0.02 ppm O3); O3 (ambient CO2, 385±4 ppm+elevated O3, 72±5 ppm O3 for 7 h d\(^{-1}\), 9.00–16.00h) and elevated CO2+O3 (elevated CO2, 714±16 ppm+elevated O3, 72±5 ppm for 7 h d\(^{-1}\)). Overall, elevated CO2 significantly \((P<0.05)\) increased \( F_{\text{m}} \) and \( F_{\text{v}} \) in the young leaf. Elevated CO2 considerably \((P<0.01)\) increased \( F_{\text{v}}/F_{\text{m}} \) in both matured and young leaves. Exposure to O3 decreased \( F_{\text{m}} \) and \( F_{\text{v}} \) in the matured leaf, but increased \( F_{\text{v}}/F_{\text{m}} \) in the young leaf. High O3 decreased \( F_{\text{v}}/F_{\text{m}} \) in the mature \((P<0.001)\) and young \((P<0.1)\) leaves of wheat cultivars.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( F_{\text{0}} )</th>
<th>( F_{\text{m}} )</th>
<th>( F_{\text{v}} )</th>
<th>( F_{\text{v}}/F_{\text{m}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf 3</td>
<td>Leaf 4</td>
<td>Leaf 3</td>
<td>Leaf 4</td>
</tr>
<tr>
<td>Control</td>
<td>248±12</td>
<td>237±19</td>
<td>1356±48</td>
<td>1299±88</td>
</tr>
<tr>
<td>CO2</td>
<td>246±13</td>
<td>216±21</td>
<td>1345±53</td>
<td>1236±87</td>
</tr>
<tr>
<td>O3</td>
<td>252±15</td>
<td>261±22</td>
<td>1209±55</td>
<td>1303±97</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>242±14</td>
<td>277±21</td>
<td>1246±50</td>
<td>1605±81</td>
</tr>
</tbody>
</table>

(a) Beijing 6 (1961)

(b) Zhongmai 9 (1997)

Elevated CO2 significantly \((P<0.05)\) increased \( V_{\text{cmax}} \), \( J_{\text{max}} \), and \( J_{\text{max}}/V_{\text{cmax}} \) in the young leaf of wheat cultivars \((\text{Fig. 1})\). 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 \( J_{\text{max}} \) and \( J_{\text{max}}/V_{\text{cmax}} \) than the old cultivar. The old cultivar displayed a higher \( J_{\text{max}}/V_{\text{cmax}} \) value than the modern one at elevated CO2 \((\text{variety×CO2, } P<0.05)\). 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 PPFD at the CO2 concentration of 700 ppm in the leaf chamber \((\text{Fig. 2})\). 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 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...
Light saturated rate of net assimilation (A sat), stomatal conductance (g s), intercellular CO2 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 CO2 and/or O3 for 21 d in OTCs. Chlorophyll fluorescence parameters were recorded simultaneously with gas exchange measurements: control (CO2, 385 ± 4 ppm+CF4, 4 ± 0.02 ppm O3); elevated CO2 (CO2, 714 ± 16 ppm+CF4, 4 ± 0.02 ppm O3); O3 (ambient CO2, 385 ± 4 ppm+elevated O3, 72 ± 5 ppm O3 for 7 h d−1, 9.00–16.00 h); O3 (ambient CO2, 385 ± 4 ppm+elevated O3, 72 ± 5 ppm O3 for 7 h d−1, 9.00–16.00 h)). Overall, elevated CO2 significantly increased A sat (P < 0.01) and g s (P < 0.01) in both matured and young leaves, but decreased WUE int in the young leaf. Exposure to O3 significantly decreased A sat (P < 0.01), but increased C i (P < 0.1) in the matured leaf. Elevated O3 did not alter A sat but decreased g s (P < 0.05) and increased WUE 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>
<thead>
<tr>
<th>Treatment</th>
<th>A sat (µmol m−2 s−1)</th>
<th>g s (mol m−2 s−1)</th>
<th>C i (ppm)</th>
<th>WUE int (µmol mol−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf 3</td>
<td>Leaf 4</td>
<td>Leaf 3</td>
<td>Leaf 4</td>
</tr>
<tr>
<td>(a) Beijing 6 (1961)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.68 ± 0.63a</td>
<td>14.73 ± 0.85ab</td>
<td>0.20 ± 0.02a</td>
<td>0.28 ± 0.01bc</td>
</tr>
<tr>
<td>CO2</td>
<td>8.31 ± 0.60a</td>
<td>14.38 ± 0.76ab</td>
<td>0.19 ± 0.02a</td>
<td>0.28 ± 0.01bc</td>
</tr>
<tr>
<td>O3</td>
<td>3.75 ± 0.48a</td>
<td>13.50 ± 0.98a</td>
<td>0.13 ± 0.02a</td>
<td>0.16 ± 0.01d</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>8.55 ± 0.52a</td>
<td>16.50 ± 0.76b</td>
<td>0.17 ± 0.02b</td>
<td>0.29 ± 0.01c</td>
</tr>
<tr>
<td>(b) Zhongmai 9 (1997)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.93 ± 0.75a</td>
<td>15.55 ± 0.76b</td>
<td>0.21 ± 0.02b</td>
<td>0.32 ± 0.01e</td>
</tr>
<tr>
<td>CO2</td>
<td>10.08 ± 0.73a</td>
<td>16.70 ± 0.74a</td>
<td>0.25 ± 0.02a</td>
<td>0.32 ± 0.01e</td>
</tr>
<tr>
<td>O3</td>
<td>4.87 ± 0.53a</td>
<td>13.70 ± 0.71a</td>
<td>0.16 ± 0.02a</td>
<td>0.24 ± 0.01d</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>10.08 ± 0.60a</td>
<td>15.87 ± 0.77ab</td>
<td>0.25 ± 0.02a</td>
<td>0.37 ± 0.01e</td>
</tr>
</tbody>
</table>

