Flixweed Is More Competitive than Winter Wheat under Ozone Pollution: Evidences from Membrane Lipid Peroxidation, Antioxidant Enzymes and Biomass

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Abstract

To investigate the effects of ozone on winter wheat and flixweed under competition, two species were exposed to ambient, elevated and high [O3] for 30 days, planted singly or in mixculture. Eco-physiological responses were examined at different [O3] and fumigating time. Ozone reduced the contents of chlorophyll, increased the accumulation of H2O2 and malondialdehyde in both wheat and flixweed. The effects of competition on chlorophyll content of wheat emerged at elevated and high [O3], while that of flixweed emerged only at high [O3]. The increase of H2O2 and malondialdehyde of flixweed was less than that of wheat under the same condition. Antioxidant enzyme activities of wheat and flixweed were seriously depressed by perennial and serious treatment using O3. However, short-term and moderate fumigation increased the activities of SOD and POD of wheat, and CAT of flixweed. The expression levels of antioxidant enzymes related genes provided explanation for these results. Furthermore, the increase of CAT expression of flixweed was much higher than that of SOD and POD expression of wheat. Ozone and competition resulted in significant reductions in biomass and grain yield in both winter wheat and flixweed. However, the negative effects on flixweed were less than wheat. Our results demonstrated that winter wheat is more sensitive to O3 and competition than flixweed, providing valuable data for further investigation on responses of winter wheat to ozone pollution, in particular combined with species competition.

Introduction

Atmosphere ozone (O3) at ground level is one of the most serious air pollutants, which negatively affects crop growth and yield [1]. The tropospheric [O3] is predicted to rise globally by 40–60% by 2100 as a consequence of rapid economic development [2], suggesting O3 pollution under global change scenarios will become more serious. The potential impact of elevated O3 on agricultural productivity is particularly relevant in the south and East Asian region. For instance, it is estimated that by 2020 current day, the yield losses due to O3 damage will rise from current 13% and 23%, to 16% and 35%, respectively for cereals and soybean productions of China [3,4]. Economically, annual crop losses induced by O3 are estimated at $3–5.5 billion in China, and will likely increase by 16%–110% in the near future [5,6]. These predictions suggest that China in particular might be on the cusp of substantial reductions in grain production following the rapid economic transformation, industrialization and urbanization [7].

Among major food crops, wheat is believed to be the most sensitive to O3 [8]. Such a phenomenon has attracted attentions by a number of scientists from the view of food security [9,10]. Previous studies have found that O3 negatively influence the growth and development of plants [11,12]. Acute and chronic exposure to O3 can pressurize plants to bring a series of changes, such as appearances of visible symptoms, premature senescence, yield losses and alterations of plant community structure [13]. It is well known that ozone affects primarily growth of plant by producing reactive oxygen species (ROS), which severely compromise the integrity of metabolically important membranes [14]. The concept that antioxidant enzymes act as a barrier against toxic oxygen derivatives, as initially suggested in the 1970s, promise the integrity of metabolically important membranes [14]. Therefore, antioxidant enzymes which have the ability of detoxifying ROS, plays important roles in engendering tolerance to O3 in different species [16].

Formerly, most of the researches revealed the response of plants to O3 at the level of individual species. There are few investigations on the indirect impact of O3 pollution on growth and yield performance under the competition from weed. Flixweed (Descurainia Sophia) is the most troublesome annual weed, widely occurred in the major wheat planting regions of China. It is a vigorous competitor with prolific seed production, with the ability to quickly invade wheat fields. More seriously, it has
developed strong resistance to herbicides, becoming a great threat to wheat production [17,18]. The presence of weeds with other crops decreases grain yields owing to competition for light, moisture and minerals [19]. Allelopathic functioning from weeds is another important factor to cause detrimental effect on the growth of the crops by releasing allelochemicals into the ambient environment [20,21].

From the above mentioned introduction, winter wheat confronts not only O₃ stress under global climate change scenarios, but also the threat caused by weeds such as flixweed. Li et al. (1999) have found that the relative competitive status of wheat competing with wild oat decreased grain production under UV-B enhancement [22]. It has been well documented that competition is an important factor affecting plant responses to ozone stress [23]. Nevertheless, we never know the performing patterns of competition from wheat and weeds under O₃ pollution. Therefore, it is urgent to assess the interactive effect on winter wheat and flixweed under O₃ stress and competition.

