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Effects of Elevated CO_2 and O_3 on Phenolic Compounds in Spring Wheat and Maize Leaves

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Abstract C_3 crops are generally considered more sensitive than C_4 crops to the elevated CO_2 and O_3, but it is unclear whether the concentrations of phenolic compounds in them are affected. In this paper, an enrichment experiment with open-top chamber was conducted to examine the effects of elevated CO_2, O_3, and their combination on the contents of total phenolic compounds and flavone in the leaves of spring wheat (C_3 crop) and maize (C_4 crop). The results showed for spring wheat, the total phenolic contents in its leaves at jointing stage was significantly higher under elevated CO_2 and/or O_3, with the sequence of CO_2 plus $O_3 > O_3 > CO_2 >$ ambient, while at grain-filling stage, the total phenolic content was lower under CO_2 plus O_3 than under CO_2, O_3, and ambient. The total phenolic content in maize leaves at jointing stage had the similar variation trend with that for wheat, but at grain-filling stage, the total phenolic content was slightly affected by elevated CO_2 and/or O_3. The flavone content in spring wheat leaves was significantly lower under CO_2 and/or O_3 stress at jointing stage, but had lesser difference at grain-filling stage under the stress. The same variation trend was observed in the flavone content in maize leaves at jointing and grainfilling stages, i.e., CO_2 plus $O_3 > CO_2 >$ ambient $> O_3$. C_3 plant was more sensitive than C_4 plant to the CO_2 and/or O_3 stress.

Keywords Elevated CO_2 and O_3 · Phenolic compounds · Spring wheat and maize

In recent years, the concentrations of atmospheric CO_2 and O_3 have a rapid increase. Elevated atmospheric CO_2 is associated with the stimulation of plant photosynthesis and growth (Kimball et al. 2002), while elevated O_3 inhibits plant photosynthesis and other physiological processes, causing significant growth- and yield losses (Mauzerall and Wang 2001; Adams and Horst 2003; Mills et al. 2007).

Both CO_2 and O_3 affect plant chemistry (Booker et al. 2005). Under elevated CO_2, the contents of phenolic compounds including flavone, catechin, and gallic acid in ginseng are affected significantly (Ali et al. 2005), and the total phenolic content in *Dactylis* and *Bromus* was increased by 15% and 87%, respectively, while no significant interactions between CO_2 and genotype were observed in either of these two species (Castells et al. 2002). Sgarbi et al. (2003) reported that the phenol metabolism in two cell lines from grape leaf is affected by elevated O_3.

C_3 crops are generally considered more sensitive than C_4 crops to the elevated CO_2 and O_3 (Fuhrer, 2003; Mera et al. 2006), but it is unclear whether the concentrations of phenolic compounds in C_3 and C_4 crops are affected. In this paper, an enrichment experiment with open-top chamber was conducted to examine the effects of elevated CO_2, O_3, and their combination on the contents of total phenolic compounds and flavone in the leaves of spring wheat (C_3 crop) and maize (C_4 crop).

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Materials and Methods

The enrichment experiment with open-top chamber was conducted at the Shenyang Ecological Experimental Station (41°31′N, 123°24′E) in the Shenyang suburb of Northeast China. The annual mean air temperature is 7–8°C, annual mean precipitation is 700 mm, and non-frost period is about 147–164 days. Meadow brown soil is the main soil type for agricultural production in this area.

Four treatments were installed, i.e., control (ambient air), elevated CO_2 (550 μmol CO_2 mol^{-1}), elevated O_3 (80 nmol O_3 mol^{-1}), and elevated CO_2 plus O_3 (550 μmol CO_2 mol^{-1} plus 80 nmol O_3 mol^{-1}), which were arranged in a randomized complete block design with three replicates. The open-top chamber was 3 m in diameter and 2.8 m in height. CO_2 and O_3 were provided 24 h a day and 8 h at daytime (8:00–12:00 am, 14:00–18:00 pm), respectively, from 27 April to 27 June for spring wheat and from 13 July to 17 August for maize. The CO_2 and O_3 concentrations in open-top chamber were respectively inspected by ES-D infrared CO_2 sensor and S-900 O_3 analyzer, with a variance ±5.4% for CO_2 and ±12.5% for O_3.

