THE INDIVIDUAL AND COMBINED EFFECTS OF OZONE AND SIMULATED ACID RAIN ON CHLOROPHYLL CONTENTS, CARBON ALLOCATION AND BIOMASS ACCUMULATION OF ARMAND PINE SEEDLINGS

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Abstract. The seedlings of armand pine (*Pinus armand* Franch.) were applied to exposure, alone or in combination, to charcoal filtered air (CF) or ozone (O_3) at 300 ± 15 nl/l(ppb) for 8 h a day, 6 days a week, and simulated rain of pH 6.8, 3.0 or 2.3, six times a week, alone and in combination, for 14 weeks from June 15 to September 20, 1993. No significant interactive effects of O_3 and simulated acid rain were observed on chlorophyll contents, carbon allocation and biomass accumulation of the seedlings in the present study. The O_3 caused reductions in biomass accumulation of whole-plant and below-ground parts, but not that of above-ground parts without an acute visible foliar injury. At the same time, the O_3 reduced R/S ratio, but raised F/C ratio. Therefore, O_3 also altered carbon allocation pattern. On the other hand, chlorophyll contents were increased by simulated acid rain, but other determined parameters were not altered.

Keywords: Ozone; acid rain; chlorophyll contents; carbon allocation; biomass accumulation.

1. Introduction

Forest declines were observed in Europe and North America (Ashmore, 1985; Johnson and Siccama, 1983; Krause *et al.*, 1986; Thornton *et al.*, 1994). In China, similar problem, for example, the decline of armand pine (*Pinus armand* Franch.) has been also reported (Ma, 1988). Gaseous air pollutants such as O_3 and SO_2, and acid rain are considered to be possible contributors or causes of dieback of trees and forest decline (Ashmore, 1985; Johnson and Siccama, 1983; Feng *et al.*, 1986; Ma, 1988; Totsuka, 1993; Thornton *et al.*, 1994). Many researchers have already investigated the effects of O_3 and acid rain, alone and in combination, on seedlings of forest trees mainly grown in America and Europe (Chappelka *et al.*, 1985; Edwards *et al.*, 1992; Reich *et al.*, 1986). However , there are few studies on the effects of both stresses on trees grown in Asian countries (Miwa *et al.*, 1993). Therefore, it is necessary to clarify their effects on growth and physiological functions of more trees in Asia in further detail.

Ozone produced by photochemical reactions is a phytotoxic air pollutant. Many areas in China are experiencing the elevated levels of ozone with the dramatical increase of the traffic and petrochemical industry. Especially, in Lanzhou, Northwestern China, the peak concentration, maximum hourly average concentration and summer daily

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average concentration recorded in 1982 were 450, 332 and 51 ppb (nl. l-1), respectively (Tang *et al.*, 1987). In Beijing, a peak concentration of O_3 at 160 ppb was reported (Tang *et al.*, 1987). On the other hand, acid rain has been observed in many areas of China since acid rain monitoring begun in 1979 (Zhao and Sun, 1986). Since they co-occur in most of polluted areas, it is necessary to study their effects on trees in single or combined situation. Armand pine was selected as the test species because it widely distributed in mid-high altitude areas and northern China. In the present study, the exposure of armand pine seedlings to relatively high concentration of O_3 was performed because no growth or foliar nutritional effects on either mature or juvenile scions of red spruce that had been exposed to O_3 concentration as high as 300 $\mu g \cdot m^{-3}$ above ambient for two growing seasons (Rebbeck *et al.*, 1993). Moreover, significant damage to Japanese cedar, which also is a wood species of gymnosperae as same as armand pine, by three month exposure to 300 ppb O_3 was not observed in our laboratory (Miwa *et al.*, 1993) and high concentrations of O_3 were documented as stated above.

2. Materials and Methods

Seeds of armand pine (*Pinus armand* Franch.) originated from Yunnan province, China were sown in 300 ml plastic pots containing forest surface soil (Umbric Andosols). The seedlings were grown in a greenhouse (one seedling per pot). After eleven weeks , 60 seedlings (average height at the beginning of the exposure: 3.7 cm) were used for the experiments mentioned below.