In the present study, we investigated the effects of O₃ on winter wheat and flixweed under competition. The objectives of this investigation are: 1) to determine the effects of O₃ on growth and yield of winter wheat and flixweed; 2) to investigate the physiological mechanism of their responses to O₃ and competition. This research may provide some valuable information for developing the necessary weed management strategies and theoretical foundation for future wheat breeding.

Materials and Methods

Plant culture and O₃ fumigation

Winter wheat (T. aestivum, cv Liangxing99) and flixweed (Descurainia Sophia) were selected in the experiment. Flixweed grows as a common and competitive weed occurring in wheat fields of North China, with a growth season icing from October to May. On 12 October 2010, 15 seeds of winter wheat or 15 seeds of flixweed were sowed in each pot, with total of 54 plastic pots (25 cm in diameter, 28 cm in height). For the competition experiment, 15 wheat seeds together with 15 seeds of flixweed were planted in each of the 54 plastic pots. Pots were all filled with clay soil containing organic C, total N, available P and K at the rate of 1.3 g/kg, 0.73 g/kg, 67 mg/kg, and 157 mg/kg, respectively. No chemical fertilizers were applied either as basal or topdressing. Monocultures of winter wheat and flixweed were both thinned to ten per pot after their emergence. Mixcultures had ten wheat seedlings and ten flixweed seedlings per pot.

On 1 April 2011, six pots of either monoculture or mixculture were moved to each of nine open-top chambers (OTC, 2.6 m in diameter, 2.4 m in height) built in the open field. Plants were allowed to adapt to chamber environments for 7 days before O₃ exposure. During this adaptation period, all plants received ambient air with an O₃ concentration of less than 40 ppb. The gas dispensing system of the OTCs was conducted according to Uprety (1998) [24]. Ozone was added to the open air entering three of the chambers to maintain an O₃ concentration of 80±5 ppb for 7 h day⁻¹ (10:00−17:00 hours) for 30 days. Another three chambers were set up the same way but with higher O₃ concentration of 120±10 ppb. Meanwhile, three chambers were ventilated with ambient air as the control. The injected O₃ was quickly frozen in liquid nitrogen then transferred to an ultra-freezer at −80°C until the time of assay.

Plant sampling

After one day of O₃ fumigation, the most recently expanded leaves on the main stem of wheat and flixweed from differential treatments were collected from each chamber per treatment for biochemical measurements. Then the most recently expanded leaves were sampled every ten days. Leaf samples were immediately frozen in liquid nitrogen then transferred to an ultra-freezer at −80°C until the time of assay.

Chlorophyll content

Chlorophyll was extracted from fresh leaf samples (0.2 g) in 95% ethanol in the dark for 4 h at 4°C. The extract was then assayed for chlorophyll with the absorption spectra provided by Arnon (1949) [25].

Determination of H₂O₂ and malondialdehyde (MDA)

Hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) in leaves were measured to assess the effect of O₃ on oxidative damage of wheat and flixweed. H₂O₂ content was measured according to the method described by Alexieva et al. (2001) [26]. MDA was determined according to the method of Kramer et al. (1991) [27].

Determination of the activity of antioxidant enzymes

Leaf samples (0.5 g) were homogenized in a precooled mortar and pestle placed on ice with 3 mL 0.1 M potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at 1,200 g for 20 min at 4°C and the supernatant was used to determine enzymes activities.

Superoxide dismutase (SOD) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium described by Giannopolitis and Ries (1977) [28]. One unit of SOD is defined as the amount of enzyme that inhibited the nitroblue tetrazolium reduction by 50%. Catalase (CAT) activity was assayed using the method of Aebi (1984) [29]. Unit of CAT activity was defined as variation of absorbance per minute per gram total protein. Peroxidase (POD) was determined through measuring the oxidation of guaiacol [30]. Total soluble protein was measured by the method of coomassie brilliant blue [31].

