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Effects of ozone pollution on yield and quality of winter wheat under flixweed competition

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ABSTRACT

The concentration of ozone (O_3) is believed to continually increase due to anthrogenic activity, which might affect severely the growth and grain quality of winter wheat (*Triticum aestivum*). Flixweed (*Descurainia sophia*) is one of the most troublesome annual dicot weeds in wheat fields. As a vigourous competitor, the presence of flixweed in wheat field is a major factor to reduce wheat production. However, few studies have investigated the effects of ozone on wheat growth and yield under competition with flixweed. In the present study, physiological and ecological responses of wheat to O_3 stress with different density of flixweed competition were reported. Chlorophyll concentration and leaf area were reduced by elevated O_3. Gas exchange of wheat was suppressed by both elevated O_3 and the presence of flixweed. Moreover, exposure of wheat to elevated O_3 induced accumulation of Proline due to the up-regulation of *pyrroline-5-carboxylate synthetase* at transcriptional level, suggesting that Proline may confer tolerance to oxidative stress induced by O_3 pollution. Finally, elevated O_3 had significant adverse impacts on wheat above-ground biomass, grain yield and harvest index, and these impacts were intensified by the presence of flixweed. Exposure of wheat to elevated O_3 led to a significant increase in crude protein concentration of grain, while decreases in mineral element concentrations of grain were observed. Our findings indicate that effects of ozone and flixweed competition on winter wheat should be considered for the future breeding and cultivation.

1. Introduction

The near ground atmospheric ozone (O_3) is a deleterious secondary air pollutant produced by complex atmospheric reactions. The nitrous oxides, carbon monoxide, and volatile organic compounds contributed to O_3 formation. O_3 concentration at ground level has approximately doubled since the beginning of the industrial revolution, and it is believed to continually increase in future (Akimoto, 2003; Morgan et al., 2006). Being the most significant air pollutant, the damaging effects of O_3 on carbon assimilation, stomatal conductance, and plant growth feed forward to reduce crop yields (Ainsworth, 2008; Biswas et al., 2008; Emberson et al., 2009). Numerous regions of the globe are now suffering from O_3-induced crops yield loss, with economic costs reaching several billion dollars per annum in regions such as the US, EU and East Asia (Sitch et al., 2007).

Wheat (*Triticum aestivum*) is one of the most important crops worldwide, billions of people in the world rely on it as their primary staple food. However, wheat is also known to be one of the most O_3-sensitive crops as shown in plentiful fumigation experiments (Sarkar and Agrawal, 2010; Wahid, 2006). Previous studies have found that O_3 pollution has negative impacts on the growth and development of plants (Ashmore, 2005; Jin et al., 2001). Ozone primarily enters plants through the stomata where it can further react in the apoplastic liquid. It can directly react with the plasmalemma through ozonolysis and also be converted into reactive oxygen species (ROS), which damage cell membranes, leading to cell death and leaf senescence (Fiscus et al., 2005). Attempts have been made to breeding crops with greater capacity for ROS scavenging (Ainsworth et al., 2012). However, modifications in metabolic responses of plants under O_3 stress are a complex process with energy consumption, which may in turn suppresses growth and leads to yield losses (Singh et al., 2010; Wang et al., 2012).

Plant growth and development are also frequently affected by other biotic and abiotic factors. Numerous studies have demonstrated that several environmental factors can interact with O_3 to

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affect growth, yield and quality of crops (Wang et al., 2007; Pleijel and Uddling, 2012; Zheng et al., 2012). In addition to the environmental factors, weed is also an important factor to reduce crop yield and production by competing resources with crops. For example, it has been reported that the presence of weeds in the crop fields reduces grain yields owing to competition for light, moisture and mineral nutrients (Woldeamlak et al., 2001; Xu et al., 2010). Flixweed (*Descurainia sophia*) is the most troublesome annual weed, widely occurs in the major wheat planting regions in China. It has developed strong resistance to herbicides, imposing a great threat to wheat production in China (Cui et al., 2008; Xu et al., 2010). However, few studies have taken into account the influence of O_3 pollution on crop yield and quality in the presence of weeds in the field.

Wheat grains are important sources of protein, carbohydrates and minerals for human nutrition (Hogy et al., 2013; Shewry, 2009). The nutritional quality of wheat-based products is determined by these grain quality parameters. Many studies have shown that exposure to O_3 enhances crude protein concentration in grains (Feng et al., 2008; Piikki et al., 2008; Pleijel et al., 2006), while a few studies have reported lower protein concentration in grains by O_3 treatment. Synthesis of carbohydrates in grains depends primarily on concurrent carbon fixation. Grain starch concentration under O_3 stress is reduced owing to lower carbon fixation (Mishra et al., 2013). Nevertheless, whether and how O_3 stress impacts mineral nutrients in grains have not been studied.

