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Effects of Elevated Atmospheric CO_2 and O_3 on Hill Activity and Ca^{2+}/Mg^{2+}-ATPase Activity of *Pinus tabulaeformis* Carr.

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Abstract The main photo-physiological characteristics of *Pinus tabulaeformis* Carr. were analyzed in open-top chambers under elevated carbon dioxide and ozone concentrations. The results indicated that the leaves net photosynthetic rates ($p < 0.05$), Hill activity, Ca^{2+}/Mg^{2+}-ATPase activity, soluble sugar and starch contents all increased under elevated carbon dioxide concentration in whole growing season. While under elevated ozone concentration, the leaves net photosynthetic rates, Hill activity, Ca^{2+}/Mg^{2+}-ATPase activity, soluble sugar and starch contents all decreased. Under elevated carbon dioxide and ozone concentration, the leaves net photosynthetic rates, Hill activity, soluble sugar and starch contents all increased, but Ca^{2+}-ATPase activity increased during the earlier growing season, decreased in later growing season, while Mg^{2+}-ATPase activity responded contrarily.

Keywords Elevated CO_2 and O_3 concentration · Hill activity · Ca^{2+}/Mg^{2+}-ATPase activity · *Pinus tabulaeformis* Carr.

Carbon dioxide is the most important anthropogenic greenhouse gas. The global atmospheric concentration of carbon dioxide has increased from a pre-industrial value of about 280–379 ppm in 2005 (IPCC 2007), and it will reach 550 μmol/mol in 2050, while exceed 700 μmol/mol in this century end (IPCC 2001). The response of net photosynthetic rate and leaf dry mass per unit of area to elevated CO_2 was a mean increase (James 2005). Biomass growth in Prunus increased an average of 56% at elevated CO_2 (Hattenschwiler and Korner 2003), but there were no effects of CO_2 treatment on tissue or total biomass on *Pinus taeda* L. (Hussain et al. 2001). Tropospheric ozone, a secondary pollutant generated in the downwind of major metropolitan areas from nitrogen oxides and volatile organic compounds reacting in the presence of sunlight. According to statistic, the earth exterior concentration of ozone has increased from a pre-industrial level of about 10 nL L^{-1} to average 60 nL L^{-1} now. Both biomass allocation below ground and inhibits whole-plant growth rate were significantly reduced by O_3 (Grantz et al. 2006). Ozone as a general stress facto influenced the photosynthetic parameters (Eichelmann et al. 2004) and exposure to O_3 common during the growing season can increase water loss in Populus saplings, but this effect might be offset by decreased foliar biomass (Maarten et al. 2001).

The interactive effect of carbon dioxide and ozone is important to the ecosystem. The inhibitory effects of added O_3 on biomass production could be largely compensated by elevated CO_2 (Booker et al. 2005). Wood properties of young silver birch trees were altered under elevated CO_2 in both clones, whereas the effects of O_3 depended on clone (Katri et al. 2006). Catherine et al. (2003) addressed high CO_2 concentration protects, to some extent, against O_3 via providing additional carbon and energy through increased net assimilation.

Pinus tabulaeformis Carr. is one of main urban forest species in Shenyang and its main photosynthetic characteristics were investigated in open-top chambers under elevated CO_2 and O_3 concentration in this paper, such as

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Hill activity, Ca^{2+}/Mg^{2+}-ATPase activity. The effects of different treatments on photosynthesis of *P. tabulaeformis* Carr. were studied to analyze its photosynthetic response mechanisms to elevated CO_2 and O_3 concentration and predict the feasibility of *P. tabulaeformis* Carr. as one of composing tree species of Shenyang urban forest under future climate change.

Materials and Methods

The study was conducted in 12 open-top chambers at the Shenyang Arboretum of Chinese Academy of Sciences (41°46′N, 123°26′E) in an urban environment. The OTCs imitated the Heagle design (Heagle et al. 1973), were 4 m in diameter and 3 m in height with a 45° sloping frustum, and were placed on 4 m centers (north–south and east–west) to avoid mutual shading. The minimum distance between any two chambers is 4 m.

