

## BRIEF COMMUNICATION

**Influence of elevated CO<sub>2</sub> and O<sub>3</sub> on IAA, IAA oxidase and peroxidase in the leaves of ginkgo trees**X.-M. LI\*,\*\*<sup>1</sup>, X.-Y. HE\*, L.-H. ZHANG\*\*\*, W. CHEN\* and Q. CHEN\*\**Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, P.R. China\***College of Chemical and Life Science, Shenyang Normal University, Shenyang 110034, P.R. China\*\***College of Resources and Environmental Science, Liaoning University, Shenyang 110036, P.R. China\*\*\****Abstract**

This study examined the impacts of elevated CO<sub>2</sub> or O<sub>3</sub> on indole-3-acetic acid (IAA) content, activities of IAA oxidase (IAAO) and peroxidase (POD) in *Ginkgo biloba* leaves. Plants grown in open-top chambers were exposed to ambient atmosphere (control; C), elevated CO<sub>2</sub> and elevated O<sub>3</sub> from 1 June to 30 September. An increase in IAA content and decrease in IAAO and POD activities were observed in plants exposed to elevated CO<sub>2</sub> compared with C. Elevated O<sub>3</sub> had no significant effect on IAA content and IAAO activity, but increased POD activity during the early days. When trees pre-exposed to elevated CO<sub>2</sub> were transferred to elevated O<sub>3</sub> or C, the increase in IAAO activity resulted in the decrease in IAA content. When trees pre-exposed to elevated O<sub>3</sub> were transferred to elevated CO<sub>2</sub> or C, IAA content, IAAO and POD activities showed no significant changes. The influence of POD activity on the IAA activity was low.

*Additional key words:* *Ginkgo biloba*, carbon dioxide, open-top chamber, ozone.

Atmospheric carbon dioxide (CO<sub>2</sub>) concentration is increasing rapidly and is expected to double by the end of the current century. Global atmospheric ozone concentration has risen 36 % since pre-industrial times. These increases are primarily due to emissions of oxidized nitrogen (NO<sub>x</sub>) and volatile organic compounds from fossil fuel combustion (Ryerson *et al.* 2001). Elevated CO<sub>2</sub> has been found to promote growth (Druart *et al.* 2006, Lambrevia *et al.* 2006). In contrast to the beneficial effects of elevated CO<sub>2</sub>, O<sub>3</sub> is generally detrimental to tree growth and metabolism (Kaakinen *et al.* 2004, Degl'Innocenti *et al.* 2007). The interactive effects of CO<sub>2</sub> and O<sub>3</sub> on growth and productivity of trees have been investigated, however, conflicting results have been reported, even for a given species (Wustman *et al.* 2001). Plant height and leaf area were the growth parameters most significantly affected by elevated CO<sub>2</sub> or O<sub>3</sub> (Noormets *et al.* 2001).

Growth in elevated CO<sub>2</sub> or O<sub>3</sub> changes plant structure through its effects on primary and secondary meristems of shoots and roots (Pritchard *et al.* 1999). Plant growth and development are closely related to the concentration of

some endogenous plant growth regulators, such as indole-3-acetic acid (IAA). Here we want to show that the effects of elevation in CO<sub>2</sub> and O<sub>3</sub> concentration on ginkgo growth are related to IAA catabolism modification and changes in the leaf IAA content.

Since peroxidase (POD) participates in IAA catabolism, it can modify the hormonal balance in plants, thus leading to a modulation of morphogenesis (Ros Barceló and Muñoz 1992). On the other hand, it has been proposed that IAA oxidase (IAAO) activities are similar to POD and may help regulate IAA content (Mohamed-Yasseen and Splittstoesser 1990). Thus IAA content, POD and IAAO activities were followed in relation to the elevation of CO<sub>2</sub> and O<sub>3</sub> alone and in combination. The following experiments were designed to see the effects of elevated CO<sub>2</sub> or O<sub>3</sub> on IAA content and IAA catabolism in ginkgo leaves and whether elevated CO<sub>2</sub> ameliorates the impact of elevated O<sub>3</sub>.

The experiment was conducted in Shenyang Arboretum, Chinese Academy Science, located in the populated central area of Shenyang (41°46'N, 123°26'E),

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*Abbreviations:* IAA - indole-3-acetic acid; IAAO - indole-3-acetic acid oxidase; POD - peroxidase.

