Tropospheric ozone reduces resistance of Japonica rice 
(*Oryza sativa* L., cv. Koshihikari) to lodging

Masahiro YAMAGUCHI$^{a*}$, Yuriko NISHI$^b$, Sayaka KAWADA$^a$ and Kentaro NAKASHIMA$^a$

($^a$ Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan 
$^b$ Faculty of Environmental Science, Nagasaki University, Nagasaki 852-8521, Japan)

Abstract

To clarify the effects of ozone (*O$_3$*) on the resistance of Japonica rice (*Oryza sativa* L.) to root lodging, the Japanese major rice cultivar Koshihikari was exposed to four levels of *O$_3$* in open-top chambers (mean *O$_3$* concentration: 17.3, 32.4, 51.1, and 66.3 nl l$^{-1}$). We evaluated the resistance to root lodging by measuring pushing resistance at 15 days after heading (DAH) and the dry masses of plant organs such as roots at harvest (35 DAH). Exposure to *O$_3$* significantly reduced pushing resistance per panicle, that the *O$_3$* reduced resistance to root lodging. Because the degree of *O$_3$*-induced reduction in pushing resistance per panicle correlated with that of *O$_3$*-induced reduction in root dry mass per panicle, the *O$_3$*-induced reduction in the resistance to root lodging could be caused by *O$_3$*-induced reduction in root development. There was a significant negative correlation between relative value of pushing resistance per panicle and *O$_3$* concentration or dose, indicating that resistance to root lodging decreases with increasing *O$_3$* concentration. In East Asia, because the *O$_3$* concentration is projected to increase in the near future, lodging issues in the rice cultivation could become serious.

**Key words:** Photochemical oxidant, Pushing resistance, Root development, Root lodging

1. Introduction

The concentration of tropospheric ozone (*O$_3$*), the main component of photochemical oxidants, has been increasing globally, especially in Europe, North America, and East Asia (Cooper et al., 2014). Since 1950s, it has been recognized that *O$_3$* has negative impacts on vegetation such as visible foliar injury, inhibition of net photosynthesis, and reductions in growth and yield (Heggstad and Middleton, 1959; Ainsworth et al., 2012; Emberson et al., 2013). Because of its phytotoxicity, the current levels of *O$_3$* are sufficiently high to reduce the yield of major staple crops (Fuhrer, 2009). Van Dingenen et al. (2009) estimated that present-day global *O$_3$*-induced crop yield losses were more than 10% for *O$_3$*-sensitive crops, such as wheat (*Triticum aestivum* L.) and soybean (*Glycine max*). Although rice (*Oryza sativa* L.) is classified as being moderately resistant to *O$_3$* and global *O$_3$*-induced yield losses of rice were less than those of wheat and soybean (Van Dingenen et al., 2009), the current levels of *O$_3$* in Japan and China have been high enough to significantly reduce the rice yield (Yamaguchi et al., 2014; Feng et al., 2015). Additionally, Sawada et al. (2016) reported *O$_3$*-induced deterioration of the grain quality of Japonica rice without a significant reduction in the yield after exposure to *O$_3$*. To estimate the risk of *O$_3$* on crop production, it is necessary to clarify the effects of *O$_3$* on parameters related to food productivity besides the yield.

Lodging, the bending of plant stems from their vertical position, reduces canopy photosynthesis, crop yield, and mechanical harvesting efficiency in many cereals such as rice, wheat, and barley (Setter et al., 1997; Berry et al., 2004). There are two types of lodging: stem lodging, which results from the bending or breaking of culm internodes, and root lodging, which results from root anchorage failure (Berry et al., 2004). Terashima et al. (1994) suggested that rice cultivars tolerant to root lodging develop more roots than those susceptible to lodging, suggesting that the dry matter partitioning to roots could be an important factor for increasing the resistance to root lodging. On the contrary, it is well known that exposure to *O$_3$* reduces the allocation of carbohydrates to roots, resulting in reduced dry matter partitioning to roots (Grantz et al., 2006). These results suggest that the exposure to *O$_3$* reduces the resistance to root lodging through reduction in the dry matter partitioning to roots.