Simulated acid rain was prepared with deionized water and a mixture of 8:1 molar ratio of nitric and sulfuric acids. The solution pH was adjusted to 3.0 or 2.3 because similar acidity levels of simulated acid rain treatments were used (Takemoto *et al.*, 1988; Miwa *et al.*, 1993; Reich *et al.*, 1986) and several reports showed pH of ambient acid rain as low as pH 2.3 (Cape, 1993). The target pHs 5.60-simulated rain contained the deionized water only and had an actual pHs of 6.80 (control rain). Simulated rain was applied to the seedlings using a sprayer above the top of the seedlings for 10 minutes from 8:30, 6 times per week, for 14 weeks from June 15 to September 20, 1993. The rainfall of a rain event was 3.3 mm and total precipitation for 14 weeks was 277.2 mm.

After each treatment with simulated rain, the seedlings were exposed to O_3 at 300$\pm$ 15 nl/l (ppb) or charcoal-filtered air (CF, control) for 8 h per day from 9:00 to 17:00, 6 days a week, for 14 weeks in the gas exposure chambers placed in a laboratory. Air temperature, relative humidity and light intensity at the top of the seedlings placed in the chambers were maintained at 30 $\pm$3 °C, 70$\pm$10% and 410$\pm$20 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The O_3 was generated with a silent electrical discharge O_3 generator (Nihon Ozone Co., Model O-1-2), and O_3 concentrations in the chambers were continuously monitored with a UV absorption O_3 detector (Dashibi Co., USA, Model DY-1500). At the end of the daily treatment, all seedlings were returned to the naturally-lit phytotrons where they were kept until 8:30 of the following day. The air temperature and relative humidity in the phytotrons were maintained at 30/22 °C (day/night), 70$\pm$10%, respectively. Air entered the phytotrons as charcoal filtered air.

At the end of the exposure period of 14 weeks, leaves were sampled in the middle storey of seedling canopies. The 100 mg fresh leaf samples were used for every measurement. The chlorophyll was extracted from leaves with a mixture of acetone, ethanol and deionized water in a ratio of 4.5:4.5:1. Absorption of the extract was measured at 663 and 645 nm with a spectrophotometer (Shimadzu Co. , Model UV-1200), and chlorophyll $_{a+b}$ contents were calculated from the formulae proposed by Arnon (1949). Then, all the seedlings were harvested (final harvest) and dried at 80 °C for one week. The dry weights of above-ground parts and below-ground parts were measured.

3. Results

The O_3 did not induce the acute foliar injury symptom, bronzing of leaves throughout the exposure period, but premature senescence with yellowing of basal oldest leaves became evident in the early September (the 12th week for exposures). No visible foliar injury of the seedlings was produced by simulated acid rain of pH 2.3 or 3.0.

The O_3 significantly reduced chlorophyll contents. On the contrary, simulated acid rain significantly increased chlorophyll contents (Figure 1). However, no significant interactive effects of O_3 and simulated acid rain on chlorophyll contents were observed (ANOVA results not shown here).

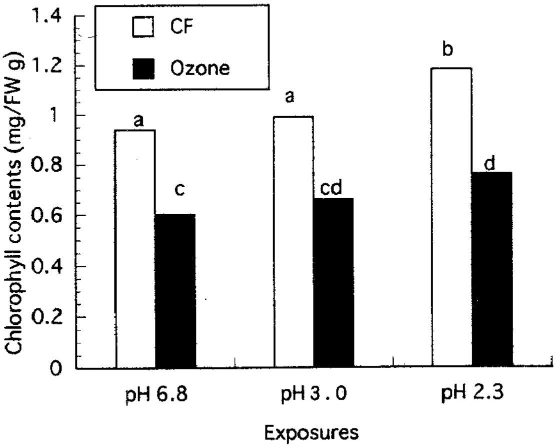

Fig 1. Effects of O_3 and simulated acid rain on chlorophyll contents of armand pine seedlings. Each value represents the mean of 10 determinations. Means followed by different letters are significantly different according to Duncan's multiple range test ($p<0.05$).

The interactive effects of O_3 and simulated acid rain on biomass accumulation of above-ground parts, below-ground parts and whole-seedling, and carbon allocation were not significant (Table I and II).

As shown in Table I, total biomass accumulation of the seedlings exposed to O_3 reduced by 11.9% of that of the seedlings exposed to charcoal-filtered air (CF). The biomass accumulation of below-ground parts of the O_3-exposed seedlings also reduced by 27.4% of that of the seedlings exposed to CF. However, that of above-ground parts were not affected by O_3.