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Liaoning province, China. Six-year-old ginkgo (*Ginkgo biloba* L.) 1.2 - 1.5 m in height came from the same maternal plant. All trees were planted individually in pots (50 cm diameter × 40 cm height) that contained fertile soil. A factorial design of 9 open-top chambers (OTCs, diameter 4 m, high 3 m and distance between neighbor OTCs is 4 m), containing high CO<sub>2</sub>, high O<sub>3</sub> and ambient air treatment was randomized in three blocks; each treatment had 3 replicates. At the beginning of April 2005 trees were transferred to the OTCs and randomized. From 1 June to 30 September trees in the OTCs were exposed to control (ambient O<sub>3</sub>, 55 nmol mol<sup>-1</sup> + ambient CO<sub>2</sub>, 350 μmol mol<sup>-1</sup>, C), elevated CO<sub>2</sub> (high CO<sub>2</sub>, 700 μmol mol<sup>-1</sup> + ambient O<sub>3</sub>, EC) and elevated O<sub>3</sub> (high O<sub>3</sub>, 80 ± 8 nmol mol<sup>-1</sup> + ambient CO<sub>2</sub>, EO). From 1 August to 30 September, one-third trees pre-exposed to elevated CO<sub>2</sub> were transferred to elevated O<sub>3</sub> (EC-EO) and one-third to control (EC-C); one-third trees pre-exposed to elevated O<sub>3</sub> were transferred to elevated CO<sub>2</sub> (EO-EC), one-third to control (EO-C). Ozone was produced from bottled oxygen by electrode discharge (O<sub>3</sub> generator, model GP-5J, Shenyang, China) and pure CO<sub>2</sub> was injected into the chambers from cylinders. An O<sub>3</sub> transducer (S-900, Auckland, New Zealand) and CO<sub>2</sub> infrared transducer (*SenseAir*, Delsbo, Sweden) were used to monitor O<sub>3</sub> and CO<sub>2</sub> concentrations inside the chambers. Elevated CO<sub>2</sub> was applied for 24 h d<sup>-1</sup>. Elevated O<sub>3</sub> was applied for 8 h d<sup>-1</sup> in the daytime (09:00 - 17:00). Healthy *Ginkgo biloba* leaves were collected at 09:00 every 20 d.

The collected leaves were frozen in liquid nitrogen and stored at -70 °C until use. The method for quantification of IAA by ELISA was as described Yang *et al.* (2001). The mouse monoclonal antigens and antibodies against IAA and immunoglobulin G-horseradish peroxidase (IgG-HRP) used in ELISA were produced at the Phytohormones Research Institute (China Agricultural University). The IAA oxidase activity was measured following the method described by Bryant and Lane (1979). The peroxidase activity was determined by the method of Scalet *et al.* (1991). All the determinations of IAA, IAAO and POD were done for samples collected from 3 repeated OTCs in the same treatment. ANOVA was used to analyze the results and the means were compared by the Tukey test at 5 % probability levels.

After 40 d exposure to elevated CO<sub>2</sub>, the IAA content in leaves was significantly increased compared with the control and maximum was observed at 100 d after exposure ( $P \leq 0.05$ ; Fig. 1). Whereas elevated O<sub>3</sub> had no significant effect on the IAA content through the whole experimental period. When trees pre-exposed to elevated CO<sub>2</sub> were transferred to elevated O<sub>3</sub> (EC-EO) or control chamber (EC-C), there was a marked ( $P \leq 0.05$ ) reduction of IAA content and EC-EO showed more apparent effect (Fig. 1). The reverse changes were shown when trees pre-exposed to elevated O<sub>3</sub> were transferred to elevated CO<sub>2</sub> (EO-EC) or control chamber (EO-C).

IAAO activity was decreased by elevated CO<sub>2</sub> exposure over the whole season. At 40 d exposure to elevated CO<sub>2</sub>, IAAO activity was slightly reduced

compared with control, but did not reach the level of statistical significance, then sharply decreased and minimum was observed after 60-d exposure ( $P \leq 0.05$ ). IAAO activity remained unchanged under elevated O<sub>3</sub> treatment compared with control (Fig. 1). IAAO activities of EC-EO or EC-C were enhanced and the enhancement was more apparent in the trees transferred to elevated O<sub>3</sub>. When trees pre-exposed to elevated O<sub>3</sub> were transferred to elevated CO<sub>2</sub> for a long period (60 d), the IAAO activity was decreased (Fig. 1).

POD activity was significantly ( $P \leq 0.05$ ) decreased compared with the control by exposure to elevated CO<sub>2</sub> in the whole season. The difference was more apparent as early as after 20 d exposure and then in the late season beginning from day 80. The effect of elevated O<sub>3</sub> on enhancing POD activity was seen only after 40 d and longer exposure resulted in diminishing of the effect (Fig. 1). POD activities of EC-EO and EC-C were very low, and the maximum POD activity of EC-EO was observed after 60 d treatment (Fig. 1). When trees exposed to elevated O<sub>3</sub> were transferred to elevated CO<sub>2</sub>, there was a reduction of POD activity after 40 d transfer, but for trees transferred to control, there was an increase.

It is well known that sugars are the most readily available form of energy in plant body and plant hormone metabolism is dependent on the supply of sugars. Elevated CO<sub>2</sub> increases photosynthesis, and thus increases the sugar concentration in the plants, which in turn may stimulate plant growth and maybe also affected hormone concentrations. Our unpublished results showed that the growth increment of ginkgo terminal shoot in EC was increased by 44 % compared with C from 20 d to 90 d of exposure. The total leaf area measured on 30 September was increased by 5 %. The result was consistent with our findings that the amount of IAA was significantly increased in ginkgo leaves in EC chambers. A study on effects of elevated CO<sub>2</sub> on IAA in the expanding leaves and root tips of orchid found that IAA content increased under CO<sub>2</sub> enrichment (Li *et al.* 2002). Of course, the enhancement in growth by EC exposure, besides the IAA effect, should also be related to the action and interaction of other phytohormones (Yong *et al.* 2000, Teng *et al.* 2006).