Spring wheat (*Triticum aestivum* L.) cultivar Liaochun 17 was sown on 2 April 2007 and harvested on 22–27 June 2007, and maize (*Zea mays* L.) cultivar Shennuo was transplanted on 3 July and harvested on 25 August 2007. Wheat samples were collected at jointing (9 May) and grain-filling (6 June) stages, and maize samples were collected at vigorous jointing (25 July) and grain-filling (8 Aug) stages.

The determinations of plant phenolic compounds and flavone were performed with Folin-D method (Luthria et al. 2006) 0.25 g fresh leave was ground (0°C) and placed in a 15-mL centrifuge tube with 6 mL of the solvent mixture MeOH/H_2O (80:20, %v/v). The vials were then placed in a sonicator bath (Model KQ-250DE, Ultrasonic Corporation, Kunshan, CT) at ambient temperature for 30 min. The mixture was centrifuged at 3,000 rpm for 5 min and the supernant was transferred into a 50-mL volumetric flask. The residue was resuspended in 6 mL of MeOH/H_2O (80:20, %v/v), gently mixed manually and sonicated for an additional 30 min followed by centrifugation. The supernant was combined with the initial extract and the volume of combined supernant was made up to 50 mL volumetric flask with the extraction solvent. This was followed by addition of 30 mL of water. This mixture was vortexed for 10–20 s and 2 mL of Folin-D reagent was added. The mixture was vortexed for an additional 20–30 s and after 5 min 2 mL of filtered 20% sodium carbonate solution was added. This was recorded as time zero; after 30 min at ambient temperature, the absorbance of the colored reaction product was measured at 765 nm using a Specord 50 spectrophotometer (Analytik Jena AG, Germany). A calibration curve was created using different concentrations of standard gallic acid solutions, each time an analysis was run. The level of total phenolic in the extract was calculated from the standard calibration curve. Results were expressed on the basis of mg of Gallic Acid Equivalent per gram (mg GAE g^{-1}) of fresh weight.

Plant samples for total flavone analysis were processed as $NaNO_2$–$Al(NO_3)_3$ method (Wei and Wang 2003; Li et al. 2006). 0.25 g dry leaves were ground for powder and placed in a 50 mL little beaker with 30 mL of the solvent mixture Ethanol/H_2O (60:40, %v/v), marinated for 24 h. The breakers were then placed in a sonicator bath (Model KQ-250DE, Ultrasonic Corporation, Kunshan, CT) at ambient temperature for 1 h. The mixture was filtrated into a 50-mL volumetric flask, made up to the scale with the extraction solvent. About 1 mL of the solvent was put into 10 mL volumetric flask, 0.4 mL of 5% $NaNO_2$, 6 min later with 0.4 mL 10% $Al(NO_3)_3$ and 4 mL 4.3% NaOH. This was recorded as time zero; after 15 min at ambient temperature, the absorbance of the colored reaction product was measured at 500 nm using a Specord 50 spectrophotometer (Analytik Jena AG, Germany). A calibration curve was created using different concentrations of standard rutin solution. Results were expressed on the basis of mg of rutin gram (mg 100 g^{-1}) of dry weight.

All data were subjected to statistical analysis of variance (ANOVA) in the SPSS statistical package.

Results and Discussion

Table 1 shows that for spring wheat, the total phenolic content in its leaves at jointing stage was significantly higher under elevated CO_2 and/or O_3, with the sequence of CO_2 plus O_3 > O_3 > CO_2 > ambient, while at grain-filling stage, the total phenolic content was lower under CO_2 plus O_3 than under CO_2, O_3, and ambient, because of the changed biosynthesis or the changed efficiency of converting carbohydrates into harvestable yield (Rudorff et al. 1996).

As for maize, the total phenolic content in its leaves at jointing stage had the similar trend with that in spring wheat leaves, but at grain-filling stage, the total phenolic content was slightly affected by elevated CO_2 and/or O_3. The total phenolic content in maize leaves was lower at grain-filling stage than at jointing stage, perhaps more carbohydrates being transferred into tillers to meet the sink demands during grain-filling (Rudorff et al. 1996).

The comparison of total phenolic content in spring wheat and maize leaves showed that maize was not sensitive to CO_2 and/or O_3 stress, because C_4 plants are saturated with carbon, and thus, the carbohydrates in them are slightly affected.

