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Impact of Elevated CO_2 and O_3 Concentrations on Biogenic Volatile Organic Compounds Emissions from *Ginkgo biloba*

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Abstract In natural environment with ambient air, ginkgo trees emitted volatile organic compounds 0.18 μg g^{-1} h^{-1} in July, and 0.92 μg g^{-1} h^{-1} in September. Isoprene and limonene were the most abundant detected compounds. In September, α-pinene accounted for 22.5% of the total. Elevated CO_2 concentration in OTCs increased isoprene emission significantly in July ($p < 0.05$) and September ($p < 0.05$), while the total monoterpenes emission was enhanced in July and decreased in September by elevated CO_2. Exposed to elevated O_3 increased the isoprene and monoterpenes emissions in July and September, and the total volatile organic compounds emission rates were 0.48 μg g^{-1} h^{-1} (in July) and 2.24 μg g^{-1} h^{-1} (in September), respectively. The combination of elevated CO_2 and O_3 did not have any effect on biogenic volatile organic compounds emissions, except increases of isoprene and Δ3-carene in September.

Keywords Elevated CO_2 and O_3 · Biogenic volatile organic compounds

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Global atmospheric CO_2 concentration has risen by nearly 30% since preindustrial times, largely because of the industrial emissions, especially for urban atmospheric environment. Similarly, background concentrations of tropospheric ozone concentration related to emissions of nitrogen oxides and volatile organic compounds (VOCs) from fossil fuel, such as thermal generation and transportation, have increased from 10 to over 40 nmol mol^{-1} (IPCC 2001). Such changes of tropospheric atmospheric environment are expected to affect the capacity of plants to emit BVOCs (Constable et al. 1999; Llusia et al. 2002).

More than 1150 Tg C y^{-1} of biogenic volatile organic compounds (BVOCs) are released into the atmosphere, almost double the anthropogenic emission. It would be rapidly oxidized in the atmosphere leading to the formation of ozone and other secondary pollutants when anthropogenic nitrogen oxides and sunlight are present, thus affecting the atmospheric composition (Rosenstiel et al. 2003; Calfapietra et al. 2007). BVOCs increases the atmospheric lifetime of greenhouse gas methane by competing for OH radicals, the principal atmospheric oxidizing compounds of methane, then have an indirect impact on global changes (Sallas et al. 2001; Loreto et al. 2001a). Moreover, the emission of volatile organic compounds represents some percents of carbon loss, which were significant for the missing sink.

Rosenstiel et al. (2003) found that increased atmospheric CO_2 concentration (to 800 μmol mol^{-1} and 1,200 μmol mol^{-1}) reduced agriforest ecosystem isoprene production by 21% and 41%, while Constable et al. (1999) estimated that isoprene emission will increase by 80% by simulating an increase in temperature coupled with a doubling in atmospheric CO_2 concentration. Loreto and Velikova (2001b) suggested that isoprene and monoterpenes have antioxidant properties and can protect plant for

ozone damage. Velikova et al. (2005a) demonstrated that O_3 exposure increased BVOCs emission from reed, and then decreased the concentration of nitric oxide in leaves, which may be a mechanism to control dangerous compounds formed under abiotic stress conditions. The studies investigating the single effects of elevated CO_2 or O_3 concentration on the BVOCs emission of plants are common, but there were little about the interactions of CO_2 and O_3 in terms of their effect on BVOCs emissions from plants, especially for urban trees. Vuorinen et al. (2005) reported the interactive effects of elevated CO_2 and O_3 concentrations. They found that elevated CO_2 and O_3 in combination did not have any effect on BVOCs emissions from silver birch clones in an open top chamber.

Urban vegetation is often characterized by the presence of exotic species interspersed with natural vegetation. The different management and the stressful conditions to which urban trees are subjected may affect BVOCs emission rates (Centritto et al. 2005). Urban tree canopies in some parts of the world may influence urban ozone chemistry in a significant way. Here, we analyze the BVOCs emission rates and emission patterns of *Ginkgo biloba*, one of the dominant urban tree species in Shenyang, in open top chamber. The aim of this paper is to understand the effects of elevated tropospheric CO_2 and O_3 concentrations on BVOCs emissions.

Materials and Methods

The measurements were performed in Shenyang (41°46′31.29″ N, 123°26′27.51″ E) in the northeastern China. Five-year-old *Ginkgo biloba* trees were planted on ground (loamy soil, no extra fertilizer) of twelve open top chambers (OTCs), in May 2007. Three repeats for ambient air (CK), elevated CO_2 (EC), elevated O_3 (EO) and elevated $CO_2 + O_3$ (EC + EO). The trees were randomly distributed among the chambers, 20 trees per chamber.