Expression patterns of antioxidant enzyme related genes

The response of genes to environmental change was much quicker than physiological reaction, so the expression of antioxidant enzyme related genes were determined after one day exposure by real-time quantitative PCR (RT-qPCR). Total RNAs were extracted from harvested leaves of wheat and flixweed with Trizol reagent (Invitrogen) after one day fumigating. The total RNAs were reversely transcribed into first-strand cDNA with PrimeScript® RT reagent Kit With gDNA Eraser (TaKaRa), and the cDNAs obtained were used as templates for PCR amplification with specific primers. The full genome of flixweed haven’t been sequenced, primers of flixweed were designed according to Arabidopsis thaliana which is in the same family—Cruciferae. Gene-specific primers used for RT-qPCR were: 5’-TTT TAG GTC GCT GGT TTC-3’ and 5’-CCA AGT CCA CGG TTC ATA G-
3’ for *TaSOD* (U69536.1); 5’-AGT TGG ACG GAT GGT ACT GA-3’ and 5’-AAG ACG GTG CCT TTG GGT-3’ for *TaCAT* (X94352.1); 5’-GAC GCC TGA ATG GTT GAA-3’ and 5’-AAT GCC TTC TGCG TCC TCT-3’ for *TaAPX* (AF387739.1); 5’-AAC TAC CGG CTC TTC TGC TGC-3’ and 5’-GCC TTC GGC TGT GTG-3’ for *TaGPX* (AJ104553.1); 5’-TGA AGA GTC GAG GTG-3’ and 5’-GAG TTG GGT CCT CTA AGA GG-3’ for *DcSOD* (NM_100757.3); 5’-CTC TTC CCT CAC CAT CCG-3’ and 5’-TGG AGA ACG GGA CAA TAA CG-3’ for *DcCAT* (NM_001035995.3); 5’-GGT CCG ATG GGA CTC AAT-3’ and 5’-AGC GCC TTG GCT GTG GGT-3’ for *DcAPX* (NM_111798.3); 5’-GGT GGA TGT GGA CCG TAA G-3’ and 5’-CCA ACG CAG TAT GAA TGA CTT-3’ for *DcGPX* (NM_128714.3). In addition, *Actin* was used as internal control: 5’-CTA TTC TTT TCG TGT ACG TT-3’ and 5’-AGC GAT CCT TTC TTT ATG TAT-3’ for *TakActin* (AB181099.1); 5’-TGT TCT TCC CCT CTG GCA TTA GGG G-3’ and 5’-GAT TAG TCC CTT CAC TGG TTT CGG T-3’ for *DcActin* (NM_112046.3). RT-qPCR was performed using Stratagene MX3000P™ instrument. Each reaction contained 7.5 μl 2×SYBR Green Master Mix reagent (*TaKara*), 0.5 μl cDNA samples, 0.6 μl 10 mM gene-specific primers and 0.3 μl 50×ROX in a final volume of 15 μl. The thermal cycle was used as follows: 95°C for 2 min; 40 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s. The relative expression level was analyzed by the comparative Ct method and the value of *Actin* was normalized to one.

Biomass and grain yield

Five plants per pot for each species and each treatment (n = 15) were harvested to determine the biomass and grain yield. Plants were dried to constant weight in an oven at 72°C. Grains were removed from each ear or pod by hand. Yield per plant was determined for sundried seeds.

Statistical analysis

The experiment was arranged as a split plot with three replications. O3 concentration levels (ambient [O3], 10 ppb, high [O3] 120 ppb) represent the subplot. One-way ANOVA of SPSS package (Ver. 17, SPSS, Chicago, USA) with Tukey-Kramer multiple comparison tests were performed to test for significant differences between the control and other treatments. Differences among treatments were considered significant if *P* ≤ 0.05.

Results

Chlorophyll content

As shown in Figure 1A, at the absence or presence of flixweed, the chlorophyll contents of wheat decreased in varying degrees compared with those growing singly in ambient air (AS). On the second day of O3 exposure, the contents of chlorophyll were significantly decreased by 18%, 23%, 31% and 36% for wheat planted singly in elevated [O3] (ES), planted in mixture in elevated [O3] (EM), planted singly in high [O3] (HS) and planted in mixture in high [O3] (HM) against AS, respectively (*P* ≤ 0.01, Figure 1A). However, there were no significant differences between those planted in mixture in ambient air (AM) and AS treatments. Along with the exposure time, the adverse effect induced by O3 was enforced gradually.

The present of O3 also reduced the chlorophyll contents of flixweed. At the end of exposure, elevated [O3] (80 ppb) significantly reduced the chlorophyll content of flixweed by 27% and 31%, respectively for ES and EM treatments against AS (*P* ≤ 0.05, Figure 1B). While in the case of the high [O3] (120 ppb), the chlorophyll content was dramatically being 30% and 52% lower in HS and HM than that of AS (*P* ≤ 0.01, Figure 1B).