Table 1 Effects of elevated CO_2 and/or O_3 on total phenolic content in spring wheat and maize leaves

Treatment	Wheat (mg g^{-1} fresh weight)		Maize (mg g^{-1} fresh weight)	
	Jointing stage	Grain-filling stage	Jointing stage	Grain-filling stage
Ambient	1.01 ± 0.06 a	1.12 ± 0.07 a	1.35 ± 0.03 a	1.28 ± 0.03 a
CO_2	1.17 ± 0.06 b	1.25 ± 0.12 b	1.42 ± 0.02 b	1.33 ± 0.03 b
O_3	1.27 ± 0.06 c	1.30 ± 0.15 c	1.41 ± 0.03 b	1.30 ± 0.04 a
CO_2 and O_3	1.32 ± 0.07 cd	1.10 ± 0.10 a	1.41 ± 0.02 b	1.30 ± 0.03 a

Total phenolic content is presented with mean ± standard deviation (mg g^{-1} Fresh weight)

Different letters meant significant difference among treatments at 0.05 level ($\alpha = 0.05$) in ANOVA analysis

CO_2: elevated CO_2 concentration; O_3: elevated O_3 concentration; CO_2 and O_3: interaction of elevated CO_2 and O_3

Table 2 Effects of elevated CO_2 and/or O_3 on flavone content in spring wheat and maize leaves

Treatment	Wheat (mg g^{-1} dry weight)		Maize (mg g^{-1} dry weight)	
	Jointing stage	Grain-filling stage	Jointing stage	Grain-filling stage
Ambient	13.33 ± 2.05 c	15.29 ± 4.66 c	19.57 ± 2.71 b	18.86 ± 1.39 a
CO_2	9.49 ± 1.04 b	14.07 ± 1.25 b	19.91 ± 2.49 b	21.36 ± 3.19 b
O_3	9.52 ± 1.36 b	11.94 ± 1.34 a	17.03 ± 1.18 a	17.41 ± 2.24 a
CO_2 and O_3	7.18 ± 1.60 a	15.28 ± 1.64 c	21.19 ± 3.32 c	21.98 ± 2.33 b

Content of flavone is presented with mean ± standard deviation (mg g^{-1} Dry weight)

Different letters mean significant difference among treatments at 0.05 level ($\alpha = 0.05$) in ANOVA analysis

CO_2: Elevated CO_2 concentration; O_3: Elevated O_3 concentration; CO_2 and O_3: Interaction of elevated CO_2 and O_3

Dizengremel (2001) reported that after a long-term exposure to elevated O_3, a general increase in phenylpropanoid metabolism, the stimulation of lignin's biosynthetic pathway, is observed with the production of more phenolic compounds. Our results also showed an obvious increase (1.9–25.8%) of total phenolic content in maize and wheat leaves.

Flavone is one of the phenolic compounds, but its response to the environment condition is different from total phenols. As one of the carbohydrates excreted from plant body, the biosynthesis process of flavone is affected by plant C/N ratio and environment stress.

Table 2 shows that for spring wheat, the flavone content in its leaves at jointing stage was significantly lower under CO_2 and/or O_3 stress, but at grain-filling stage, the flavone content had lesser difference under the stress. The flavone content in maize leaves had the same variation trends at jointing and grain-filling stages, i.e., CO_2 plus $O_3 > CO_2 >$ ambient $> O_3$.

The comparison of flavone content in spring wheat and maize leaves showed that elevated O_3 had more evident effects than elevated CO_2 and its combination with O_3. The flavone content under O_3 stress was significantly lower, demonstrating that there existed phenolic disbalance and *de nove* biosynthesis of compounds (Kanoun 2001). Enriched CO_2 could compensate O_3 injury, and the compensation degree was higher for maize than for spring wheat.

It is well-known that stress conditions can induce metabolic changes. Our study showed that short-term CO_2 and/or O_3 stress could significantly modify the total phenolic compounds accumulation in wheat leaves. As observed by Rudorff et al. (1996), wheat (C_3 crop) was more sensitive than corn (C_4 crop) to CO_2 and/or O_3 stress. Sild et al. (2002) indicated that O_3 stress induced leaf- and plant senescence, associated with the changes in carbohydrate concentration.