Mean temperature is about 25°C (Maxair = 35.7°C, Minair = 11.4°C) and mean relative air humidity is 50.2% in OTCs. The trees were exposed to high CO_2 (700 μmol mol^{-1}) for 24 h day^{-1} in EC and EC + EO chambers, and exposed to high O_3 (80 nmol mol^{-1}) for 9 h (08:00–17:00) day^{-1} in EO and EC + EO chambers. The fumigation periods were from 15 June to 1 October in 2007.

To collect air samples, transparent plastic bags were used to cover well-lighted branches and the base of each bag was carefully closed. Background BVOCs emission was checked in empty plastic bags and the emission was found negligible. Care was taken to avoid BVOCs release due to "rough handling", for damaged or crushed foliages which usually increased BVOCs emission remarkably.

The samples were collected from inside of the bags on glass adsorbent tubes (11.5 cm long and 0.4 cm internal diameter) filled with Tenax-TA, Carboxen 1000 and Carbosieve SIII (Supelco Inc., Bellefonte, PA, USA) using a constant-flow type pump. The flow rates were 100 mL min^{-1} and the sampling time was 5 min. Samples were replicated four times. Sample tubes were pre-cleaned prior to use, and kept at 4°C until analysis.

After sampling, the leaves enclosed in the bags were removed from the trees, and placed in a drying oven at 60°C for 48 h. The dry weights were used for normalization of BVOCs emission rate to unit leaf mass.

Biogenic volatile organic compounds were separated and detected by a gas chromatographic with flame ionization detection. Desorption and analysis of BVOCs was carried out using a thermal disorber sample injection system (ACEM 9300, CDS, USA) connected by a thermal transfer line maintained at 250°C to a gas chromatograph (14B, Shimadzu, Japan) with FID. The pre-concentrated samples were thermally desorbed a 250°C for 5 min at 30 mL min^{-1}, and secondary desorption was at 275°C for 2 min. The trapping and desorption efficiency of liquid and volatilized standards was practically 100%. A fused silica capillary (60 m × 0.32 mm × 0.25 μm) DM-1 column was used to detect and quantify BVOCs. An initial oven temperature of 40°C was maintained for 5 min, and then increased to 120°C at 2°C min^{-1} followed by an increase at 20°C min^{-1} to 200°C for 10 min.

BVOCs emission measurements were repeated 4 times. Data are shown as means ± standard errors. Mean separation and statistical differences between treatments were assessed with one way analysis of variance (ANOVA) and differences statistically significant at $p < 0.05$.

Results and Discussion

Emission of isoprene and 6 monoterepenes were quantified. Isoprene and limonene were the most abundant compounds in July and September. In September, α-pinene accounted for 22.5% of the total BVOCs (Table 1). In control chambers, the BVOCs emission rates were affected by the time of the growing season, emissions being significantly higher in September (0.92 μg g^{-1} h^{-1}) than in July (0.18 μg g^{-1} h^{-1}) ($p < 0.05$) (Fig. 1). The results were similar to our experiment on Chinese pine in Shenyang.

Elevated CO_2 concentration increased isoprene emission significantly in July ($p < 0.05$) and September ($p < 0.05$) (Fig. 1), while the total monoterpenes emission was enhanced by CO_2 in July but decreased in September. The relative abundances of BVOCs were changed by elevated CO_2 evidently (Table 1). Isoprene accounted for 49.25% (July) and 46.98% (September) of the total terpenoid in the

Table 1 Effects of elevated CO_2 and O_3 concentrations on the relative abundances (%) of the volatile organic compounds emitted from ginkgo (*Ginkgo biloba*) leaves

Date	Controls	Isoprene	α-Pinene	Camphene	β-Pinene	Carene	Limonene	Ocimene
July	CK	29.92	7.14	1.83	11.77	–	29.88	19.47
	EC	49.25	10.65	2.33	13.73	–	16.58	7.47
	EO	22.64	12.55	3.58	4.49	3.31	24.54	28.89
	EC + EO	46.88	8.59	8.40	8.56	–	8.55	19.02
Sep	CK	33.41	22.54	2.25	3.83	1.32	29.67	6.97
	EC	46.98	8.39	1.73	3.91	2.34	36.24	0.41
	EO	22.95	10.97	7.04	2.39	1.81	40.67	14.18
	EC + EO	49.98	8.49	2.16	3.15	1.65	26.72	7.85