The adverse effects of O3 on chlorophyll content were obvious for both of winter wheat and flixweed, as well as competition existed in mixculture treatments. However, the effects of competition on chlorophyll content of wheat emerged at elevated and high [O3], while that of flixweed emerged only at high [O3].

H2O2 and MDA contents

Hydrogen peroxide (H2O2) is one kind of ROS induced by adverse stress. Ozone significantly increased H2O2 content both in winter wheat and flixweed leaves (Figure 2A, B). For winter wheat, at the end of exposure period, the H2O2 contents were notably increased by 17%, 19%, 20%, 22% and 40%, respectively for AM, ES, EM, HS and HM against AS (Figure 2A). Nevertheless, in absence or presence of wheat, the increase of H2O2 contents from flixweed leaves was only found to be significant in EM (10%), HS (11%) and HM (21%) treatments against AS (*P* ≤ 0.05, Figure 2B). Meanwhile, species competition between wheat and flixweed growing in mixculture, significantly enhanced the content of H2O2 for both wheat and flixweed at high [O3] (*P* ≤ 0.05).

Increase of malondialdehyde (MDA) content, which has been widely recognized as a parameter for lipid peroxidation, was observed in wheat plants exposed to O3 pollution (Figure 2C). Compared with the AS treatment, O3 significantly enhanced MDA contents by approximately 50%, 150%, 200%, and 300% respectively for ES, EM, HS and HM at the end of exposure. Similar change tendency of MDA content was detected in flixweed plants. MDA contents were notably increased by 78%, 102%, 138% and 160% for ES, EM, HS and HM relative to AS in flixweed (Figure 2D). However, the increase of flixweed was less than that of wheat under the same condition.

Antioxidant enzymes activity

To investigate the response of antioxidant system to O3 and explain the physiological mechanisms by which plants accumulate different H2O2 and MDA under O3 fumigation, we continually monitored the dynamic patterns in the activities of SOD, CAT and POD, which are the key enzymes responsible for removing ROS induced by oxidative stress. For wheat, O3 significantly affected the activities of SOD, CAT, and POD in varying degrees during the 30 exposure days (Figure 3). As shown in Figure 3A, the SOD activity of wheat elevated at the beginning and declined afterward with the increasing [O3]. The activity of POD expressed a similar trend after fumigation of one day (Figure 3E). However, CAT activity was significantly depressed at each O3 level (*P* ≤ 0.05, Figure 3C).

In the absence or presence of wheat, the activity of CAT in flixweed increased at elevated [O3], but reduced at high [O3] during the whole fumigating phase (Figure 3D). The activities of SOD and POD displayed a downward trend along with the time of fumigation (Figure 3B, F).

Expression of antioxidant enzymes related genes

In order to give an accurate explanation of the changing trend of antioxidant enzymes activities, the effect of ozone on the expression level of antioxidant enzymes related genes were examined at transcriptional levels by real-time qPCR after one day of O3 exposure. As shown in Figure 4A, elevated [O3] up-regulated the expression of SOD and GPX related genes in winter wheat. Meanwhile, competition from weed depressed them. For flixweed, O3 significantly up-regulated the transcript level of CAT related gene by 8.5 or 3.2 folds, respectively for ES and HS.
Figure 1. Effects of different ozone concentrations on chlorophyll contents. Contents of chlorophyll in leaves of winter wheat and flixweed were shown in panel A and B, respectively. AS and AM represent winter wheat or flixweed grown singly and in mixture in ambient air, respectively. ES and EM represent winter wheat or flixweed grown singly and in mixture in elevated O₃ concentration, respectively. HS and HM are winter wheat or flixweed planted singly and in mixture in high O₃ concentration, respectively. Error bar indicates SE. n = 9. T bars with different letters are significantly different between treatments at P≤0.05 at the same sampling time.

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Figure 2 Effects of ozone on hydrogen peroxide and malondialdehyde contents. H₂O₂ contents in leaves of wheat and flixweed were shown in panel A and B, respectively. Malondialdehyde contents in leaves of wheat and flixweed were shown in panel C and D, respectively. Columns with error bars are mean±SE of six replicates. T bars with different letters are significantly different between treatments at P≤0.05 at the same sampling time.

