

EFFECTS OF ELEVATED CO₂ AND O₃ ON LEAF AREA, GAS EXCHANGE AND STARCH CONTENTS IN CHINESE PINE (*PINUS TABULAEFORMIS* CARR.) IN NORTHERN CHINA

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Abstract

Elevated carbon dioxide (CO₂) and tropospheric ozone (O₃) are considered to substantially influence forest ecosystem health worldwide. *Pinus tabulaeformis* Carr. is dominant species of coniferous forest in northern China. The present study investigated the effects of elevated CO₂ and O₃ on leaf area and gas exchange of *P. tabulaeformis*. The contents of starch were also studied to identify how CO₂ and O₃ affect photosynthetic function of plant. Gas exchange responses to elevated CO₂ and O₃ in terms of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (Tr) of the plant. After 90 days O₃ exposure, a significant decrease in leaf area, gas exchange and starch contents were observed. However, the photosynthesis of the plant was positively affected in terms of increased leaf area and P_n by elevated CO₂. The higher level of starch contents also confirmed that elevated CO₂ affect positively photosynthetic functions of the plant. It was also observed that elevated CO₂ could reduce damage induced by O₃ to some extent, which was indicated by the positive responses of leaf area, gas exchange and starch contents in the plant under elevated CO₂ and O₃ treatment.

Introduction

Human activities such as fossil fuel burning, wood harvesting, soil disturbance and land-use conversion are contributing to global climate change by greenhouse gas emissions (Barcelos *et al.* 2014). Carbon dioxide (CO₂) and tropospheric ozone (O₃) are two of the most important greenhouse gases in the atmosphere (IPCC 2007, Tripathi and Agrawal 2012, Kumari and Agrawal 2014). Elevated CO₂ and O₃ are considered to substantially influence forest ecosystem health (Kangasjarvi *et al.* 2005, Körner *et al.* 2005).

Atmospheric CO₂ are beneficial for plants (Bazzaz 1990). A moderate elevated CO₂ concentration will increase the growth, photosynthesis and resource allocation of plant system, derived from higher photosynthetic rate (Jablonski *et al.* 2002, Loya *et al.* 2003, Geissler *et al.* 2009). In contrast, O₃ can cause leaf damage indicated by stomata closure and net photosynthetic rate (P_n) decline (Leitao *et al.* 2008). So far, several studies have been also focused on the interactive effects of elevated CO₂ and the major living factors such as light, temperature, water and nutrients in different plant species (Allen *et al.* 1990, Idso *et al.* 1993, Hamilton *et al.* 2008, Lee 2011, Danyagri and Dang 2013, Meng *et al.* 2013, Manderscheid *et al.* 2014, Meng *et al.* 2014), however, no information is available on the effects of elevated CO₂ and O₃ in combination on coniferous trees.

Chinese pine (*Pinus tabulaeformis* Carr.) is dominant species of coniferous forest in northern China. It is also widely used in 14 northern provinces and autonomous regions, with a land cover of close to 3 million km² (Li *et al.* 2011). The plant species have the important ecological and

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economy status as indigenous coniferous tree species in northern China (Chen *et al.* 2008). Therefore, in the present study, investigation was carried out on the effects of elevated CO₂ and O₃ on leaf area and gas exchange of *P. tabulaeformis*. Also studied was the contents of starch to identify how CO₂ and O₃ affect photosynthetic function of the plant, which will provide critical insights on future research.

Materials and Methods

The study site was established at the Shenyang Botanical Garden of Chinese Academy of Sciences (42° 24' 09" N, 128° 05' 45" E), which belongs to the temperate zone with a semi-humid monsoon climate. The factorial design of open top chambers (OTCs) was described by He *et al.* (2006). The OTCs were 4 m in diameter and 3 m in height with a 45° sloping frustum. CO₂ was added to each chamber from cylinders and the concentrations of CO₂ were continuously monitored by an infrared sensor (SenseAir, Sweden). O₃ was generated from pure compressed oxygen by electric generator (GP-5J, China), and the concentrations of O₃ were continuously monitored by O₃ analyzer (S-900 Aeroqual, New Zealand).

The treatments were elevated CO₂ (700 μmol/mol, EC), elevated O₃ (EO, 80 nmol/mol), elevated CO₂ and O₃ in combination (700 μmol/mol + 80 nmol/mol, EC+EO), and the control (350 μmol/mol CO₂ + 40 nmol/mol O₃, CK). Four-year-old seedlings of *P. tabulaeformis* were transplanted into 12 OTCs in April 2010. Each treatment was repeated three times. Twenty plants were randomly distributed in each OTC, and the plants were harvested after 30, 60 and 90 days for analysis, respectively. Mean temperature is about 26.5 °C and mean relative humidity is 70% in the OTCs, which were detected by Time domain reflectometry (TDR200, Spectrum Technologies, USA). Leaf area of the plants was measured by LI-3000 leaf area meter (LI-COR, Lincoln, NE, USA). Gas exchange was determined on fully expanded leaves by a portable photosynthesis system (LI-6400, LI-COR Inc. Lincoln, NE, USA). Light intensity, leaf temperature and CO₂ concentration inside the leaf chamber were kept constant at 1000 μmol/m²/s PPFD (Photosynthetic Photon Flux Density), 25 ± 0.3°C and 400 ± 5 μmol CO₂/mol, respectively. All of the measurements were taken between 9:00 and 11:30 a.m. on fully sunny days under natural conditions and all the measurements were recorded 5 times. Starch content was determined by the method of phenol-sulfuric acid (Dubois *et al.* 1956).