Overall, elevated CO2 significantly (P < 0.001) increased RGR, RGR s, and RGR r, but did not alter K in wheat cultivars (data not shown). Elevated O3 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 CO2 (variety×CO2, P < 0.05). The old cultivar showed considerably higher RGR, RGR s, and RGR r values than the modern one at high O3 (variety×O3, P < 0.05). Elevated CO2 significantly ameliorated the negative effects of O3 on RGR, RGR s, and RGR r under elevated CO2 and O3 (CO2×O3, P < 0.05). The

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F i/F m</th>
<th>ΦPSII</th>
<th>qP</th>
<th>NPQ</th>
<th>ETR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf 3</td>
<td>Leaf 4</td>
<td>Leaf 3</td>
<td>Leaf 4</td>
<td>Leaf 3</td>
</tr>
<tr>
<td>(a) Beijing 6 (1961)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.60 ± 0.04</td>
<td>0.50 ± 0.01a</td>
<td>0.99 ± 0.01a</td>
<td>0.99 ± 0.00ab</td>
<td>0.13 ± 0.01b</td>
</tr>
<tr>
<td>CO2</td>
<td>0.62 ± 0.04</td>
<td>0.48 ± 0.01ab</td>
<td>0.97 ± 0.01a</td>
<td>0.95 ± 0.00ab</td>
<td>0.15 ± 0.01b</td>
</tr>
<tr>
<td>O3</td>
<td>0.54 ± 0.03</td>
<td>0.50 ± 0.01a</td>
<td>0.72 ± 0.01ab</td>
<td>0.84 ± 0.01bc</td>
<td>0.15 ± 0.01b</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>0.57 ± 0.03</td>
<td>0.51 ± 0.02a</td>
<td>0.70 ± 0.01de</td>
<td>0.92 ± 0.00ab</td>
<td>0.12 ± 0.01b</td>
</tr>
<tr>
<td>(b) Zhongmai 9 (1997)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.51 ± 0.04</td>
<td>0.51 ± 0.01a</td>
<td>0.52 ± 0.01c</td>
<td>0.86 ± 0.00a</td>
<td>0.10 ± 0.01b</td>
</tr>
<tr>
<td>CO2</td>
<td>0.59 ± 0.04</td>
<td>0.46 ± 0.01b</td>
<td>0.103 ± 0.01ab</td>
<td>0.84 ± 0.00bc</td>
<td>0.18 ± 0.01b</td>
</tr>
<tr>
<td>O3</td>
<td>0.56 ± 0.03</td>
<td>0.51 ± 0.01b</td>
<td>0.077 ± 0.01ab</td>
<td>0.82 ± 0.00bc</td>
<td>0.14 ± 0.01b</td>
</tr>
<tr>
<td>CO2+O3</td>
<td>0.49 ± 0.02</td>
<td>0.46 ± 0.01b</td>
<td>0.066 ± 0.01ab</td>
<td>0.98 ± 0.00ab</td>
<td>0.13 ± 0.01b</td>
</tr>
</tbody>
</table>

WUE int in the old cultivar than in the modern one relative to elevated CO2 at higher PPFs.