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treatments. The effects of competition on the transcript level of antioxidant enzymes related genes in flixweed were similar as wheat (Figure 4B).

**Biomass and grain yield**

Ozone exposure significantly depressed the biomass of winter wheat by 12%, 24%, 23% and 37% respectively for ES, EM, HS and HM, against AS treatment ($P \leq 0.05$, Figure 5A). For wheat growing without competition from flixweed, grain yield was notably reduced by 19% and 40% at elevated [O$_3$] (80 ppb) and high [O$_3$] (120 ppb), respectively. However, for those growing with flixweed, the grain yield decreased by 31% and 50% at elevated [O$_3$] and high [O$_3$], respectively against AS ($P \leq 0.05$, Figure 5C).

In the absence or presence of wheat, the biomass and grain yield of flixweed significantly declined with the increasing [O$_3$] concentration (Figure 5B, D). Against AS treatment, the biomass of flixweed was reduced by 13%, 16%, 19% and 25%, respectively for ES, EM, HS and HM. Grain yield showed similar tendency as biomass (Figure 5D).

Figure 3. Effects of ozone on activities of superoxide dismutase, catalase and peroxidase. Activities of SOD, CAT and POD of winter wheat were shown in panel A, C and E, respectively. These of flixweed were shown in panel B, D and F, respectively. Error bar indicates SE. $n = 9$. T bars with different letters are significantly different between treatments at $P \leq 0.05$ at the same sampling time.
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Discussion

Differences in contents of chlorophyll, H$_2$O$_2$ and MDA under O$_3$ and competition

Ozone-induced oxidative stress not only decreases chlorophyll synthesis but also decomposes the original chlorophyll in plant tissues [32]. In the present study, chlorophyll contents of both winter wheat and flixweed significantly declined under the conditions of both elevated [O$_3$] and high [O$_3$] (Figure 1). It is the typical symbol of O$_3$ impacts on leaf pigmentation in plants as documented by previous investigations [16,33]. However, the reduction in flixweed was less than wheat under the same treatment, suggesting flixweed was more tolerant to O$_3$ than wheat. On the other hand, the effects of competition on chlorophyll content of wheat emerged at elevated and high [O$_3$], while that of flixweed emerged only at high [O$_3$], implying the effects of competition on wheat was more obvious than flixweed. Great reductions in chlorophyll contents are likely to decrease net photosynthetic rate, and eventually leads to biomass losses (Figure 5).

Once entering into aqueous solutions of protoplasm, ozone will decompose to reactive oxygen species like hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2$) and hydroxyl radicals (‘OH) [34]. In addition, ozone is well known to affect the plasma function by disorganizing the membrane structure and altering membrane permeability through lipid peroxidation [35]. In the present study, H$_2$O$_2$ and MDA accumulation were drastically enhanced by O$_3$ pollution (Figure 2), indicating that O$_3$ intensified accumulation of ROS induced by oxidative stress and degree of lipid peroxidation of leaf issue membrane [14,36]. Nevertheless, the competition aggravated the effects of oxidative stress. Although wheat or flixweed is not directly involved in producing oxidative stress, it might be correlated with the generation of ROS because of strong competition for water and nutrient resources. Even though the biochemical indicators showed similar tendency, winter wheat exhibited greater growth potential in H$_2$O$_2$ and MDA accumulation due to its high susceptibility to O$_3$ [9].

Differences in the antioxidant activities and expression levels of antioxidant enzymes related genes

