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Arbuscular mycorrhizal fungi alter the response of growth and nutrient uptake of snap bean (*Phaseolus vulgaris* L.) to O_3

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Abstract

The effects of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* on the responses to elevated O_3 in growth and nutrition of snap bean (*Phaseolus vulgaris* L. cv Guangzhouyuan) were investigated. Exposure was conducted in growth chambers by using three O_3 concentrations (20 (CF), 80 (CFO1) and 120 nL/L (CFO2); 8 hr/day for 75 days). Results showed that elevated O_3 slightly impacted overall mycorrhizal colonization, but significantly decreased the proportional frequency of hypha and increased the proportional frequency of spores and vesicles, suggesting that O_3 had significant effects on mycorrhizal structure. Elevated O_3 significantly decreased yield, dry mass and nutrient contents (N, P, K, Ca and Mg) in both non-mycorrhizal and mycorrhizal plants. However, significant interactive effects were found in most variables due to that the reduction by O_3 in the mycorrhizal plants was less than that in the non-mycorrhizal plants. Additionally, AMF increased the concentrations of N, P, Ca, and Mg in shoot and root. It can be concluded that AMF alleviated detrimental effects of increasing O_3 on host plant through improving plant nutrition and growth.

Key words: arbuscular mycorrhizal fungi; nutrition status; mycorrhizal colonization; elevated O_3; snap bean

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Introduction

Tropospheric ozone (O_3), the most important secondary air pollutant, is regarded to be one of the most potent and pervasive phytotoxic gaseous pollutants in the atmosphere (Krupa et al., 2001). Although the increasing trend in O_3 concentration has slowed down or has been decreasing in the USA and Europe (IPCC, 2007), surface O_3 concentration in peri-urban areas of East Asia is still rising due to rapid industrialization and urbanization (The Royal Society, 2008). In China, the means of daily O_3 concentration has been reported to reach more than 150 nL/L in some developed regions, such as the Beijing-Tianjin Region, Yangtze Delta, and Pearl Delta (Shao et al., 2006; Wang et al., 2007a), where ambient O_3 concentration has been shown to have a significantly negative impact on crop yields (Feng et al., 2003; Wang et al., 2007b).

The most obvious effect of ozone on vegetation is an accelerated senescence, accompanied by a decrease of photosynthetic rate, alteration of carbon allocation and biomass partitioning, yield loss, and seed quality alteration (Scebba et al., 2006; Feng et al., 2008, 2010; Feng and Kobayashi, 2009). These detrimental effects may attribute partly to the deterioration of plant nutrition status induced by O_3 stress. However, information on the effects of elevated O_3 on macronutrients other than nitrogen in herbaceous species is scarce and inconsistent (Tingey et al., 1986; Heagle et al., 1998; Fangmeier et al., 2002; Piikki et al., 2007). For example, O_3 exposure decreased Ca and Mg concentrations in the leaves of snap bean (*Phaseolus vulgaris* L.), while increased foliar concentration of Ca in soybean (*Glycine max* L. Merr.), and slightly changed K and P concentrations in the leaves of both crops (Tingey et al., 1986; Heagle et al., 1998).

Arbuscular mycorrhiza (AM) symbiosis is the most widespread mycorrhizal association type with plants that have true roots (Read et al., 2000). About 80%–90% land plants in natural, agricultural and forest ecosystems live in symbiosis with AM (Brundrett, 2002). Their effects were well-documented on the enhancement of the phosphorus uptake, and on other macro- and micro-elements, in stressful environment (Berta et al., 1995). However, few studies have addressed the influence of AMF on plant response to increasing O_3. Among the few studies, Brewer and

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Heagle (1983) reported that mycorrhizal soybean were less sensitive to O_3 than non-mycorrhizal soybean in a field experiment. In a greenhouse experiment, mycorrhizal tomato (*Lycopersicon esculentum* Mill cv Heinz 1350) plants were more sensitive than non-mycorrhizal plants when tomato plants exposed to 300 nL/L O_3 (O_3 fumigation lasted 3 hr each time, once a week for 9 weeks in total) (McCool and Menge, 1983, 1984). Similar result was also found in clover inoculated with *Glomus margarita* (Miller et al., 1997). Thus, it is difficult to make generalizations about the interactive effects of O_3 and AM symbiont on plants.

The objective of this study was to investigate whether AMF affects growth and nutrient uptake of snap bean (*Phaseolus vulgaris* L. cv Guangzhouyuan) under elevated O_3 conditions. We hypothesized that elevated O_3 affected significantly mycorrhizal colonization and plant growth due to carbon limitation to the AM symbiont.

1 Materials and methods

1.1 Soil preparation

The soil was collected from sub-surface (0–20 cm) in suburb, Haidian District, Beijing, China. The basic soil properties were as follows: fine-loamy, 0–20 cm depth, pH 8.0, organic matter 2.18%, N 0.13%, extractable P 37.2 mg/kg, available K 123.5 mg/kg. After air drying, the soil samples for cultivation were passed through 2 mm mesh sieves (Huier Instruments Ltd., Hangzhou, China). All soil samples and part of the AM inoculum were steamed at 121°C for 30 min on three consecutive days to eliminate indigenous mycorrhizal fungi. Polyvinylchlorid (PVC) pots (diameter 11 cm, depth 30 cm) were filled with 2 kg sterilized soil. At the bottom of pot, there were three drain holes covered by two gauze layers.

