Effects of Ozone Stress on Rice Growth and Yield Formation under Different Planting Densities - A Face Study

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Abstract

The rising tropospheric ozone concentration inhibits rice growth and causes yield loss, but whether ozone effects can be ameliorated by cultivation measures such as planting density regulation was unclear, especially under fully open-air field conditions. An experiment was conducted with rice to address this issue by using free-air gas concentration enrichment (FACE) facility stored in paddy field of China. A conventional indica cultivar Yangdao 6 (2011) and a super hybrid indica cultivar IY084 (2012) were grown at ambient or elevated ozone concentration (target at 50% above ambient, E-O3) under low (LD, 16 hills m−2), medium (MD, 24 hills m−2) or high planting density (HD, 32 hills m−2) from tillering until maturity. E-O3 greatly accelerated phenological development and reduced plant height of rice LY084. The results from the final harvest showed that E-O3 reduced grain yield of Yangdao 6 and IY084 by 16% and 27%, respectively. The yield loss was attributed to a great reduction in sink size (the number of spikelets per panicle or unit area, -22%), secondly the decline in grain-filling capacity shown by decreases in filled grain fraction (-13%) and filled grain weight (-5%) but increases in empty and lightened grain fractions. Although yield components were regulated by changing planting density in general, no significant ozone effect by planting density interaction was found for any of the measured parameters, which indicated that planting measures could not ameliorate ozone damage, at least under the current experimental conditions. It was concluded that yield loss of hybrids were greater than conventional rice cultivars, the future projection must include hybrid rice or/super rice not to underestimate the effects of ozone stress on rice productivity. © 2018 Friends Science Publishers

Keywords: Rice; Ozone; FACE (Free Air ozone Concentration Enrichment); Planting density; Yield

Introduction

In many Asian countries including China and India, the rapid economic development and population growth in recent years has led to increased emissions of air pollutants (NOx) and volatile organic compounds (VOC), these precursors are converted to ozone in photochemical reactions, which resulted in a rapid rise in tropospheric ozone concentrations (Ohara et al., 2007; Fowler et al., 2008; Emberson et al., 2009; Lei et al., 2012).

Ozone is a strong oxidant and enters plant through leaf stomata and decomposed into active oxygen in the apoplast (Kobayashi et al., 1995). These reactive oxygen damage protein, lipids and DNA, or trigger the programmed cell death, cause leaf damage and lead to reduced photosynthesis, growth inhibition and yield loss (Kobayashi et al., 1995; Fiscus et al., 2005; Ainsworth et al., 2012). Rice is one of the major staple in South and East Asia and rice cultivation regions often experience high ozone concentration (Ohara et al., 2007; Alexandratos and Bruinsma, 2012; Frei, 2015). Furthermore, meta-analysis has indeed demonstrated that rice is not less sensitive to O3 than other sensitive species such as soybean and wheat (Ainsworth, 2008; Feng and Kobayashi, 2009). Therefore, it is important to study the effects of ozone stress on rice growth, yield and its mechanism, and to develop appropriate response measures to ensure regional food security in the context of global climate change. The responses of rice growth and yield formation under ozone stress have been reported extensively, but most of these studies are single factor tests focusing on ozone effects only (Ainsworth, 2008; Yang et al., 2008). The limited number of multi-factorial experiments investigating the interactions between ozone and other factors has demonstrated that the ozone effects on rice yield varied with...
fumigation methods, cultivars and environmental conditions (Olszyk and Wise, 1997; Ainsworth, 2008; Wang et al., 2014; Frei, 2015), which justify the fact that understanding ozone effect in field requires more multifactor tests. Rice production management measures including planting density, fertilizer application and water management are essential for achieving high yield. Whether proper management measures can modify ozone stress was unclear, because such research requires larger test space, and better conducted in natural field. But most of the previous studies were conducted in closed or partial closed chambers with relatively small space (diameter less than 2 m). In contrast to chamber, Free Air gas Concentration Enrichment (FACE) platform has a relatively larger operation space (diameter more than 14 m), is closer to the real-world situation than chamber, provides better opportunity for studying interactions between ozone and other factors (Long et al., 2005; Ainsworth et al., 2012). In 2007, for the first time with rice in the world, the full-size (14-m diameter) FACE system was set up in farmer fields in Jiangsu Province, China (Tang et al., 2011).