Rice is a primary agricultural crop in Japan. However, the current levels of *O$_3$* are high enough to reduce the yield of rice (Yamaguchi et al., 2014). Furthermore, the concentration of *O$_3$* has been increasing due to transboundary air pollution and reduced NO titration in Japan (Aikimoto et al., 2015), and the concentration of *O$_3$* in East Asia is expected to increase due to an increase in the emission of its precursors (Wild et al., 2012). Kobayashi et al. (1995) reported the *O$_3$*-induced reduction in dry matter partitioning to leaf sheath and culm of Japonica rice (cv. Koshihikari) and *O$_3$*-induced severe lodging, which might be stem lodging. Because they also observed *O$_3$*-induced reduction in the dry matter partitioning to roots, it is possible that the exposure to *O$_3$* reduces resistance of rice to root lodging. To evaluate the adverse effects of *O$_3$* on rice productivity, it is necessary to clarify the effects of *O$_3$* on the resistance of Japonica rice to root lodging, in addition to its effects on yield and grain quality. In the present study, we hypothesized that exposure to *O$_3$* reduced dry matter partitioning to roots and consequently reduced...
resistance of Japonica rice to root lodging. To test the hypothesis, we conducted an experimental study on the effects of O$_3$ on the resistance of Japonica rice cv. Koshihikari to root lodging.

2. Materials and methods

2.1. Plant material

The Japonica rice cultivar “Koshihikari” was used as the plant material because it is the most widely cultivated variety in Japan. Two- to three-leaf seedlings of Koshihikari were planted in 1/2000a Wagner’s pots (ø256 mm × 297 mm in height, approximately 14 L) filled with flooded andosols (12 L) at three hills per pot and one seedling per hill on 1 May 2015. The seedlings were grown on the roof floor of the Nagasaki University (Nagasaki, Japan) until 16 May in order to ensure the rooting and were subsequently grown in 16 open-top chambers (OTCs): 60 cm in width, 120 cm in height, 82.5 cm in depth) placed on the roof floor without any covers. Three pots were assigned to each chamber, for a total of 48 pots. To acclimatize the plants to the chamber, the ambient air was directly introduced into each OTC from 16 to 20 May. NPK (15:15:15) fertilizer was applied at 2.25 g per pot, which corresponds to 6.75 g N m$^{-2}$, before planting.

2.2. O$_3$ exposure

The rice plants in the OTCs were exposed daily to O$_3$ removal-filtered air (FA), non-filtered air (NF), NF supplemented with approximately 20 nl l$^{-1}$ O$_3$ (NF+) or 35 nl l$^{-1}$ O$_3$ (NF++). The chambers assigned to the FA treatment were equipped with an O$_3$ removal filter, a honeycomb-structured filter coated with a catalyst for O$_3$ decomposition reaction (HONEYCLE-ZV, No. 8803ZV, NICHIAS Corporation, Tokyo, Japan), and the chambers assigned to NF, NF+, and NF++ treatments were equipped with a non-coated filter (No. 8801, NICHIAS Corporation). The ambient air firstly passed the filter, was drawn by the FAN, and was blown into the chambers. Supplementary O$_3$ was generated by an electrical discharge generator (SO-03UN, Hamanetsu Co., Shizuoka, Japan). The generated O$_3$ was passed through a water trap to remove nitrogen by-products before being injected into the NF+ and NF++ chambers. The O$_3$ concentrations in the chambers assigned to the NF+ and NF++ treatments were controlled by a flow rate of O$_3$ injection using handy valve. Supplementation of O$_3$ was constant and lasted throughout the exposure period (i.e., 24 h/day). Four chamber replications were included for each gas treatment, for a total of 16 OTCs. Pots were rotated within and among the chambers at 7- to 10-day intervals to minimize variation among the chambers. The gas treatments lasted for 109 days, from 21 May to 7 September 2015, except during the rotations and when a typhoon struck Japan on 24 and 26–27 July.