The 10-year-old *P. tabulaeformis* Carr. seedlings were transplanted to chambers in April 2006, and continuously air exposed to open from 17 June to September 30, 2006. *P. tabulaeformis* Carr. leaves were collected at 9:00 a.m. every 20 days for immediate analysis.

The experiment involved four treatments with ambient air, elevated CO_2, elevated O_3 or a combined treatment of elevated CO_2 and O_3 and three replications for each treatment. The elevated CO_2 concentration was 700 ± 20 μmol mol^{-1}, 24 h/day with ambient O_3 concentration, and the elevated O_3 concentration was 80 ± 8 nmol mol^{-1}, 8 h/day with ambient CO_2 concentration, respectively. The combined treatment of elevated CO_2 and O_3 was the elevated 700 ± 20 μmol mol^{-1}, 24 h/day CO_2 concentration with elevated 80 ± 8 nmol mol^{-1}, 8 h/day O_3 concentration. Target airs were delivered via a computer controlled system modified from Siemens Germany (PLC, LT/ACR-2002).

Net photosynthetic rates were measured with the LI-COR 6400 in a purpose-built leaf chamber attached to a rapid-kill apparatus.

Hill activity was measured according to Tang (1999). The 0.1 mL chloroplast suspend liquid in a 0.5 mmol/L Tris HCL (pH 8.0), buffer containing 0.05 mmol/L $MgCl_2$, 0.1 mmol/L NaCl, 0.01 mmol/L Potassium hexacyanoferrate (III) and distilled water. One group was illuminated and the other was in dark. After 1 min, 0.2 mL 10% Trichloroacetic was added which centrifuged at 3,000 r/min for 2 min. Then 0.7 mL centrifugal liquid was extracted to add into feedback liquid, placed for 10 min in dark, in turn measured OD_{520} in spectrophotometer.

Ca^{2+}/Mg^{2+}-ATPase activity were measured by following the procedures described by Huang (1985). Ca^{2+}-ATPase activity: 0.1 mL chloroplast suspend liquid in a 0.25 mmol/L Tris HCL (pH 8.0), buffer liquid containing 0.02 mmol/L EDTA Na_2, 0.01 mmol/L ATP, 2 mg/mL Trypsin. In 64°C water bathing was heated for 4 min and extracted 0.5 mL and kept in 37°C for 10 min.

Mg^{2+}-ATPase activity: 0.1 mL chloroplast suspend liquid in a 0.5 mmol/L Tris HCL (pH 8.0), buffer containing 0.5 mmol/L NaCl, 0.05 mmol/L $MgCl_2$, 0.05 mmol/L DTT. Activate it by illumination for 5 min. About 0.1 mL ATP was added and kept in 37°C for 5 min. Above all, 0.2 mL 20% Trichloroacetic was added and centrifuged at 3,000 r/min for 5 min. Then 0.5 mL centrifugal liquid was extracted and added into feedback liquid, placed for 30 min, which measured OD_{660} in spectrophotometer.

Soluble sugar and starch contents were measured according to Zou (2000). About 0.5 g leaves were heated in 100° water for 30 min, then filtrated the percolate hold to 50 mL. Distilled water and 9.2 mol/L HCl_4 to surplus in turn were added and heated in a water bath for 15 min, filtrate and the percolate hold in 100 mL. Extracted liquid and anthrone heated for 10 min, then measured OD_{630} in spectrophotometer.

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

Results and Discussion

The responses of net photosynthetic rates (Pn) of *P. tabulaeformis* Carr. leaves to treatments are different (Fig. 1). Elevated CO_2 improved the photosynthetic ability of *P. tabulaeformis* Carr. and increased Pn significantly ($p < 0.05$), but the effects of elevated O_3 are negatively and Pn is decreased by 7.39%–54.52%. However, net photosynthetic rate of leaves under elevated CO_2 and O_3 is ameliorated significantly ($p < 0.05$) and was increased by 14.37%–70.95% compared to ambient air.