Because of its great yield potential and strong stress resistance in general, hybrid rice has become the rice type with the largest planting area in China (>60%), plays an important role in ensuring food security (Yuan, 1999). However, in our previous studies, we found the yield loss of hybrid rice under ozone stress that was significantly higher than conventional rice (Shi et al., 2009; Wang et al., 2012). This brings pessimism for the future rice production if ozone level can’t be controlled during rice growth period. Therefore, finding proper management measures targeting ozone damage is an urgent issue to secure rice production. Optimizing planting density is an important field management technology, which regulates crops yield and quality. Changing planting density will modify the robustness of individual plant as well as population quality. Meanwhile, changing planting density also changes the free space for individual plant and it may influence stomatal ozone uptake of leaves, especially those at the lower position of a plant. These factors together will determine the final yield response of rice to ozone. The objective of this O$_3$-FACE experiment is to study the rice yield response to E-O$_3$ under different planting densities, and its relation with the growth processes. Understanding this interaction, especially under a natural field conditions, will help us to formulate adaptation strategies of rice production systems under high [O$_3$] in the near future.

Materials and Methods

Research Site and ozone-FACE Platform

The experiment was conducted in the China O$_3$-FACE platform, located at Xiaoji town, Jiangdu County, Jiangsu province, China (119°45'E, 32°35'N). The site, at 5 m above the sea level in elevation, sits in the subtropical marine climatic zone. Throughout the period of 113 days growing period from June 21 to October 17 for the experiment in 2012, mean daily temperature was 24.5°C, mean daily integral solar radiation (or PAR) was of 13.9 MJ m$^{-2}$, and mean daytime (6:00–18:00) vapor pressure deficit was of 0.67 kPa (Tang-11). This site has been in continuous cultivation for more than 1000 years with rice–wheat or rice–rape-seed rotation. Detailed descriptions of the soil properties for the site can be found in our previous publication (Shi et al., 2009). Details of the design and performance of this O$_3$-FACE system are provided by Tang-11. In brief, the FACE system has eight 240 m$^2$ plots, of which replicate plots were exposed to elevated ozone concentration (hereinafter called E-O$_3$ plots) and four equal size plots were in ambient ozone concentration (A-O$_3$ plots). Any one of the E-O$_3$ plots was separated from other plots by at least 70 m to avoid cross contamination. The quantity and direction of the ozone release for each E-O$_3$ plot was controlled by a proportional integral derivative algorithm for computer feedback that compares achieved ozone concentration to the target ozone concentration of 1.5 times ambient ozone concentration with an ozone monitor (model 49, Thermo Environmental Instruments, MA, USA), a data logger-controller, an anemometer and a wind vane. A mixed gas consisting of about 5% ozone and 95% O$_2$ was produced by an ozone generator (KCF-BT0.2, Jiangsu Koner Ozone Co., Ltd, Yangzhou, China). The mixed gas was released in a stream of compressed air into the plots through the ABS pipes positioned at about 50 above the canopy. In the ambient plots, plants were grown under ambient ozone conditions without the ring structures. The ozone fumigation began on 1st July and continued throughout the rice season until harvest except for occasions such as system malfunction, rain or wet leaves. The fumigation was settled during day-time 7-h (09:00–16:00 Chinese Standard Time) every day. Fig. 1 shows the seasonal change in daily 7-h mean ozone concentration for A-O$_3$ and E-O$_3$ plots.