2.3. Monitoring of climatic parameters and O$_3$ concentration

The air temperature and relative air humidity inside each chamber were continuously measured using a TR-72wf Thermo Recorder (T&D Corporation, Nagano, Japan). The photosynthetic photon flux density (PPFD) outside the chamber was continuously measured using a LI-190 Quantum Sensor (LI-COR, Lincoln, NE, USA). The O$_3$ concentration inside the chambers was monitored using an UV absorption O$_3$ monitor (Model 202 Ozone Monitor, 2B Technologies, Colorado, United States) and recorded at 30-sec intervals using a voltage recorder (VR-71, T&D Corporation). To measure the O$_3$ concentration inside the 12 chambers (four chambers assigned to FA and NF treatments and eight chambers assigned to NF+ and NF++ treatments), the air inside the chambers was sampled sequentially using an electric valve system for a period of 5 min, and was introduced into the O$_3$ monitor. The average concentration during the 5-min sampling period was used as the 1-h average concentration for each chamber. The 1-h average concentration was used to calculate the average concentration, the concentration accumulated over a threshold O$_3$ concentration of 40 nl l$^{-1}$ during daylight hours (daylight AOT40), and the sum of all hourly average concentrations above 60 nl l$^{-1}$ (SUM06) (Table 1). The accumulation period for daylight AOT40 and SUM06 was from 21 May to 18 August or 7 September.

Table 1. The concentration, SUM06, and daylight AOT40 of O$_3$ in each gas treatment during the O$_3$-exposure period.

<table>
<thead>
<tr>
<th>Period</th>
<th>Treatment</th>
<th>Concentration (nl 1$^{-1}$)</th>
<th>SUM06$^a$ (µl 1$^{-1}$ h)</th>
<th>AOT40$^a$ (µl 1$^{-1}$ h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24-h</td>
<td>12-h$^b$</td>
<td>8-h$^b$</td>
</tr>
<tr>
<td>21 May-18 Aug</td>
<td>FA</td>
<td>17.3 (1.0)</td>
<td>17.6 (0.9)</td>
<td>17.9 (0.9)</td>
</tr>
<tr>
<td></td>
<td>NF</td>
<td>32.4 (0.1)</td>
<td>35.8 (0.4)</td>
<td>37.8 (0.3)</td>
</tr>
<tr>
<td></td>
<td>NF+</td>
<td>51.1 (1.1)</td>
<td>54.7 (0.8)</td>
<td>57.3 (0.6)</td>
</tr>
<tr>
<td></td>
<td>NF++</td>
<td>66.3 (2.9)</td>
<td>67.1 (2.5)</td>
<td>68.2 (2.1)</td>
</tr>
<tr>
<td>21 May-7 Sep</td>
<td>FA</td>
<td>18.3 (0.9)</td>
<td>18.8 (0.8)</td>
<td>19.1 (0.8)</td>
</tr>
<tr>
<td></td>
<td>NF</td>
<td>32.5 (0.1)</td>
<td>36.1 (0.4)</td>
<td>38.2 (0.3)</td>
</tr>
<tr>
<td></td>
<td>NF+</td>
<td>51.9 (1.6)</td>
<td>55.6 (1.3)</td>
<td>58.5 (1.1)</td>
</tr>
<tr>
<td></td>
<td>NF++</td>
<td>66.8 (1.7)</td>
<td>68.1 (1.7)</td>
<td>69.3 (1.5)</td>
</tr>
</tbody>
</table>

FA: O$_3$ removal-filtered air; NF: Non-filtered air; NF+: NF plus additional O$_3$ at approximately 35 nl l$^{-1}$. Each value is the mean of two (for FA and NF) or four (for NF+ and NF+++) chamber replicates, and the standard deviation is shown in parentheses. $^{a}$24-h: 6:00–18:00; $^{b}$8-h: 9:00–17:00. $^a$SUM06: Sum of all hourly average concentrations above 60 nl l$^{-1}$. $^a$Daylight AOT40: Concentration accumulated over a threshold O$_3$ concentration of 40 nl l$^{-1}$ during daylight hours (photosynthetic photon flux density of >230 µmol m$^{-2}$ s$^{-1}$). The accumulation period for daylight AOT40 and SUM06 was from 21 May to 18 August or 7 September.
2.4. Measurement of pushing resistance

On 19 August, the 15 days after heading, the pushing resistances of all rice plants were measured. The heading date, which was defined as the 50% of the plants emerged the panicle, was 4 August. A digital force gauge (FG-5000A, FUSO Co. Ltd, Tokyo, Japan) was set perpendicularly at 10 cm from the bottom of the plant. Pushing resistance was measured when the plant was pushed to an angle of 45° from the vertical position, which reflects the resistance to root lodging (Terashima et al., 1992).