**Plant growth and resource allocation**

Overall, elevated CO2 significantly (P < 0.001) increased RGR, RGR s, and RGR r, but did not alter K in wheat cultivars (data not shown). Elevated O3 significantly (P < 0.05) decreased RGR, RGR s, RGR r, and K in wheat cultivars.
combined gas treatment resulted in a greater reduction in RGR, RGR<sub>r</sub>, and K in the modern cultivar than in the old one relative to elevated CO<sub>2</sub> (variety×CO<sub>2</sub>×O<sub>3</sub>, P < 0.05; Table 5).

### Discussion

**Visible symptoms of O<sub>3</sub> damage in the two cultivars of winter wheat as affected by elevated CO<sub>2</sub>**

Scoring of visible symptoms in two wheat cultivars exposed to four treatment combinations of O<sub>3</sub> and CO<sub>2</sub> for 21 d revealed that only elevated O<sub>3</sub> 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<sub>3</sub>-induced oxidative stress was higher in old and mature leaves, despite their lower O<sub>3</sub> 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<sub>3</sub> 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<sub>2</sub>, elevated CO<sub>2</sub> and O<sub>3</sub>, CFA, as found in an O<sub>3</sub>-sensitive spring wheat cultivar exposed to elevated CO<sub>2</sub> and/or O<sub>3</sub> (Cardoso-Vilhena et al., 2004).
Photosynthetic and growth responses of an old and modern winter wheat cultivar to elevated CO2

Elevated CO2 is expected to increase the productivity of C3 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 CO2. Overall, elevated CO2 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 CO2 also produced a larger increase in $A_{sat}$ in the mature leaf (41%) than in the young leaf (10%). Exposure to elevated CO2 decreased WUE$_{int}$ in the young leaf due to a higher relative increase in $g_s$ (26%) at the CO2 concentration of 400 ppm in the leaf chamber. However, elevated CO2 increased both $g_s$ and WUE$_{int}$ in the young leaf at the CO2 concentration of 700 ppm in the leaf chamber. This indicated that elevated CO2 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 CO2, as found in this study, is consistent with previous reports (Norby and O’Neil, 1991; Pettersson and McDonald, 1992; Wang et al., 2000). Elevated CO2 significantly increased $\Phi_{PSII}$, 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 CO2. Overall, elevated CO2 significantly decreased NPQ in the mature leaf but did not alter NPQ in the young leaf. We found that a 10% increase
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$_r$, RGR$_s$, and NAR in 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$_2$.