EC treatments. According to the source-sink balance theory, increased carbon resource at elevated CO_2 may result in carbon allocation to secondary metabolites, and lead to increased emissions of BVOCs. However, the results about effect of high CO_2 fumigation on BVOCs emissions in field and potted experiments were conflict and difficult to be interpret. Constable et al. (1999) estimated that isoprene emission will increase by 80% by simulating an increase in temperature coupled with a doubling in atmospheric CO_2 concentration. On the other hand, a decrease of isoprene emission rates was observed under elevated CO_2 (Rosenstiel et al. 2003). The emission of from *Quercus ilex* plants grown at elevated CO_2 in open top chambers (OTC) was generally lower (Loreto et al. 2001a). The decrease in emission rate has been attributed to a decrease in the availability of precursors or inhibition of synthase activity in the isoprene and monoterpenes synthesis pathways occurring under elevated CO_2. However, the estimations at large scale may differ from the effects at the leaf level because of the indirect effects of global change on leaf area index, canopy temperature, water and nutrient availability and length of growing season (Calfapietra et al. 2007).

Exposure to elevated O_3 increased the isoprene and monoterpenes emission rates significantly in July ($p < 0.05$) and September ($p < 0.05$) (Fig. 1), and the total emission rates were 0.48 μg g^{-1} h^{-1} (in July) and 2.24 μg g^{-1} h^{-1} (in September), respectively. In July, Δ3-carene was detected only in EO treatment (1.56%) (Table 1). Our result is similar to the report of Velikova et al. (2005a), which indicates that exposure to high O_3 caused a significant increase of isoprene emission from reed. At the same time, in laboratory experiments, ozone fumigation was found to stimulate emission of monoterpenes in *Quercus ilex* (Loreto et al. 2004). In contrast, Velikova et al. (2005b) reported a decrease emission of isoprene in *Quercus pubescens*. Calfapietra et al. (2007) investigated the isoprene synthase protein levels and isoprene emission in aspen trees exposed to elevated ozone in FACE experimental site, and then found that the drop in isoprene synthase protein levels lead to a drop in the isoprene emission rate. A species-specific effect was reported in monoterpene emission rates from Mediterranean tree species exposed to elevated O_3 concentrations in open top chambers (Llusia et al. 2002). The presence of isoprenoids in the leaves has a strong influence on the sensitivity to O_3, Loreto et al. (2004) concluded that monoterpenes produced by *Q. ilex* leaves share the same biosynthetic pathway and function as isoprene, and demonstrated that leaves fumigated with ozone are stimulated to emit monoterpenes. Penuelas and Llusia (2002) had shown that monoterpene fumigation decreases photo-damage when photorespiration is inhibited and speculated that this may indicate antioxidant activity of these compounds. In addition, leaves emitting isoprene showed less damage by high O_3 concentrations than non-emitting leaves (Loreto and Velikova 2001b). Isoprenoids may stabilize membranes, making them resistant to denaturation caused by heat, ozone and perhaps other stresses, or they may react with reactive molecules in the mesophyll, indirectly decreasing the rate of formation of reactive oxygen species that can irreparably damage the photosynthetic apparatus.

There was not significant difference between EC + EO and CK treatments on BVOCs emission rates, except isoprene ($p < 0.05$) and Δ3-carene ($p < 0.05$) in September (Fig. 1). Even less was known about the joint effect of elevated CO_2 and O_3 on the rates and patterns of BVOCs emission by plants. Our result indicated that the combination of elevated CO_2 and O_3 had special-specific effect on BVOCs emissions (Fig. 1). Similar observation was made by Vuorinen et al. (2005), who found that growing under $CO_2 + O_3$ fumigation did not have any effect on emissions of total monoterpenes, sesquiterpenes, or individual quantified VOCs of silver birch clones in an open top chamber. Some researches about interactions on plants secondary metabolic found that elevated CO_2 can mitigate the effects of elevated O_3. However, Calfapietra et al. (2007) reported that elevated CO_2 could not alter the effect of elevated O_3 on isoprene synthase transcript levels,