2.5. Measurement of whole-plant growth and root-to-shoot ratio

On 8 and 9 September (35 days after heading), all the rice plants were harvested to determine the whole-plant growth and dry mass ratio of roots to shoots (root-to-shoot ratio). The harvested plants were divided into panicle, leaf blade, leaf sheath, and root parts. The separated plant organs were dried in an oven at 80°C for 5 days and then weighed. Whole-plant dry mass was calculated as the sum of the dry masses of all plant organs. The root-to-shoot ratio was calculated as the ratio of root dry mass to the sum of the dry masses of the panicle, leaf blade, and leaf sheath.

2.6. Statistical analysis

The mean values of three pots in each chamber were used for the statistical analysis (n = 4 for each treatment). All statistical analyses were performed with IBM SPSS Advanced Statistics 19. Analysis of variance (ANOVA) was used to test the significance of O$_3$ effects on the growth and pushing resistance at $p < 0.05$. Because there were no significant interactions between O$_3$ and chamber replication, the chamber replication was classified into error term. When a significant effect of O$_3$ was observed, Tukey’s HSD test was used to clarify the significant difference among the four treatments ($p < 0.05$). The association of pushing resistance per panicle to root dry mass per panicle was analyzed by Pearson’s correlation test, and that to O$_3$ indices was analyzed by linear regression.

3. Results and discussion

During the exposure period from 21 May to 7 September 2015, average daily mean, daily maximum, and daily minimum air temperature inside the OTCs were 25.5°C, 30.2°C, and 22.2°C, respectively. Average daily mean and daily minimum relative air humidity during the exposure period inside the chambers were 77.2% and 56.8%, respectively. Average daily mean and daily maximum PPFD and highest 1-h value of PPFD outside the chambers during the period were 347, 1236, and 2024 μmol m$^{-2}$ s$^{-1}$.

The mean 24-h O$_3$ concentrations from the beginning of the exposure to 15 days after heading (when the pushing resistance was measured) in the FA, NF, NF+, and NF++ treatments were 17.3, 32.4, 51.1, and 66.3 nl l$^{-1}$, respectively (Table 1). Although the mean O$_3$ exclusion efficiency in the FA treatment was not so high (<45%), the AOT40 and SUM06 during the experimental period in the FA treatment was 0.0 μL l$^{-1}$ h, suggesting that the air provided to the plants was clean. The difference in the concentration between daytime (8-h) and daily (24-h) average was nonsignificant (Table 1). In general, however, the O$_3$ concentration is high during daytime and peaks in the afternoon, resulting in the much higher daytime average concentration of O$_3$ than the daily average concentration of O$_3$ (e.g., Akimoto et al., 2015). In Nagasaki, unlike the situation around the metropolis, the concentration of O$_3$ is strongly influenced by Asian continental outflow air masses and relatively high O$_3$ concentration is observed in the spring and autumn during both daytime and nighttime (e.g., Kanaya et al., 2016). As a result, the difference between daytime and daily average concentrations of O$_3$ in the present study site was less than that observed around the metropolis.

Table 2 shows the effects of O$_3$ on whole-plant dry mass, root-to-shoot ratio, panicle number per plant, and pushing resistance per plant. There were no significant effects of O$_3$ on the whole-plant dry mass, root-to-shoot ratio, and panicle number per plant. The pushing resistance per plant in the NF+ and NF++ treatments was significantly lower than that in the FA and NF treatments. Although the effect of O$_3$ on panicle number per plant was not statistically significant, the panicle number obviously affected the pushing resistance per plant and caused considerable variation in the resistance. Because of this, we evaluated the resistance to root lodging by pushing resistance per panicle. The pushing resistance per panicle in the NF++ treatment was significantly lower than that in the FA and NF treatments (Fig. 1). This result indicates that relatively high concentration of O$_3$ reduces the resistance of Japonica rice to root lodging. It is well

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole-plant dry mass (g)</th>
<th>Root/shoot ratio (g g$^{-1}$)</th>
<th>Panicle no. per plant (plant$^{-1}$)</th>
<th>Pushing resistance (N plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>27.9 (0.5)</td>
<td>0.172 (0.012)</td>
<td>5.8 (0.1)</td>
<td>9.59 (0.21) a</td>
</tr>
<tr>
<td>NF</td>
<td>28.0 (0.3)</td>
<td>0.164 (0.010)</td>
<td>6.0 (0.1)</td>
<td>9.60 (0.10) a</td>
</tr>
<tr>
<td>NF+</td>
<td>26.6 (0.6)</td>
<td>0.152 (0.005)</td>
<td>6.0 (0.1)</td>
<td>8.41 (0.24) b</td>
</tr>
<tr>
<td>NF++</td>
<td>26.5 (0.5)</td>
<td>0.143 (0.006)</td>
<td>6.0 (0.1)</td>
<td>7.53 (0.30) b</td>
</tr>
</tbody>
</table>