The activities of antioxidant enzymes can be considered as a parameter of tolerance of oxidative stress. We found that O$_3$ exposure caused remarkable changes activities of SOD, CAT and POD in both winter wheat and flixweed after one day exposure (Figure 3). A well-known mechanism to suppress excessive production of ROS is through the SOD which converts superoxide radicals into H$_2$O$_2$ and molecular oxygen. H$_2$O$_2$ can be further neutralized by organelle specific CAT [37]. Meanwhile, CAT metabolizes H$_2$O$_2$ into water and O$_2$, while POD decomposes H$_2$O$_2$ by oxidation of co-substrates such as phenolic compounds and/or antioxidants. For winter wheat, activities of SOD and POD were stimulated in elevated [O$_3$] (80 ppb), but decreased in high [O$_3$] (120 ppb) after one day exposure (Figure 3A, E). These results indicated that the O$_3$-induced accumulation of ROS triggered the activities of SOD and POD in wheat exposed to O$_3$ at 80 ppb, which were considered to be ROS scavenging enzymes. On one hand, the production of large amounts of ROS might lead to the reduction in the activities of SOD and POD in wheat exposed to O$_3$ at 120 ppb. On the other hand, the activity of CAT in winter wheat was significantly
reduced by the exposure to O3 at either 80 or 120 ppb, which was coincident with that obtained in the previous study on two Japanese winter wheat cultivars [38]. To explore the mechanisms of antioxidant varieties under ozone pollution, we investigated the expression levels of related genes that are known to be responsible for enzyme production. According to the transcript levels of antioxidant enzymes related genes, associated with the synthesis of these enzymes, SOD and POD positively resisted the damage induced by O3 stress (Figure 4A). Wherefore, they are much more sensitive to O3 and might play more important role in eliminating ROS induced by O3 stress than CAT in winter wheat.

Differential exhibitions of antioxidant enzymes were obvious in flixweed, the common weed in wheat fields. Although the activities of SOD and POD were not significantly different between O3 and control treatment after one day exposure (Figure 3B, F), CAT was dramatically inactivated to scavenging ROS induced by O3 stress. The expression of CAT related gene was also significantly up-regulated in O3 treatments as compared with the control, which could provide explanation to the variable trend of CAT activity (Figure 4B). Furthermore, the increased expression of CAT related gene in flixweed was much higher than that of SOD and POD. This result suggested that CAT is more sensitive to oxidative stress induced by O3 and might play more important role in removing H2O2 for flixweed. With the increasing exposure time, antioxidant enzymes activities decreased gradually in both wheat and flixweed. This study revealed that antioxidant activities were developmentally regulated and markedly affected by O3 [39]. Such responses, showed a course of dynamic change under O3 from short-term moderate treatment to perennial severe treatment.

Biomass and yield in response to O3 and competition

Ozone generates reactive oxygen species (ROS), therefore accelerates lipid peroxidation, photosynthetic pigment decomposition, and decreases CO2 assimilation and biomass accumulation [40]. The O3-induced reduction in biomass has been reported for a wide range of crop species, such as soybean, rice, cotton and wheat [14,41–43]. However, little is known about how those species respond to O3 pollution when weed competes with crops. In this study, winter wheat (growing either singly or in mixture) displayed less dry biomass, and grain yield under O3 treatments against the control. Such effects may be owing to a reduction in net photosynthesis, alterations in the source-sink balance and assimilate partitioning [42,44,45]. We found that wheat grain yield was strongly affected by both elevated [O3] and high [O3] (Figure 5), which was similar to the results of many reports [14,46]. Other side, the biomass and grain yield of flixweed were also reduced by O3 pollution (Figure 5B, D), however, they were much less than that of wheat. Ozone not only depressed the growth and development of wheat and weed, but also altered the competitive pattern between wheat and flixweed, being in favor of flixweed. The competition effects of wheat on biomass and grain yield emerged significantly under both elevated [O3] and high [O3], but that of flixweed emerged only under the high [O3]. Alterations of the competitive balance expressed by total biomass and grain yield were similar to Li et al. (1999) [22], who argued that higher
antioxidant capacity and strong survivability to O₃ were the main causes of stress adaption and species competition superiority. In many weedy species, short life cycles, as well as prolific production and dispersal, will accelerate adaptation to higher ambient O₃ concentration [33]. From the above mentioned findings, we believe that the higher negative response of wheat is due to the competition co-existed between wheat and flixweed, and to higher susceptibility of wheat to O₃ stress during the seed filling stage [47].

Under the normal condition, the competition between the weed and wheat was effective, and the weed was more competitive than the wheat. Moreover, under the O₃ fumigation, the loss of wheat caused by the competition was more than that of the weed compared to the normal condition (Figure 5). Therefore, there were interaction between ozone pollution and competition, and ozone intensified the adverse effect of competition on plants.

For the past decades, yield of wheat cultivars has been improved by a great degree at the expenses of their resistances to environmental stress [14,43]. Consequently, other factors were overlooked in the process of crop breeding, such as O₃ pollution, competition from weeds and so on. Actually, competition has been demonstrated to be an important factor affecting plant responses to O₃ stress [23]. In the agro-ecosystem, wheat production is not only confronted with O₃ pollution but also competition from weed, which has exhibited moderate to high levels of tribenuron resistance [17]. Therefore, adversity resistance should be fully considered during wheat breeding as well as high yield and quality.