Plant Material and Cultivation

The cultivars were Yangdao 6 (2011) and II You 084 (2012). Rice seeds were sown on 21 May; on which the seedlings grew under ambient air, and manually transplanted into the A-O$_3$ and E-O$_3$ plots with one seedling per hill on 21 June 2012. Planting density was 16, 24 and 32 hills m$^{-2}$ for low, medium and high density, equivalent to the spacing of hills 25×25, 16.7×25 and 12.5×25, respectively. Nitrogen (N) was supplied as urea (N = 46%) and compound chemical fertilizer (N: P$_2$O$_5$: K$_2$O = 15:15:15) at a rate of 15 g N m$^{-2}$. Of the total N, 50% was applied as the basal dressing on 20 June, 10% was top-dressed on 7 July (tiller stage) and 40% top-dressed on 7 August (panicle initiation stage). Both phosphorus (P) and potassium (K) were applied as compound chemical fertilizer at equal rates of 7 g m$^{-2}$ as the basal dressing at 1 d before transplanting. Standard cultivation practices as commonly performed in the area.
were followed in all experimental plots. In brief, the paddy fields were submerged with water of about 3 in depth from 21 June to 4 July, and then the fields were subjected to wet–dry cycles through natural drainage and intermittent irrigation. At 10 d before harvest, irrigation was terminated to allow paddy fields to dry for final harvesting. Pesticides and fungicides were applied when necessary throughout the experiment.

**Plant Phenology, Height and Tillering Dynamics**

The date for heading when 50% of plant headed and grain maturity was recorded in each subplot. Plant height and tiller numbers were measured for fixed 10 hills in each treatment at 5–10 days intervals until heading. The productive tiller ratio (%) was expressed as the panicle number per square meter/ the maximum tiller number per square meter x 100.

**Grain Yield and Yield Components**

Actual grain yield was determined of 60 hills from a 4, 3, and 2 m² patch in each LD, MD and HD subplot, respectively. The grains were air dried and weighted to obtain actual grain yield.

Grain yield components, i.e., the number of panicles per square meter, the number of spikelets per panicle, filled spikelet fraction, and individual grain mass, were determined at maturity with six hills per plants in each subplot. Grains were soaked in tap water (specific gravity=1.0) and the number of sunken and floated grains was counted to determine the filled spikelet fraction. The floated grains were dried and then separated into empty and lightened grains, each of which was counted and the empty grain percentage and lightened grain percentage were calculated. The sunken (filled) grains were oven-dried at 80°C until constant weight achieved. The theoretical grain yield was obtained by adjusting dry weight of filled grains to a moisture content of 0.14 g H₂O g⁻¹ fresh weight. Average grain mass = Total grain mass ×1000/Total grain number.

**Statistical Analysis**

A split-plot design was employed with [O₃] as main-plot treatment and planting density as the split-plot treatment. Analysis of variance (ANOVA) was performed using the software Statistical Product and Service Solutions (SPSS Inc., Chicago, IL, USA) to determine the main effects of [O₃], planting density and the effects of their interaction. Treatments were compared by Tukey’s test and differences were declared statistically significant (P<0.05). Pearson’s correlations were calculated to determine the relationships between the different parameters of rice quality.

**Results**

**Effect of E-O3 on Phenology and Plant Height**

E-O₃ greatly accelerated phenological development of rice LY084. The period from transplanting to heading stage and from heading to maturity stage were reduced by 3 and 4 days, respectively, resulting in one-week earlier maturity by E-O₃ (Fig. 2). The reduction in plant height by E-O₃ increased with plant growth process: with no effect on plant height at tillering stage, but reduced it by 3.1% (2.4, P <0.1), 6.7% (7.0, P <0.01) and 7.8% (8.6, P <0.01), at jointing, heading and maturity stages, respectively (Table 1). No effect of planting density or O₃ by planting density interaction was detected on phenology and plant height.