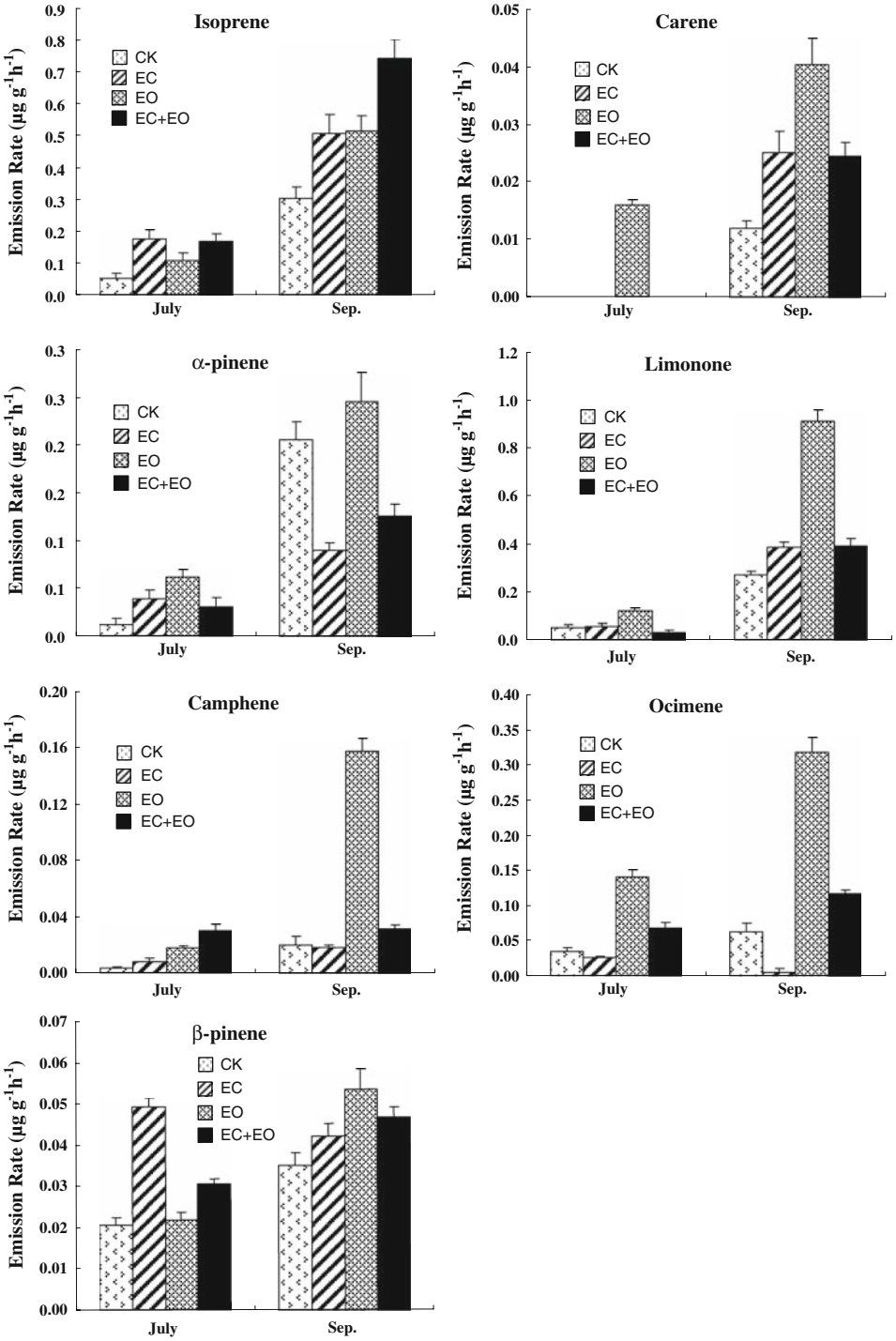

Fig. 1 Emission rates of volatile organic compounds from ginkgo *(Ginkgo biloba)* grown at ambient air (CK), elevated CO_2 (EC), elevated O_3 (EO) or at elevated $CO_2 + O_3$ (EC + EO). Data shown are the means and standard deviation of four replicates (μg g^{-1} h^{-1})

isoprene synthase protein levels and isoprene emission rate of aspen trees. Sallas et al. (2001) observed that the terpenes in pine needles were not affected significantly by the combination of elevated CO_2 and O_3 concentrations, except α-pinene and limonene. Furthermore, temperature, light and herbivores also affect the BVOCs emissions, which may conceal the effects of these atmospheric gases. More researches are needed to understand the complicated biosynthesis and emission mechanisms.

In conclusion, elevated CO_2 or O_3 concentration singly has considerable effect on BVOCs emissions of ginkgo, but the effect of elevated CO_2 and O_3 in combination did not change the BVOCs emission rates significantly. Some BVOCs are believed to have antioxidant activity, which protects plants from ozone damage, but the mechanisms were unclear. Furthermore, the effect of these atmospheric gases on BVOCs emission may be affected by some abiotic and biotic

conditions, such as temperature, light, water and herbivores, especially in urban environment.

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