In summary, adverse effects of O₃ pollution on winter wheat and weed (flixweed) have been clearly found under both competition and non-competition conditions. And these effects differed from winter wheat and flixweed, which may also cause long term changes in competition pattern by disadvantaging winter wheat more than weed. Flixweed has stronger resistance to O₃ and is more competitive than winter wheat due to its stronger adaptability to environmental stresses. More effective weed management strategies and wheat breeding with adversity resistance should be developed in order to maintain ideal wheat production.

Supporting Information

Figure S1 The mean daily temperature and mean monthly precipitation of 2011 at the experimental site. (TIF)

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Author Contributions

Conceived and designed the experiments: CHL TZW GMJ. Performed the experiments: CHL TZW. Analyzed the data: CHL TZW YL YHZ GMJ. Wrote the paper: CHL TZW GMJ.

References

1. Wang X, Zheng Q, Yao F, Chen Z, Feng Z, et al. (2007) Assessing the impact of ambient ozone on growth and yield of a rice (Oryza sativa L.) and a wheat (Triticum aestivum L.) cultivar grown in the Yangtze Delta, China, using three rates of application of ethyleneurea (EDU). Environ Pollut 148: 390–395.

2. Wang G, Mills G, Hayes P, Wilkinson S, Cooper D, et al. (2012) Reduced soil water availability did not protect two competing grassland species from the negative effects of increasing background ozone. Environ Pollut 165: 91–99.

3. Aunan K, Berntsen TK, Seip HM (2000) Surface ozone in China and its possible impact on agricultural crop yields. Ambio 29: 294–301.

4. Wang XP, Mauzerall DL (2004) Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. Atmos Envir 43: 4303–4402.

5. Galanti A, Koester RP, Ainsworth EA, Hicks LM, Jez JM (2012) From climate change to molecular response: redox proteomics of ozone-induced responses in soybean. New Phytol 194: 220–229.

6. Van Dingenen R, Dentener FJ, Raes F, Krol MC, Emberson L, et al. (2009) The global impact of ozone on agricultural crop yields under current and future air quality legislation. Atmos Environ 43: 604–618.

7. Agrawal M (2003) Effects of air pollution on agriculture: An issue of national concern. Nari Acad Sci Lett 28: 93–106.

8. Herberger K, Tausz M, Wensrich A, Soja G, Sorger A, et al. (2002) Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiol Biochem 40: 691–696.

9. Emerson LD, Bueker P, Ashmore MR, Mills G, Jackson LS, et al. (2009) A comparison of North American and Asian exposure-response data for ozone effects on crop yields. Atmos Environ 43: 1945–1953.

10. Long SP (2012) Virtual Special Issue on food security - greater than anticipated impacts of near-term global atmospheric change on rice and wheat. Global Change Biol 18: 1409–1490.

11. Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. Plant Cell Environ 28: 949–964.

12. Jin MH, Feng ZW, Zhang FZ (2001) Impacts of ozone on the biomass and yield of rice in open-top chambers. J Environ Sci 13: 233–236.

13. Krupa S, McGrath MT, Andersen CP, Booker FL, Burkey KO, et al. (2001) Interactive effects of drought and ozone stress on the antioxidant defence systems of the higher plant Chenopodium rubrum. New Phytol 149: 381–389.

14. Uprety DC (1998) Carbon dioxide enrichment technology: Open top chambers - a new tool for global climate research. J Sci Ind Res 57: 266–270.

15. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24: 1–15.

16. Avela K, Raunio K, Wilén K, Puolanne E, Pukkala E, et al. (2006) The impact of ozone on growth, yield and water use efficiency in soybean. New Phytol 171: 51–63.

17. Liu Y, Yue M, Wang XL, Hu ZD (1999) Competition and sensitivity of wheat and wild oat exposed to enhanced UV-B radiation at different densities under field conditions. Environ Exp Bot 41: 47–55.

18.Novak K, Schaub M, Fuhrer J, Shelly JM, Frey B, et al. (2008) Ozone effects on visible foliar injury and growth of Fagus sylvatica and Vehurnum lantana seedlings grown in monoculture or in mixture. Environ Exp Bot 62: 212–220.