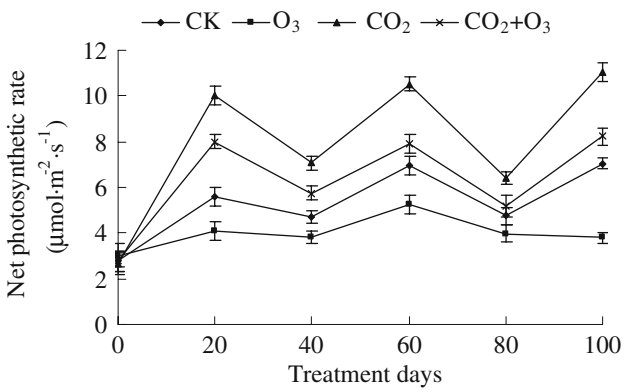

Fig. 1 Variations of net photosynthetic rate in *Pinus tabulaeformis* Carr. leaves under different treatments

The effect trends of different treatments on Hill reaction activity of *P. tabulaeformis* Carr. leaves are similar to net photosynthetic rates (Fig. 2). In the elevated CO_2 treatment and combination treatment, Hill reaction activities are 19.43%–45.66% and 6.43%–31.03% higher than control, respectively, but the highest Hill reaction activity on 80th day is under combination treatment; by contrast, the elevated O_3 treatment reduced the Hill reaction activity 8.49%–13.87%. The results further showed that photosynthetic capacity of plant is stimulated by elevated CO_2 and elevated O_3 suppressed photosynthetic capacity of plants.

Both Ca^{2+}/Mg^{2+}-ATPase contents are increased by elevated CO_2, but are reduced by elevated O_3 (Figs. 3, 4). The highest content under elevated CO_2 and the lowest content under elevated O_3 of Ca^{2+}-ATPase are +42.57% and −20.40% compared to control, while the highest content under elevated CO_2 and the lowest content under elevated O_3 of Mg^{2+}-ATPase are +18.03% and −15.03%, respectively. In combination treatment, Ca^{2+}/Mg^{2+}-ATPase contents are higher than control except that on 80th day, Ca^{2+}-ATPase activity is lower than control, while

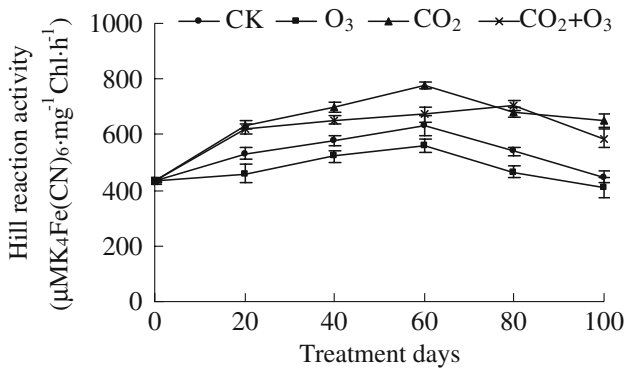

Fig. 2 Effects of different treatments on Hill reaction activity in *Pinus tabulaeformis* Carr. leaves

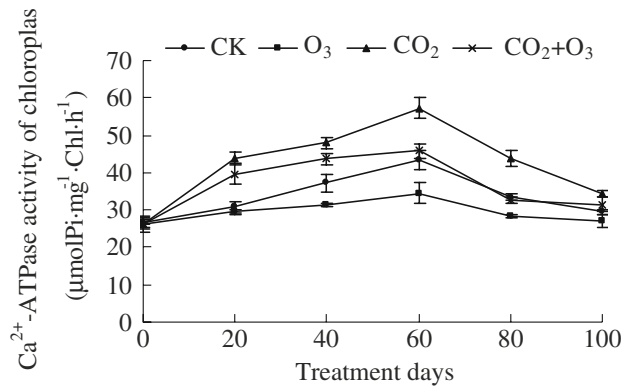

Fig. 3 Effects of different treatments on Ca^{2+}-ATPase activity in chloroplast of *Pinus tabulaeformis* Carr. leaves