**Effect of E-O3 on Grain Yield**

Final theoretical (TGY) and actual grain yield (AGY) of hybrid LY084 showed that increasing planting density from 16 to 24 hill m⁻², TGY and AGY increased by 14.5% and 5.9%, respectively but higher density level of 32 hill m⁻² did not result in further increases in grain yield (Fig. 3).
Table 1: Plant height (cm) at different growth stages of hybrid LY084 exposed to ambient (A-O₃) or elevated O₃ concentration (E-O₃; ambient×1.5) under low (LD, 16 hills m⁻²), medium (MD, 24 hills m⁻²) and high (HD, 36 hills m⁻²) levels of planting density

<table>
<thead>
<tr>
<th>Density</th>
<th>Ozone</th>
<th>Tillering stage</th>
<th>Jointing stage</th>
<th>Heading stage</th>
<th>Maturity Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>A-O₃</td>
<td>67.3±1.3</td>
<td>93.6±2.0</td>
<td>105.4±1.8</td>
<td>109.7±1.6</td>
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<tr>
<td></td>
<td>E-O₃</td>
<td>68.8±1.6</td>
<td>90.4±2.0</td>
<td>95.4±1.7</td>
<td>100.0±0.5</td>
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<td>MD</td>
<td>A-O₃</td>
<td>70.2±1.4</td>
<td>92.5±1.3</td>
<td>105.7±2.5</td>
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<tr>
<td></td>
<td>E-O₃</td>
<td>69.1±1.0</td>
<td>91.5±1.4</td>
<td>99.5±0.8</td>
<td>103.4±2.6</td>
</tr>
<tr>
<td>HD</td>
<td>A-O₃</td>
<td>69.6±1.1</td>
<td>99.0±1.8</td>
<td>104.4±3.1</td>
<td>109.3±2.4</td>
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<tr>
<td></td>
<td>E-O₃</td>
<td>68.1±1.1</td>
<td>95.9±2.0</td>
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<td>100.9±2.9</td>
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<td>ANOVA</td>
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<td></td>
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<td>Density</td>
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<td>0.139</td>
<td>0.017</td>
<td>0.572</td>
<td>0.360</td>
</tr>
<tr>
<td>O₃×Density</td>
<td>0.472</td>
<td>0.815</td>
<td>0.458</td>
<td>0.869</td>
<td></td>
</tr>
</tbody>
</table>

A-O₃: Ambient O₃ concentration; E-O₃: Elevated O₃ concentration

**Fig. 3:** Actual grain yield (A) and theoretical grain yield (B) at final harvest of hybrid LY084 exposed to ambient (A-O₃) or elevated O₃ concentration under low (LD), medium (MD) and high (HD) levels of planting density. These data are from harvests of ca. 60 plants (ca. 2 m²) within each replicate plot at maturation of the crop. Bars indicate ± SE (n=4). Bars not sharing the same letter differ significantly at p<0.05

E-O₃ greatly reduced the TGY and AGY of LY084. Averaged across 3 densities, E-O₃ decreased TGY from 1111 g m⁻² in the controls to 733 g m⁻² (34.0%) and AGY from 1018 g m⁻² in the controls to 739 g m⁻² (27.4%). Similar patterns of yield response to O₃ were observed for rice grown in different planting density. TGY was lower by 32.4%, 36.5% and 32.9%, and AGY by 26.9%, 25.0% and 30.0% for LM-, MM- and HM-crops, respectively showing no interaction of ozone with planting density for the two parameters. O₃ treatment decreased the actual grain yield of Yangdao 6 by 142.0 g m⁻², with 15.9% (p<0.01) compared to the control, with the decrease of 23.5% (p<0.01), 14% (p<0.05) and 9.9% (p<0.05) under low, medium and high density (Fig. 4).

**Fig. 4:** Actual grain yield at final harvest of conventional variety yangdao 6 exposed to ambient (A-O₃) or elevated O₃ concentration under low (LD), medium (MD) and high (HD) levels of planting density (the experiment in 2011)

Effect of E-O₃ on Yield Components

Significant effect of E-O₃ was detected for most yield parameters, however, no interaction between O₃ and planting density was observed for any of the yield parameters.