19. Updegraff DG (1998) Carbon dioxide enrichment technology: Open top chambers - a new tool for global climate research. J Sci Ind Res 57: 266–270.

20. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24: 1–15.

21. Alexieva V, Spera J, Mappelli S, Karanos E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 14: 1337–1344.

22. Kramer GF, Norman HA, Krizek DT, Mirecek RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry 30: 2101–2108.

23. Giampolitico CN, Ries SK (1977) Superoxide dismutases.1. occurrence in higher-plants. Plant Physiol 59: 309–314.

24. Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. Plant Cell Environ 28: 949–964.

25. Liu Y, Yue M, Wang XL, Hu ZD (1999) Competition and sensitivity of wheat and wild oat exposed to enhanced UV-B radiation at different densities under field conditions. Environ Exp Bot 41: 47–55.

26. Novak K, Schaub M, Fuhrer J, Shelly JM, Frey B, et al. (2008) Ozone effects on visible foliar injury and growth of Fagus sylvatica and Vehurnum lantana seedlings grown in monoculture or in mixture. Environ Exp Bot 62: 212–220.

27. Kramer GF, Norman HA, Krizek DT, Mirecek RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry 30: 2101–2108.

28. Giampolitico CN, Ries SK (1977) Superoxide dismutases.1. occurrence in higher-plants. Plant Physiol 59: 309–314.

29. Ashel H (1984) Catalase in vitro. Methods Enzymol 105: 121–126.

30. Rao MV, Paliyath G, Ormrod DP (1996) Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of Arabidopsis thaliana. Plant Physiol 110: 310–316.

31. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

32. Stepien P, Klobus G (2005) Antioxidant defense in the leaves of C3 and C4 plants under salinity stress. Physiol Plantarum 125: 31–40.

33. Shrestha A, Grantz DA (2005) Ozone impacts on competition between tomato and yellow mustard. Crop Sci 45: 1587–1595.
34. Laisk A, Kull O, Moldau H (1989) Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiol 90: 1163–1167.
35. Calatayud A, Iglesias DJ, Talon M, Barreno E (2003) Effects of 2-month ozone exposure in spinach leaves on photosynthesis, antioxidant systems and lipid peroxidation. Plant Physiol Biochem 41: 639–645.
36. Andersen CP (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157: 213–221.
37. Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141: 391–396.
38. Inada H, Kondo T, Akhtar N, Hosino D, Yamaguchi M, et al. (2012) Relationship between cultivar difference in the sensitivity of net photosynthesis to ozone and reactive oxygen species scavenging system in Japanese winter wheat (Triticum aestivum). Physiol Plantarum 146: 217–227.
39. Bernardi R, Nali C, Ginestri P, Pugliesi C, Lorenzini G, et al. (2004) Antioxidant enzyme isoforms on gels in two poplar clones differing in sensitivity after exposure to ozone. Biol Plantarum 48: 41–48.
40. Fiscus EL, Booker FL, Burkey KO (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell Environ 28: 997–1011.
41. Grantz DA (2003) Ozone impacts on cotton: towards an integrated mechanism. Environ Pollut 126: 331–344.
42. Morgan PB, Mies TA, Bollero GA, Nelson RL, Long SP (2006) Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytol 170: 333–343.
43. Biswas DK, Xu H, Li YG, Liu MZ, Chen YH, et al. (2008) Assessing the genetic relatedness of higher ozone sensitivity of modern wheat to its wild and cultivated progenitors/relatives. J Exp Bot 59: 951–963.
44. Crous KY, Vandermeiren K, Ceulemans R (2006) Physiological responses to cumulative ozone uptake in two white clover (Trifolium repens L. cv. Regal) clones with different ozone sensitivity. Environ Exp Bot 58: 169–179.
45. Demmoldy O, Long SP, DeLucia EH (2006) How does elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently? New Phytol 169: 145–155.
46. Pleijel H, Uddling J (2012) Yield vs. Quality trade-offs for wheat in response to carbon dioxide and ozone. Global Change Biol 18: 596–605.
47. Tingey DT, Rodecap KD, Lee EH, Hogsett WE, Gregg JW (2002) Pod development increases the ozone sensitivity of Phaseolus vulgaris. Water Air Soil Pollut 139: 325–341.