POD was measured because it is considered that an early increase in POD accounted for the subsequent decrease in IAA concentration (Hausman 1993). The results of the present study indicate that the activities of POD and IAAO in ginkgo were inhibited by EC treatment, and the enhancement of IAA may be related to the decrease in activities of IAAO and POD. We indicate that there are two dates (60 and 100 d of exposure) when the IAA contents of EC were much higher compared to those of C or EO (Fig. 1). This difference after 60-d exposure seems to be related to a lower IAAO activity, whereas after 100-d exposure to a lower POD activity (Fig. 1). But Lagrimini *et al.* (1997) showed no change in IAA content under significant overexpression or deficiency in peroxidase. The lower POD activity after 40 d of the EO-EC treatment did not probably affect IAA content.

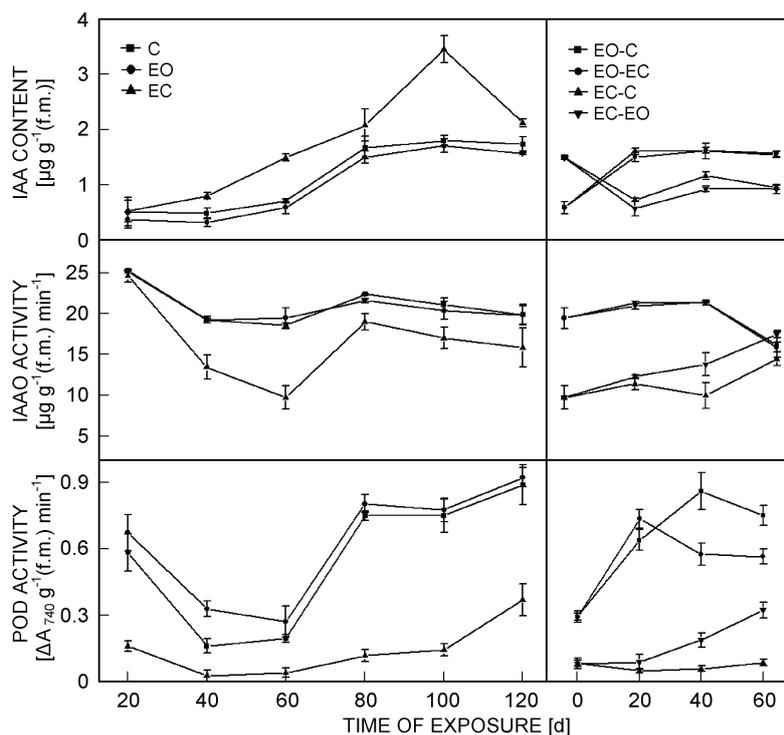


Fig. 1. IAA content, and activities of IAAO and POD in *Ginkgo biloba* leaves grown in control conditions (C), under elevated  $O_3$  (EO) or elevated  $CO_2$  (EC) for 120 d (on the left). The same parameters were measured in plants which were firstly grown in EC and then transferred into EO (EC-EO) or into control chamber (EC-C) or in plants firstly grown in EO and then transferred into EC (EO-EC) or into control chamber (EO-C) (on the right). Means  $\pm$  SE,  $n = 3 - 5$ .

The effects of POD on the catabolism of IAA may be neglected under special circumstances and require further investigation.

It has been observed that in ozone-treated leaves POD activity increases usually shortly after EO exposure to allow plants scavenging of reactive oxygen species (Hadži-Tašković and Vuletić 2007). Later, this effect might disappear, either due to morphological/anatomical adaptations of the leaves or due to exhausting of sugar pools. The ginkgo leaves after 40-d exposure to EO (Fig. 1) were well in line with the above mentioned ozone response of leaves.

In order to investigate mutual effects of elevated  $CO_2$  and elevated  $O_3$  concentrations, we designed the treatment when ginkgo trees pre-exposed to elevated  $CO_2$  were

transferred to  $O_3$  (EC-EO) or control chamber (EC-C), and those pre-exposed to elevated  $O_3$  were transferred to elevated  $CO_2$  (EO-EC) or control chamber (EO-C). Our data showed that pre-exposure to elevated  $CO_2$  increased ginkgo sensitivity of elevated  $O_3$ . For example, direct exposure to elevated  $O_3$  had no significant effect on the IAAO activity, but pre-exposure to elevated  $CO_2$  significantly increase the IAAO activity of leaves in elevated  $O_3$ . Post-exposure to elevated  $CO_2$  did not change IAA content and IAAO activity but decreased POD activity. The downregulation of POD suggests that, under a high  $CO_2$  concentration, plants suffer less from oxidative stress than when grown under an ambient  $CO_2$  concentration (Omasa *et al.* 2002).

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