![Graph](image-url)

Fig. 5: Yield components at final harvest of hybrid LY084 exposed to ambient (A-O₃) or elevated O₃ concentration under low (LD), medium (MD) and high (HD) levels of planting density. Yield components included panicle number per m²(A); spikelet number per panicle (B), spikelet number per m² (C), fulfilled grain fraction (D), and fulfilled grain mass (E), average grain mass (F), lightened grain fraction (G) and empty grain fraction (H). Bars indicate ± SD. Bars not sharing the same letter differ significantly at p < 0.05

The number of panicles per unit land area increased with the increase of plant density, but showed no response to E-O₃ (Fig. 5a). Panicle number is the product of maximum tiller number per square meter (MTN) and productive tiller ratio (PTR). The latter was unaffected by
planted under elevated O₃ conditions (25% higher than the ambient) (Fig. 2). This change in plant phenology was reported for hybrid indica SY63, but significantly greater than the other cultivars (Shi et al., 2009; Table 3).

The number of panicles had been reported to increase, decrease or unchanged under E-O₃ (Yang et al., 2008). When averaged across all enclosure studies, the number of panicles was hardly affected by ozone (Ainsworth, 2008). 

Current free-air experiment indicated no change in panicle number. Little effect on panicle number was due to the offset of its two determinants: MTN (refer to plant capacity in forming tillers) decreased but PTR (refer to pant capacity in forming panicles) increased with E-O₃. Similar increases in PTR with E-O₃ have also been reported elsewhere (Wang et al., 2012), though decrease or unchanged was reported too (Yang et al., 2008; Shi et al., 2009).

In contrast with panicles, the spikelet number per panicle showed a clear trend of decrease in response to ozone stress, and the magnitude of decrease varied with varieties in the previous chamber studies (Yang et al., 2008). The present FACE study found that E-O₃ decreased the spikelet number per panicle of LY084 independent of planting density (-22%, Fig. 5b), and this response was close to hybrids but substantially greater than conventional rice cultivars in previous FACE studies (Table 3). This large reduction in panicle size was supported by the decreases in the dry weight per stem and the ratio of spikelet number to stem dry weight (Table 2), as well as plant height at heading (Table 1). Our previous FACE study indicated that O₃-induced reduction in number of spikes resulted from both the inhibition of spikelet differentiation and promotion of spikelet degeneration (Wang et al., 2012). Spikelets per unit land area are the product of panicle number per unit area and spikelets per panicle, representing sink size of rice. Since, E-O₃ did not change panicle number; therefore, O₃-induced reduction in total sink size was driven almost totally by the decrease in panicle size rather than panicle number.

Meta-analysis on rice showed a trend of decrease in grain mass by E-O₃ (Ainsworth, 2008; Feng and Kobayashi, 2009). In the present study, filled grain fraction, filled grain mass and mean grain mass was found to decrease with the ozone stress, with the negative effect being more prominent for the filled-grain fraction, which confirmed the more growth suppression by E-O₃ after heading. The reduction in filled-grain fraction was much greater in the present study compared with the results from the previous rice-FACE studies (Pang et al., 2009; Shi et al., 2009; Wang et al., 2012), which reflected the high ozone sensitivity of LY084, especially at later growth stages. Low filled-grain fraction results from more empty or lightened grains in a panicle. The increase in empty grain percentage, especially lightened grain percentage (Fig. 5) was consistent with the down-regulation of leaf photosynthesis and plant growth in the grain-filling stage (Wang et al., 2012). Ozone-induced increase in the percentage of grain sterility of rice was also reported elsewhere (Ishii et al., 2004).

Discussion

In previous chamber studies, shortened or unchanged rice growth period by ozone stress was reported (Yang et al., 2008). In the present FACE study, heading and maturity was 3 and 7 days earlier for super rice LY084 under planting density while the former increased as planting density increased (Table 2). E-O₃ influenced the two components in opposite directions: MTN was significantly reduced by 5-11% while PTR increased by a similar amount under ozone stress for all density levels (Table 2).

Maximum tiller number, productive tiller ratio, dry weight per stem at heading and ratio of spikelet number to stem dry weight of hybrid LY084 exposed to ambient (A-O₃) or elevated O₃ concentration (E-O₃, ambient×1.5) under low (LD, 16 hills m⁻²), medium (MD, 24 hills m⁻²) and high (HD, 36 hills m⁻²) levels of planting density.