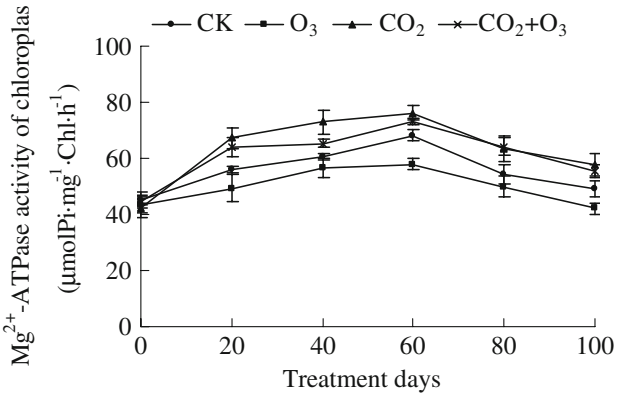

Fig. 4 Effects of different treatments on Mg^{2+}-ATPase activity in chloroplast of *Pinus tabulaeformis* Carr. leaves

Mg^{2+}-ATPase activity is higher than elevated CO_2 treatment. Elevated CO_2 also ameliorated the negative effects of elevated O_3 on Ca^{2+}/Mg^{2+}-ATPase activity in combination treatment. Similar conclusion is drawn in the research for impacts of O_3 and CO_2 concentration doubled on the soybean leaf development and biomass (Huang et al. 2005).

The leaf soluble sugar content of *P. tabulaeformis* Carr. significantly increased 5.85%–55.77% ($p < 0.05$) in elevated CO_2, while elevated O_3 caused a 1.68%–18.83% decline (Fig. 5). The effects of combination treatment changed with treatment days, and common trend is that elevated CO_2 compensated for the decline of leaf soluble sugar content is caused by elevated O_3, but leaf soluble sugar content of combination treatment is the highest on 40th day and lowest on 60th day in all the treatment.

Elevated CO_2, respectively increased 9.28%–31.69% and 4.61%–28.76% starch content in *P. tabulaeformis* Carr. leaves in ambient treatment and combination treatment except 80th day under elevated CO_2 treatment and 100th day under combination treatment (Fig. 6). By contrast, elevated O_3 reduced starch content in *P. tabulaeformis*

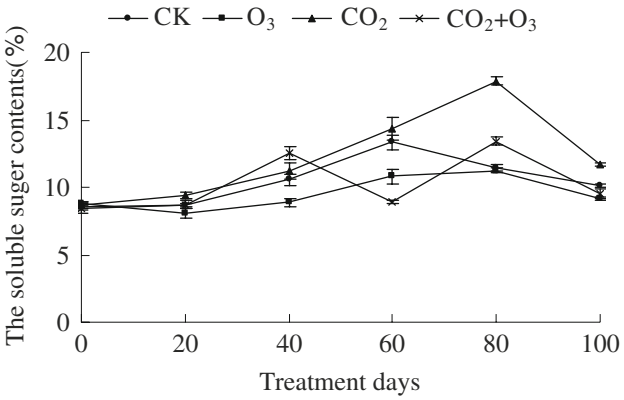

Fig. 5 Effects of different treatments on soluble sugar content in *Pinus tabulaeformis* Carr. leaves

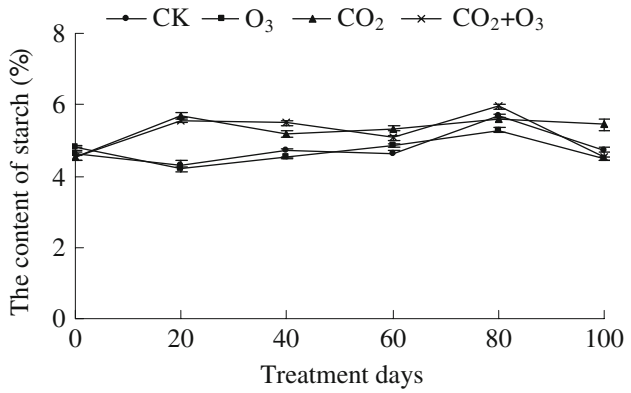

Fig. 6 Effects of different treatments on starch content in *Pinus tabulaeformis* Carr. leaves

Carr. leaves, and caused a 3.16%–6.91% decline at the whole season except on 60th day. It indicates that higher CO_2 promotes photosynthesis and accumulation of photosynthesis production. The results are consistent with the report on *Anthurium andraeanum* written by Li et al. (2005). And the contrary results under elevated ozone have been observed, which are supported by that researched on three woody plants conducted by Huang et al. (2006).