ANOVA: n.s., n.s., n.s., ***

FA: O$_3$ removal-filtered air; NF: Non-filtered air; NF+: NF plus additional O$_3$ at approximately 20 nl l$^{-1}$; NF++: NF plus additional O$_3$ at approximately 35 nl l$^{-1}$. Each value shows the mean of four chamber replications, and the standard error is shown in parentheses. ANOVA: ***p < 0.001, n.s. = not significant. Values with different letter indicate significant difference among the four treatments (Tukey’s HSD test, $p < 0.05$).
known that the $O_3$ accelerates plant senescence (e.g., Ainsworth et al., 2012). In the present study, the exposure to $O_3$ tended to accelerate heading and the heading date was one day earlier in the NF+ and NF++ treatments than in the FA and NF treatments (data not shown). Terashima et al. (1992) reported that the pushing resistance remained constant for about 20 days after heading, suggesting that the $O_3$-induced early senescence did not cause the $O_3$-induced reduction in the resistance to root lodging. On the contrary, the root dry mass per panicle in the NF++ treatment was significantly lower than that in the FA treatment (Fig. 1). Because the root lodging resistance of rice is related to root development (Terashima et al., 1994), the pushing resistance per panicle was plotted against the root dry mass per panicle (Fig. 1). As a result, the degree of $O_3$-induced reduction in pushing resistance per panicle correlated with that of $O_3$-induced reduction in root dry mass per panicle. These results indicate that the $O_3$-induced reduction in resistance to root lodging might have been caused by the reduction in root development from exposure to $O_3$.

The relative value of pushing resistance per panicle had significant negative linear relationships to the 24-h $O_3$ concentration and daylight AOT40 (Fig. 2). This result suggests that resistance to root lodging decreases with increasing $O_3$ concentration. Kobayashi et al. (1995) reported that the exposure to $O_3$ induced severe lodging of rice in the year when the conditions for the rice cultivation were conducive to lodging (e.g., low light intensity). Although there was no significant difference in the pushing resistance per panicle between the FA and NF treatments (Fig. 1), the negative linear relationship suggests that the exposure to ambient levels of $O_3$ could induce severe lodging when the condition for rice cultivation is conducive to lodging. In East Asia, furthermore, because the $O_3$ concentration is projected to increase in the near future (Wild et al., 2012), lodging issues in rice cultivation could become serious.

Yamaguchi et al. (2014) reported that $O_3$-induced yield losses of Japonica rice could be explained by exposure to $O_3$ during heading. Additionally, in the study of Sawada et al. (2016), the $O_3$-induced deterioration of Japonica rice grain quality occurred during the grain filling period. These results suggest that the $O_3$ concentration during the reproductive period is an important factor determining the yield loss and deterioration of grain quality. Conversely, because roots develop during the vegetative period, exposure to $O_3$ during the vegetative period could be related to the $O_3$-induced reduction in lodging resistance. In many cereals such as rice, lodging reduces canopy photosynthesis, crop yield, and mechanical harvesting efficiency (Setter et al., 1997; Berry et al., 2004). Therefore, $O_3$ levels during both the reproductive and vegetative periods should be considered when estimating the adverse effects of $O_3$ on rice productivity, including yield, grain quality, and lodging.

![Fig. 1. Relationship between pushing resistance per panicle and root dry mass per panicle. Circle: FA, Triangle: NF, Square: NF+, Cross: NF++. *p < 0.05. Different capital and lower-case letter above the plot indicates the significant difference among the four values in the pushing resistance per panicle and root dry mass per panicle, respectively (Tukey’s HSD test, $p < 0.05$).](image1)

![Fig. 2. Relationships between the relative value of pushing resistance per panicle and (a) 24-h average $O_3$ concentration and (b) daylight AOT40. The period for calculation of the $O_3$ indices was from the beginning of treatment to immediately before the measurement of pushing resistance (21 May–18 Aug 2015). Circle: FA, Triangle: NF, Square: NF+, Cross: NF++. **p < 0.01.](image2)
Acknowledgments

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References