Our previous studies investigated the effect of O_3 and weed competition on yield and quality of winter wheat, and found that flixweed is more competitive than winter wheat under O_3 pollution due to its more efficient enzymatic antioxidant system (Li et al., 2013). In the present study, we further elucidated the physiological mechanism underlying the interactive effects of O_3 stress and flixweed competition by measuring chlorophyll, leaf area, gas exchange, hydrogen peroxide, malondialdehyde and Proline, yield and quality properties of winter wheat. Our findings may help breeders to utilize wheat performance traits under elevated O_3 and in the presence of weed for competition resources that commonly occur in an agricultural system to breed new varieties with strong tolerance to O_3 pollution.

2. Materials and methods

2.1. Plant culture and O_3 fumigation

Winter wheat (*Triticum aestivum* L. cv Liangxing99) which is sensitive to O_3 and flixweed (*Descurainia sophia*) were selected for the present experiment. Seeds of wheat and flixweed were randomly sown in plastic pots (25 cm in diameter, 28 cm in height), 32 pots being planted for each treatment. Pots were all filled with local field top 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. Winter wheat grown in monoculture or mixculture was thinned to ten identical seedlings per pot at 14 days after their germination. For wheat production in the field, the density of wheat is fixed with varying flixweed density. In our study, flixweed grown in mixculture was thinned to 10 or 20 seedlings, respectively. Each wheat plant had two effective tillers on average. Therefore, the wheat/flixweed ratio was achieved as 30:0, 30:10 and 30:20, respectively. Plants were irrigated as required to avoid drought.

Four open-top chambers (OTCs, 2.6 m in diameter, 2.4 m in height) were randomly assigned to the two treatments, resulting in two replicates per treatment. Each of the chambers contained eight pots of plants growing in either monoculture or mixculture. Plants were allowed to adapt to chamber environments for 7 days before O_3 exposure. During this adaptation period, all plants received ambient air with an O_3 concentration of less than 40 ppb. The average O_3 concentration of ambient air in the experiment site was about 35 ppb during the exposure. The gas dispensing system of the OTCs was conducted according to Uprety (1998). Ozone was artificially added to the open air entering two of the chambers to maintain an O_3 concentration of 120 ± 10 for 7 h day^{-1} (10:00–17:00) from anthesis of wheat. During this period, wheat is quite sensitive to O_3. The exposure lasted for 20 days. Meanwhile, the other two chambers were ventilated with ambient air as the control. The injected O_3 was generated by electrical discharge using ambient air with an O_3 generator (CF-KG1, Shanmeishuimei Ltd., Beijing, China).

2.2. Determination of chlorophyll and leaf area

Green leaf area of six plants per treatment was assessed after termination of O_3 exposure. Leaf area is measured by scanner (Perfection V700 Photo, Epson, China). Chlorophyll was extracted from flag leaves and assayed with the method described by Arnon (1949). As six replicates, six fresh flag leaves from each treatment were sampled to test chlorophyll.

2.3. Measurements of gas exchange

Six flag leaves per treatment were used for measurements of gas exchange with an open infrared gas exchange system (GFS-3000, Walz, Germany). The relative humidity was maintained at 70% and the temperature at 27 °C in the leaf chamber. Air flow rate was set at 750 $\mu mol\, s^{-1}$ and CO_2 concentration maintained 380 $\mu mol\, mol^{-1}$. For gas exchange measurement, the leaf was illuminated with a PPFD (Photosynthetic Photon Flux Density) of 1500 $\mu molm^{-2}\, s^{-1}$ of internal light source of the leaf chamber. Data obtained included the area-based light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) and the related environmental variables such as air temperature and moisture.

2.4. Determination of hydrogen peroxide, malondialdehyde and Pro

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in leaves were measured to assess the effect of O_3 on membrane lipid peroxidation of wheat. To be one replicate, three fresh flag leaves after 20 days of fumigation were harvested for assaying H_2O_2, MDA and Pro, and there were six replicates.

H_2O_2 was measured according to Alexieva et al. (2001). MDA was determined following the method described by Kramer et al. (1991). Pro accumulation in winter wheat leaves was assayed by the method described previously (Bates et al., 1973).

2.5. RNA isolation and real-time quantitative PCR

Total RNAs were extracted from sampled flag leaves of wheat with Trizol reagent (Invitrogen) after ten days 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. Gene-specific primers of *TaP5CS* (AB193551.1) are 5′-TAC AGC GGT CCA CCA AGT-3′ and 5′-TGC CAC CTC TAC CAA CAC G-3′. *TaActin* (AB181991.1) was used as internal control: 5′-CTA TCC TTC GTT TGG ACC TT-3′ and 5′-AGC GAG CTT CTC CTT TAT GT-3′. RT-qPCR was performed using ABI StepOne Plus instrument. Each reaction contained 5 μL 2 × SYBR Green Master Mix reagent (TaKaRa), 0.5 μL cDNA samples, 0.6 μL 10 mM gene-specific primers and 0.2 μL 50 × ROX in a final volume of 10 μL. The thermal cycle was used as followings: 95 °C for 1 min; 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °Cfor 30 s. The expression