As shown in Table II, dry weight ratio of the assimilation organs to dissimilation

TABLE I

Effects of O_3 and simulated acid rain on biomass accumulation of armand pine seedlings [1]

	above-ground part (g)	below-ground part (g)	total (g)
Rain			
pH 6.8	0.590	0.410	0.996
pH 3.0	0.627	0.390	1.018
pH 2.3	0.609	0.367	0.976
O_3			
CF	0.610	0.449	1.060
300 ppb	0.608	0.326	0.934
ANOVA Effects[2]			
O_3	ns	***	*
Rain pH	ns	ns	ns
O_3 x Rain	ns	ns	ns

[1]The seedlings were exposed to CF or O_3 at 300 ppb and simulated acid rain of pH 2.3, 3.0, or control rain (pH 6.8), alone and in combination, for 14 weeks from June 15 to September 20, 1993. Each value represents the mean of 20 determinations.

[2]Two-factor ANOVA results: ns = not significant at $p < 0.05$; * $p<0.05$; *** $p<0.001$.

TABLE II

Effects of O_3 and simulated acid rain on carbon allocation: the dry weight ratio of assimilation parts to dissimilation parts (F/C) and that of below-ground parts to above-ground parts (R/S) of armand pine seedlings.

	F/C	R/S
Rain		
pH 6.8	0.760	0.696
pH 3.0	0.807	0.648
pH 2.3	0.870	0.600
O_3		
CF	0.688	0.748
300 ppb	0.937	0.548
ANOVA Effects[1]		
O_3	***	***
Rain pH	ns	ns
O_3 x Rain	ns	ns

[1]Two-factor ANOVA results: ns = not significant at $p < 0.05$; ** $p<0.01$; *** $p<0.001$. See legend in Table 1.

organs (F/C) were increased by 36.2%, but that of the below-ground parts to above-ground parts (R/S) were decreased by 26.7%. Significant effects of simulated acid rain on the below-ground part or the above-ground part biomass accumulation and carbon allocation of armand pine were not exhibited.

4 . Discussion

The combined effects of O_3 and simulated acid rain on plants shows inconsistency in some reports, that is, significant interactive effects (Troiano *et al.*, 1983; Takemoto *et al.*, 1988) and no interactive effects (Reich *et al.*, 1986, Edwards *et al.*, 1992) of both stresses. In the present study, there were no interactive effects of O_3 and simulated acid rain on chlorophyll contents, carbon allocation and biomass accumulation of armand pine seedlings (Table I and II, Figure 1). Therefore, we discuss the single effects of O_3 or simulated acid rain on the seedlings henceforth.

In the present study, O_3 significantly reduced chlorophyll content. Our results were in agreement with these of Sasek and Richardson (1989), Schweizer and Arndt (1990) and Violin *et al.* (1993). On the other hand, acid rain enhanced chlorophyll contents (Figure 1). The chlorophyll content increase induced by simulated acid rain may result from a fertilizer effects of NO_3^- contained in the acid rain. Similar results were reported (Eamus and Fowler, 1990; Shan *et al.*, 1994; Shan *et al.*, 1995).

The hidden damage to plant growth was induced by O_3 without visible foliar injury (Pye, 1988). In this present study, biomass accumulation of whole-seedlings and below-ground part of armand pine was reduced by O_3 in absence of acute visible foliar injury, but that of above-ground part was not affected. Therefore, less biomass accumulation was due to the reduction of below-ground biomass accumulation. In addition, O_3 reduced the R/S, but enhanced the F/C (Table II). These results show that O_3 induces not only inhibition of biomass accumulation, but also alteration of carbon partitioning in armand pine. Especially, it is noted that more photosynthates were retained in leaf, while less translocation to root. It was reported that O_3 increased the retention of assimilate in leaves (Tingey *et al.*, 1976; McLaughlin *et al.*, 1982). Miller (1987) has reported that the respiratory CO_2 produced by the root of O_3-exposed bean plants is decreased. Edwards *et al.* (1992) also reported that root respiration rate reduced in the seedlings of loblolly pine exposed to twice-ambient O_3. Miller (1987) considered that the inhibitory effects of O_3 on the respiration of roots is a consequence of reduced supply of assimilate. These and previous results indicated that the O_3-induced reduction in biomass accumulation of below-ground parts was caused by decrease of downward translocation of assimilates, rather than the increase of dry matter consumption by root respiration.

It is concluded that tropospheric O_3 poses a greater threat to armand pine growth than acid rain does at least in the short term, but it is not excluded that there are detrimental effects of acid rain in the long term. At the same time, we must state here that in this experiment, the seedlings were used and might not perfectly represent the mature tree case. More and further research is necessary for a better understanding to responses of mature tree to acid rain and ozone.

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