This study showed that photosynthesis of *P. tabulaeformis* Carr. is promoted by higher CO_2 concentration and is inhibited by higher O_3 concentration. And positive effects of elevated CO_2 alleviated negative effects of elevated O_3 with the combination of elevated CO_2 and O_3 concentration. Therefore, as a native species which has extensive adaptability, it is practical that *P. tabulaeformis* Carr. be planted in Shenyang as a response to the inevitable global climate change and to sustain our eco-friendly environment.

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References

Booker FL, Joseph EM, Edwin LF, Walter AP, Leonard AS (2005) Comparative responses of container-versus ground-grown Soybean to elevated carbon dioxide and ozone. Crop Sci 45:883–896. doi:10.2135/cropsci2004.0198

Catherine G, Nadine C, Dany A, Yves M, Normand C, Pierre D (2003) The impact of elevated ozone and carbon dioxide on young *Acer saccharum* seedlings. Physiol Plant 117:392–402. doi:10.1034/j.1399-3054.2003.00046.x

Eichelmann H, Oja V, Rasulov B, Padu E, Bichele I, Pettai H, Mols T, Kasparova I, Vapaavuori E, Laisk A (2004) Photosynthetic parameters of birch (Betula pendula Roth) leaves growing in normal and in CO_2- and O_3-enriched atmospheres. Plant Cell Environ 27:479–495. doi:10.1111/j.1365-3040.2003.01166.x

Grantz DA, Gunn S, Vu SB (2006) O_3 impacts on plant development: a meta-analysis of root/shoot allocation and growth. Plant Cell Environ 29:1193–1209. doi:10.1111/j.1365-3040.2006.01521.x

Hattenschwiler S, Korner C (2003) Does elevated CO_2 facilitate naturalization of the non-indigenous *Prunus laurocerasus* in Swiss temperate forests. Funct Ecol 17:778–785. doi:10.1111/j.1365-2435.2003.00785.x

Heagle AS, Body DE, Heck WW (1973) An open-top field chamber to assess the impact of air pollution on plants. Environ Qual 2:2031–2039

Huang ZH (1985) Experimental manual on plant physiology. Shanghai Technology press, Shanghai, pp 111–115

Huang H, Wang CY, Bai YM, Wen M (2005) Impact of O_3 and CO_2 concentration doubling on the soybean leaf development and biomass. Chin J Eco-Agric 13:52–55

Huang YY, Huang YZ, Li QX, Zhang SJ, Tang GF, Lin JH (2006) Physiological and foliar symptom response of *Pinus massoniana*, *Eucalyptus urophylla* and *Aglaia odorata* to ozone in southern China. Ecol Environ 15:674–681

Hussain M, Kubiske ME, Connor KF (2001) Germination of CO_2-enriched *Pinus taeda* L. seeds and subsequent seedling growth responses to CO_2 enrichment. Funct Ecol 15:344–350. doi:10.1046/j.1365-2435.2001.00521.x

IPCC (2001) Third assessment report. In: Houghton T, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Xiaosu D (eds) Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, UK

IPCC (2007) Summary for policymakers. In: Solomon, Qin SD, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

James AB (2005) Response of respiration of soybean leaves grown at ambient and elevated carbon dioxide concentrations to day-to-day variation in light and temperature under field conditions. Ann Bot 95:1059. doi:10.1093/aob/mci117

Katri K, Hanna J, Seija K, Pekka S, Elina V (2006) Wood properties of two silver birch clones exposed to elevated CO2 and O3. Glob Chang Biol 12:1230–1240. doi:10.1111/j.1365-2486.2006.01165.x

Li YH, Liu LN, Ye QS (2005) Effects of elevated CO_2 on the growth and photosynthesis of *Anthurium andraeanum*. J Trop Subtrop Bot 13:343–346

Maarten DJ, Schreuder, Lambertus WA, Brewer, Carol A (2001) Ozone exposure affects leaf wettability and tree water balance. New Phytol 152:443–454. doi:10.1046/j.0028-646X.2001.00272.x

Tang ZC (1999) Experimental manual on present plant physiology. Science press, Beijing, pp 108–109

Zou Q (2000) Experimental manual on plant physiology. Chinese Agriculture Press, Beijing, pp 110–114 (127–130)