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/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+CF4, 4±0.02 ppb O$_3$); elevated CO$_2$ (CO$_2$, 714±16 ppm+CF4, 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$_r$, and RGR$_s$, but did not alter $K$. Exposure to O$_3$ significantly ($P<0.05$) decreased RGR, RGR$_r$, RGR$_s$, and $K$ in wheat cultivars. Results are shown as means±1 standard error (n=10). Means with the same letter were not significantly different.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RGR (g g$^{-1}$ d$^{-1}$)</th>
<th>RGR$_s$ (g g$^{-1}$ d$^{-1}$)</th>
<th>RGR$_r$ (g g$^{-1}$ d$^{-1}$)</th>
<th>$K$</th>
<th>SLA (cm$^2$ g$^{-1}$)</th>
<th>NAR (g m$^{-2}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Beijing 6 (1961)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.065±0.003$^a$</td>
<td>0.068±0.003$^a$</td>
<td>0.056±0.003$^a$</td>
<td>0.83±0.06$^a$</td>
<td>603±34$^b$</td>
<td>2.34±0.13</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>0.067±0.002$^a$</td>
<td>0.070±0.003$^a$</td>
<td>0.059±0.003$^a$</td>
<td>0.84±0.05$^a$</td>
<td>548±32$^b$</td>
<td>2.54±0.13</td>
</tr>
<tr>
<td>O$_3$</td>
<td>0.062±0.002$^a$</td>
<td>0.066±0.003$^a$</td>
<td>0.050±0.003$^a$</td>
<td>0.76±0.04$^a$</td>
<td>658±30$^b$</td>
<td>2.17±0.12</td>
</tr>
<tr>
<td>CO$_2$+O$_3$</td>
<td>0.066±0.002$^a$</td>
<td>0.069±0.003$^a$</td>
<td>0.056±0.003$^a$</td>
<td>0.82±0.04$^a$</td>
<td>571±30$^b$</td>
<td>2.37±0.12</td>
</tr>
<tr>
<td>(b) Zhongmai 9 (1997)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.075±0.002$^a$</td>
<td>0.077±0.003$^b$</td>
<td>0.064±0.003$^b$</td>
<td>0.84±0.04$^a$</td>
<td>592±30$^b$</td>
<td>2.43±0.12</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>0.081±0.002$^a$</td>
<td>0.084±0.003$^a$</td>
<td>0.071±0.003$^a$</td>
<td>0.84±0.05$^a$</td>
<td>527±32$^b$</td>
<td>2.76±0.13</td>
</tr>
<tr>
<td>O$_3$</td>
<td>0.054±0.002$^b$</td>
<td>0.058±0.003$^b$</td>
<td>0.040±0.003$^b$</td>
<td>0.71±0.04$^a$</td>
<td>665±30$^b$</td>
<td>1.98±0.12</td>
</tr>
<tr>
<td>CO$_2$+O$_3$</td>
<td>0.076±0.002$^c$</td>
<td>0.083±0.003$^a$</td>
<td>0.065±0.003$^b$</td>
<td>0.78±0.04$^b$</td>
<td>492±30$^b$</td>
<td>2.48±0.12</td>
</tr>
</tbody>
</table>

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., 2008a; 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 O₃ had no effect on \( V_{\text{cmax}} \), \( J_{\text{max}} \), and \( J_{\text{max}}/V_{\text{cmax}} \) in the young leaf. This might be the cause underlying the non-significant reduction in \( A_{\text{up}} \) 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, RGRs, 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 \( C \) 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 \( g_i \) in both leaves of modern cultivar compared with the old cultivar at high O₃ (Biswas et al., 2008a, b). Consequently, the old cultivar demonstrated higher WUEim in the young leaf than the modern one at high O₃. However, higher O₃-induced physiological impairment resulted in greater reductions in RGR, RGRs, 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 \( F_0 \) and \( F_m \) 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 \( g_i \) compared with the old cultivar at elevated CO₂ and O₃. Consequently, the combined gas treatment showed larger decreases in \( \Phi_{\text{PSII}} \) and \( q_P \) 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 WUEim 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, RGRs, 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).

Our results also suggested that the beneficial effects of elevated CO₂ on the old cultivar were sustained due to lower O₃ uptake and lower O₃-induced photoinhibition under elevated CO₂ and O₃. In addition, a greater O₃-induced loss of the positive effects of elevated CO₂ on the modern cultivar suggests that elevated CO₂-induced growth stimulation in the recently released wheat cultivar attributed to higher energy capture and electron transport rate could be compromised by its higher O₃ uptake and greater O₃-induced photoinhibition under elevated CO₂ and O₃ conditions.

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.

Acknowledgements

The authors wish to thank Handling Editor, Dr. Elizabeth Ainsworth and two anonymous reviewers for their valuable suggestions on an earlier version of the manuscript. D.K.B thanks Protima Biswas for her continuous inspiration and
acknowledges funding of the Chinese Academy of Sciences and China Scholarship Council. This is a joint contribution between Institute of Botany of the Chinese Academy of Sciences and Agriculture Agri-Food Canada (AAFC). AAFC-ECORC contribution No. 12-358. This study was co-funded by the Shandong Province Taishan Scholarship (no. 00523902), the Innovative Group Grant of Natural Science Foundation of China (no. 30521002), the State Science and Technology Supporting Project Eco-agriculture Technological Engineering (2012BAD14B07-8), and National Natural Science Foundation of China (no. 30900200).

References


Elevated concentrations of atmospheric CO2 protect against and increase productivity to rising temperature by atmospheric CO2 concentrations: has its importance been underestimated? Plant, Cell and Environment 42, 729–739.