The number of spikelets per panicle was not changed by density, but responded significantly and negatively to ozone stress (Fig. 5b). E-O₃ significantly decreased the spikelet number per panicle by 22% on average, with the decrease of 16%, 28% and 22% under LD, MD and HD treatments, respectively. The spikelet number per panicle can be factorized into dry weight per stem and the ratio of spikelet number to stem dry weight (Table 2). Averaged across all planting density, E-O₃ significantly decreased dry weight per stem and the ratio of spikelet number to stem dry weight by 13% and 11%, with the range of decrease being 9-17% and 5-14%, respectively (Table 2). Although planting density affected the spikelet number per panicle, it had no impact on its two components.

Similar to the number of panicles, the spikelet number per unit area increased with the increase of planting density, 13% and 20% increases were found for MD and HD compared with LD (Fig. 5c). E-O₃ decreased the spikelet number per unit land area by 22% (P<0.01) on average, with 20% (P<0.01), 26% (P=0.01) and 19% (P=0.08) decrease at LD, MD and HD, respectively.

There was a trend of decrease in filled grain fraction, filled grain mass and average grain mass with the increase of planting density, and the effects were statistically significant for the latter two parameters (Fig. 5d, e and f). Averaged across all planting densities, E-O₃ decreased filled grain fraction, filled grain mass and average grain mass by 12% (P<0.01), 5% (P<0.01) and 10% (P<0.01), respectively. Ozone-induced reduction among different planting densities was very close for each parameter, in the range of 11-13%, 4-5% and 8-11% for filled grain fraction, filled grain mass and average grain mass, respectively. With respect to lightened grain fraction (Fig. 5g) and empty grain fraction (Fig. 5h), planting density had no significant influence, but E-O₃ substantially increased these two fractions with 74% and 253% for lightened and empty grain fraction, respectively, in the range of 53-88% and 180-477% at different planting densities.
Table 2: Maximum tiller number per square meter, productive tiller ratio, dry weight per stem at heading and ratio of spikelet number to stem dry weight of hybrid LY084 exposed to ambient (A-O3) or elevated O3 concentration (E-O3, ambientx1.5) under low (LD, 16 hills m–2), medium (MD, 24 hills m–2) and high (HD, 36 hills m–2) levels of planting density

<table>
<thead>
<tr>
<th>Density</th>
<th>Ozone</th>
<th>Maximum tiller number per m² (%)</th>
<th>Productive tiller ratio</th>
<th>Dry weight per stem at heading stage (g)</th>
<th>Ratio of spikelet number to stem dry weight</th>
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</thead>
<tbody>
<tr>
<td>LD</td>
<td>A-O3</td>
<td>372.8±4.0</td>
<td>84.1±0.9</td>
<td>3.1±0.1</td>
<td>39.6±1.1</td>
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<td></td>
<td>E-O3</td>
<td>344.8±2.9</td>
<td>87.4±1.2</td>
<td>2.8±0.2</td>
<td>37.7±1.9</td>
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<tr>
<td>MD</td>
<td>A-O3</td>
<td>431.5±5.0</td>
<td>76.5±1.0</td>
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<td></td>
<td>E-O3</td>
<td>383.5±4.1</td>
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<td>36.6±1.9</td>
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<tr>
<td>HD</td>
<td>A-O3</td>
<td>460.5±5.2</td>
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<td>E-O3</td>
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<td>2.7±0.2</td>
<td>38.3±2.2</td>
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ANOVA (p O3 value) Density (D) 0.001 <0.001 <0.001 <0.001

Table 3: Summary of FACE results Summary of experimental background, absolute response in phenology and relative responses (%) in plant height, grain yield and its components of rice grown at E-O3 relative to A-O3 in the Chinese Ozone-FACE experiments