All statistical analyses were carried out with SPSS11.0. The significant difference was set among treatments at $p < 0.05$ or $p < 0.01$. Multiple comparisons were made by the LSD (least significant difference) test. All data were presented as mean ± Sd.

Results and Discussion

After 60 days exposure to elevated CO₂ (EC), *P. tabulaeformis* did not show any visual symptoms and kept good growth. Under elevated O₃ (EO) treatment, the chlorosis on coniferous edge was observed. After 90 days exposure to EC, there were significant differences in leaf area of *P. tabulaeformis* compared with the control (Fig. 1). Leaf area under EC treatment showed a significant increased trend, indicating high concentration CO₂ could improve the growth of *P. tabulaeformis*. The same phenomenon has been found for studying the enhancement of CO₂ to plant growth by Barnes *et al.* (1997), the explanation of which contained increased photosynthesis efficiency. Leaf area decreased significantly by 21.75% in leaves relative to the control after 90 days exposure to EO, which in accordance with the changes of leaf exposed to EO.

Gas exchange responses to elevated CO₂ and O₃ in terms of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (Tr) of *P. tabulaeformis* are shown in Fig. 2.

During 90 days exposure to EC+EO period, the same trend as the inducing effect of EC or EO, the increased Pn showed firstly and then turned to decline, all can be seen in *g_s* and *Tr* of the plant, which may result from the difference of growth conditions such as temperature and humidity with seasonal changes.

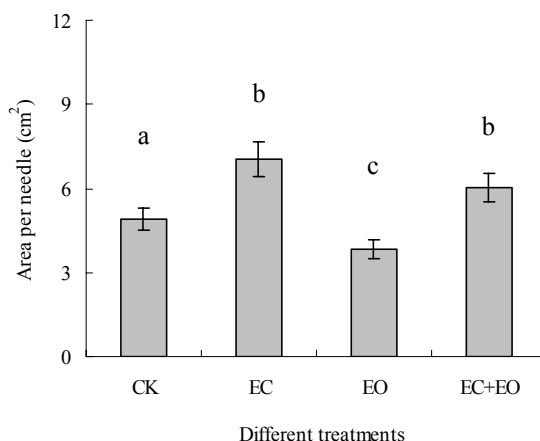


Fig.1. Effects of elevated CO₂ and O₃ concentrations on leaf area of *P. tabulaeformis*. Values represent mean \pm Sd. Different letters indicate significant differences at the 5% level according to the LSD test. CK, the control; EC, Elevated CO₂; EO, Elevated O₃; EC+EO. Elevated CO₂ and O₃.

It is known that CO₂ is both important environmental cue and substrate for photosynthesis (Kaplan *et al.* 2012). In the present study, the maintenance of high Pn in *P. tabulaeformis* under EC treatment was observed (Fig. 2A). After 30, 60 and 90 days exposure to EC, Pn increased significantly by 47.80, 51.10 and 43.15% compared with the control. This may be related to increased RuBp (Ribulose 1, 5 bisphosphate) carboxylase substrates by high CO₂ concentrations and decreased Rubisco competitive oxidation by O₂. After 30, 60 and 90 days exposure to EO, the significant decrease of Pn was shown, and Pn was decreased by 18.36, 24.74 and 42.89%, which indicated that O₃ had a negative effect on photosynthesis. This is in agreement with the study reported by Niu *et al.* (2014). It is commonly considered that Rubisco is one of the main damages induced by O₃ (Fontaine *et al.* 1999, Noormets *et al.* 2001). Decreased Rubisco in quantity and activity may be contributed to the significant decrease of Pn after 30, 60 and 90 days exposure to EO.

In the early stages of all treatments, there were not significant differences in *g_s* of *P. tabulaeformis* compared with the control. After 60 d EC or EO treatment, *g_s* was decreased significantly compared with the control, and showed the difference: EC > EC + EO > EO (Fig. 2B). This is related to the stomatal closure caused by high concentration O₃. In contrast with *g_s*, *Tr* of the plant showed a decreased trend under EO treatment, and there was significant decrease in *Tr* during 30 - 90 days exposure to EO (Fig. 2C). Under EC or EC+EO treatment, no significant decrease was observed. It could be seen that there was a positive correlation between *g_s* and *Tr*.