Our study was conducted with open-top chamber, in which, the CO_2 and O_3 were distributed uniformly, and their effects were more evident. More researches are needed, due to the complicated biosynthesis paths and physiological mechanisms.

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References

Adams RM, Horst RL (2003) Future directions in air quality research: economic issues. Environ Int 29:289–302. doi:10.1016/S0160-4120(02)00187-3

Ali BM, Hahn EJ, Paek KY (2005) CO_2-induced total phenolics in suspension cultures of *Panax ginseng* C.A. Mayer roots: role of antioxidants and enzymes. Plant Physiol and Biochem 43:449–457. doi:10.1016/j.plaphy.2005.03.005

Booker FL, Prior SA, Torbert HA, Fiscus EL, Pursley WA, Hu SJ (2005) Decomposition of soybean grown under elevated concentrations of CO_2 and O_3. Global Change Biol 11:685–698. doi:10.1111/j.1365-2486.2005.00939.x

Castells E, Roumet C, Penuelas J, Roy J (2002) Intraspecific variability of phenolic concentrations and their responses to elevated CO_2 in two mediterranean perennial grasses. Environ Exp Bot 47:205–216. doi:10.1016/S0098-8472(01)00123-X

Dizengremel P (2001) Effects of ozone on the carbon metabolism of forest trees. Plant Physiol Biochem 39:729–742. doi:10.1016/S0981-9428(01)01291-8

Fuhrer J (2003) Agroecosystem responses to combinations of elevated CO_2, ozone, and global climate change. Agr Ecosyst Environ 97:1–20. doi:10.1016/S0167-8809(03)00125-7

Kanoun M, Goulas MJP, Biolley JP (2001) Effect of chronic and moderate ozone pollution on the phenolic pattern of bean leaves (*Phaseolus vulgaris* L. *cv Nerina*): relations with visible injury and biomass production. Biochem Syst Ecol 29:443–457. doi:10.1016/S0305-1978(00)00080-6

Kimball BA, Zhu JG, Cheng L (2002) Responses of agricultural crops to free CO_2 enrichment. Chinese J Appl Ecol 13:1323–1338

Li HN, Liu HH, Tang YL (2006) Study the procedures of ultrasonic wave and ethanol extraction for extracting total flavones from longan seed. J Youjiang Medical College 28:168–169

Luthria DL, Mukhopadhyay S, Krizek DT (2006) Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. J Food Compos Anal 19:771–777. doi:10.1016/j.jfca.2006.04.005

Mauzerall DL, Wang XP (2001) Protecting agricultural crops from the effects of tropospheric ozone exposure: reconciling science and standard setting in the United States, Europe, and Asia. Annu Rev Energ Environ 26:237–268. doi:10.1146/annurev.energy.26.1.237

Mera R, Niyogi D, Bohl G, Wilkerson G, Semazzi F (2006) Potential individual versus simultaneous climate change effects on soybean (C_3) and maize (C_4) crops: an agrotechnology model based study. Global Planet Change 54:163–182. doi:10.1016/j.gloplacha.2005.11.003

Mills G, Buse A, Gimeno B, Bermejo V, Holland M, Emberson L, Pleijel H (2007) A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. Atmos Environ 41:2630–2643. doi:10.1016/j.atmosenv.2006.11.016

Rudorff BFT, Ulchi CL, Lee EH, Rowland R, Pausch R (1996) Effects of enhanced O_3 and CO_2 enrichment on plant characteristics in wheat and corn. Environ Pollut 94:53–60. doi:10.1016/S0269-7491(96)00050-4

Sgarbi E, Fornasiero RB, Lins AP, Bonat PM (2003) Phenol metabolism is differentially affected by ozone in two cell lines from grape (*Vitis vinifera* L.) leaf. Plant Sci 165:951–957. doi:10.1016/S0168-9452(03)00219-X

Sild E, Pleijel H, Selldén G (2002) Elevated ozone (O_3) alters carbohydrate metabolism during grain filling in wheat (*Triticum aestivum* L.). Agric Ecosyst Environ 92:71–81. doi:10.1016/S0167-8809(01)00270-5

Wei X, Wang ZY (2003) Determination of Flavonoids in *Portulaca Oleracea* L. Chinese J Spectro lab 20:128–129