<table>
<thead>
<tr>
<th>Items</th>
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<th>2008</th>
<th>2012</th>
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<tr>
<td>Test cultivars</td>
<td>WJ15</td>
<td>SY63</td>
<td>LY084</td>
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<tr>
<td>Genotypes</td>
<td>Inbred japonica, Inbred indica, Hybrid indica, Hybrid japonica</td>
<td>Inbred indica, Hybrid indica</td>
<td>Hybrid indica (Super rice)</td>
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<tr>
<td>N application levels (g m⁻²)</td>
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<td>20</td>
<td>15</td>
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<tr>
<td>Planting density (hills m⁻²)</td>
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<td>24</td>
<td>16, 24 or 32</td>
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<td>Phenology (d)</td>
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<td>Heading</td>
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<td>Maturity</td>
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<td>Plant height at maturity</td>
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<td>Grain yield</td>
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<td>Grain yield components</td>
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<td>Panicule number per m²</td>
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<td>Spikelet number per panicle</td>
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</table>

The above results indicate that E-O3 not only reduced the sink size of LY084, it also decreased grain-filling capacity of individual spikelet. These negative effects of ozone could not be eliminated by regulating planting density. Therefore, how ozone stress affects the processes of rice fertilization and grain filling requires further research, and special attention should be given to super rice.

Elevated O3 decreases rice grain yield was found in most cases of previous studies. A meta-analysis of chamber studies by Ainsworth (2008) suggested a 14% decrease in grain yield, when exposed to 62 ppb ozone compared with plants grown in clean air. In this FACE study, a 25% increase form the ambient O3 ca. 38 ppb to ca. 47 ppb caused a 27% yield loss of hybrid LY084, which substantially greater than the expectations from chamber studies (Ainsworth, 2008; Feng and Kobayashi, 2009) and also greater than the response of conventional rice cultivars yangdao 6, but very close to hybrids (Table 3). The significant decreases in yield components of spikelets per unit area, filled-grain fraction, grain mass, significant increase of empty and lightened grain percentage (Fig. 5), indicates the substantial yield loss of LY084 at E-O3 was related with ozone-induced inhibition in spikelets formation, fertilization and grain filling processes, and its inhibition was greater than conventional rice cultivars (Table 3).

We didn’t find any regulation effect of planting density on the response of rice to ozone stress, and ozone by density interaction was not detected for any parameters measured. Based on growth dynamics of this cultivar and the previous ozone studies on rice, we give the following explanation that higher planting density reduced the air contact of leaves at the lower position of plant population which led to lower ozone contact and uptake of these leaves, thereby amelioration of ozone damage. On the other hand, higher planting density affected the growth and development of the stem, especially in grain filling stage, which made the population quality worse, especially for the rice varieties with strong tillering ability and thin stems, and enhanced exacerbating the effects of E-O3. These opposite effects might counteract each other, resulted in no regulation effect on ozone damage by changing planting density in this experiment.
**Conclusion**

To our knowledge, it was the first time that the interactive effects between ozone and planting density on rice growth and yield were studied under fully open-air field conditions. The results showed that E-O$_3$ decreased the final productivity of super rice LY084, and the reduction was unaffected by planting density, as reflected in no interaction of ozone with planting density. The O$_3$-induced yield loss was mainly associated with the depression in reproductive growth, as shown by the negative effects on spikelet formation, fertilization and grain filling processes. The results from the present study provide important information for model predication and adaptation strategies formulation of future rice production. Firstly, because the yield loss of hybrids was much greater than conventional rice cultivars, the future prediction must include hybrids and/or super rice to avoid the underestimation of ozone stress on rice production. Secondly, the important adaptation strategy for hybrid rice to grown under ozone stress is to avoid photosynthesis depression at late growth stages in order to counteract the O$_3$-induced inhibition of spikelet and grain formation, and thus minimize the yield loss under E-O$_3$. The present study showing no clear regulation of changing planting density on ozone effects in rice converted an alarm that little potential for crop management practices to reduce the ozone damage; therefore, other adaptation strategies like developing tolerant cultivar need be reinforced.

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