In the present study, a significant stimulating effect of elevated CO₂ on Pn was showed, which provoked the enhancement of gas exchange, as indicated by the increase in *g_s*. The increase in Pn may indicate an improved growth after 90 days exposure to EC. It was reported that the decrease

of Pn could be associated with stomatal and non-stomatal factors (Ainsworth and Rogers 2007). Under EO treatment, Tr showed a decreased trend with stomatal closure by the decrease of g_s . Since decreased transpiration levels could be responsible for a partial recovery of water balance of the plant tissues, Pn was still decreased. Therefore, non-stomatal factors, such as the deactivation of Rubisco and the inhibitory effects on the electron transport system, may be a main reason of Pn decrease in the latter stages of the treatment (Niu *et al.* 2014). Under EC+EO treatment, Pn kept a sustained increase compared with the control, and the trend decreased gradually, which suggested that the positive effect of CO₂ almost counterbalance the negative effect of O₃ in the later stage of the treatment.

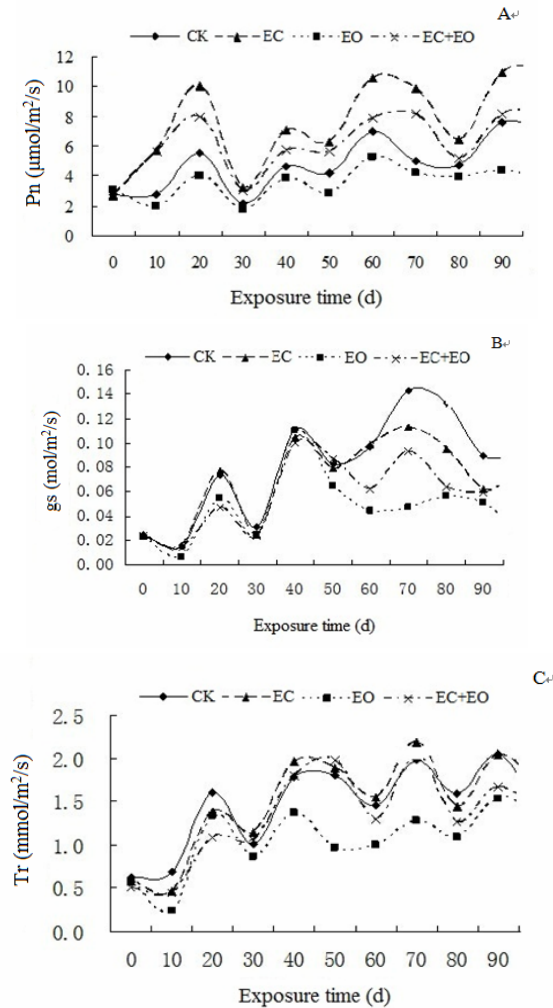


Fig. 2. Effects of elevated CO₂ and O₃ concentrations on net photosynthetic rate (Pn), stomatal conductance (g_s) and transpiration rate (Tr) of *P. tabulaeformis*. Values represent mean \pm Sd. CK, the control; EC, elevated CO₂; EO, elevated O₃; EC+EO, elevated CO₂ and O₃.

Starch is an important energy storage material (Hoekstra and Buitink 2001), which is directly generated by photosynthesis in most plants and plays an important part in improving the resistance

of plants to stress (Donnelly *et al.* 2001). In the present study, the effects of elevated CO₂ and O₃ concentrations on starch in *P. tabulaeformis* related to exposure time were shown in Table 1.

After 90 days exposure to EC, starch contents were increased significantly by 26.9% compared with the control after 30 days exposure to EC. The result indicated that elevated CO₂ had a positive effect on the increase of starch contents in *P. tabulaeformis*, which may be derived from the more accumulated photosynthetic products by increased photosynthetic capacity. It is in good agreement with other study by Reddy and Zhao (2005). It was shown an increased breakdown of starch responsible for the demand of rapid growth in the plant, which is in accordance with the study on Arabidopsis plants (Calatayud *et al.* 2002, Kaplan *et al.* 2012). In the early stages of EO treatment, there were not significant differences in starch contents of the plant compared with the control ($p > 0.05$), with the treatment time extended, a significant decrease in starch contents was observed ($p < 0.05$). It may be due to the reason that O₃ had a negative effect on photosynthesis. After 60 days exposure to EC+EO, starch contents had a significant increase compared with the control, and no significant difference was observed in the later stages of the treatment, which suggested that the positive responses of starch were enough to balance the negative effect by O₃ exposure.

Table 1. Effects of elevated CO₂ and O₃ concentrations on starch in *P. tabulaeformis* related to exposure time from univariate analysis of variance (ANOVA).

Treatments	Exposure time (d)			
	0 d	30 d	60 d	90 d
EC	NS	↑**	NS	↑*
EO	NS	NS	↓*	↓*
EC+EO	NS	↑**	↑*	NS

EC, elevated CO₂; EO, elevated O₃; EC+EO, elevated CO₂ and O₃; NS, not significant; ↑, increased; ↓, decreased; *, $p < 0.05$; **, $p < 0.01$.

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