1.2 Arbuscular mycorrhiza inoculum

AMF *Glomus mosseae* was used. The inoculum was a mixture of spores, mycelium, sandy soil, and root fragments. One hundred gram inoculum contained about 800 spores. Dosages of AM inoculum or sterilized AM inoculum were 5% of the soil weight. Inoculum was uniformly mixed with soil. This created mycorrhizal (+M) and non-mycorrhizal (–M) treatment.

1.3 Host plant

Snap bean was selected as the host plant for this experiment. The seeds were surface sterilized with 10% H_2O_2 (Analytical reagent, Sinopharm Chemical Reagent Beijing Co., Ltd., China) solution for 10 min, then washed thoroughly with deionized water and germinated at 25°C in the dark for 48 hr. Four germinated seeds were sown in each pot. Plants were thinned to one seedling per pot 5 days after emergence (DAE). Pots were randomly moved to three growth chambers (1.2 m in length, 1.2 m in width, and 2.1 m in height), which were specially designed for air pollutants fumigation experiments. Each growth chamber contained six pots including three mycorrhizal plants and three non-mycorrhizal plants. The growth chambers were illuminated by fluorescence light (T12, Dongguang Fluorescent Lamp Factory, Shangyu, China) providing a photosynthetic photon flux density (PPFD) of approximately 280 μmol/(m^2·sec) at canopy height during 14 hr photoperiod. The temperature in growth chambers was between 20–25°C during daylight and between 15–20°C at night. Relative humidity was controlled at 50%–85%, which simulated the growth of snap bean in the field in late spring and early summer. During the experiment, plants were watered daily with a fine mist of water to avoid drought stress.

1.4 Ozone fumigation

O_3 was generated from pure O_2 by electric discharge (ozone generator, QHG-1, Yuyao, China) and then mixed with charcoal filtered air to obtain the concentration of (20 ± 5) (CF), (80 ± 5) (CFO1) and (120 ± 10) nL/L (CFO2). The plants were allowed to grow till 7 DAE to adapt to chamber environment before starting O_3 treatment. O_3 fumigation was kept for 8 hr/day (09:00 to 17:00) for 75 days. Mass flow controllers (Yikechuangjing Instruments Ltd., Beijing, China) were used to regulate the flow of O_3-enriched air to the growth chambers. O_3 concentration in the growth chamber was continuously monitored using an O_3 analyzer (ML 9810B, Teledyne Monitor Labs, Englewood, USA).

1.5 Experimental design

A two-way factorial experimental design with three levels of O_3 (CF, CFO1 and CFO2) and two levels of AMF (–M and +M) was used. AMF treatment in each chamber has three replicates. In each chamber, plants were rotated every day to minimize possible position effects. To reduce microclimate differences in chambers (radiation, temperature and humidity), plants were rotated among three chambers every week. This procedure should have reduced the chamber effects significantly, and thus the pot-to-pot variation within a chamber was used as a surrogate of true error variation.

1.6 Harvest and sample analysis

When plants were harvested, the root system was separated from the shoot, and carefully washed by deionized water to remove adhering soil particles.

Sub-samples of fresh root were cleared in 10% KOH (analytical reagent, Sinopharm Chemical Reagent Beijing Co., Ltd., China) and stained with Trypan Blue (analytical reagent, Sinopharm Chemical Reagent Beijing Co., Ltd., China). Percentage of root colonization was estimated through a grid-intersect method by examination of 100 intersects under a compound microscope (BSA124S-CW, Chongqing Optical & Electrical Instrument Co., Ltd., China) at 200× magnification (McGonigle et al., 1990). All determinations were carried out by the same person to minimize possible observation errors. Root-intersects that contained vesicles, arbuscules or hyphae were scored as mycorrhizal. The decision to score hyphae as mycorrhizal was based on the associated presence of vesicles, arbuscules and spores. Roots that did not have cortex were

excluded from the analysis.

Other parts of the fresh root and the entire shoot as well as the seeds were dried at 80°C for 72 hr, and then determined when the weight was constant. Notably, the left fresh root other than one used for colonization was weighted firstly before oven-drying to calculate the whole root dry weight based on relative water content. Dry samples were milled and digested by acid digestion using a mixture of H_2SO_4 and $HClO_4$ (7:1, *V*/*V*) (analytical reagent, Sinopharm Chemical Reagent Beijing Co., Ltd., China). K, Ca, Mg, and P were analyzed in the dissolved samples by atomic emission spectrometry with inductively coupled plasma (ICP-AES, Prodigy, USA). Total N was determined by the Kjeldahl method. Nutrient concentrations were on a dry weight basis and nutrient content (the quantity of nutrients accumulated by specific organs) was the product of organ biomass and its nutrient concentration.

1.7 Statistical analysis

Analysis of variance (ANOVA) with general linear model procedure of SPSS (Ver. 13, SPSS, Chicago, USA) was employed to detect the effects of O_3, AMF and their interactions. When significant interaction between O_3 and AM was detected, Duncan's HSD test was performed to identify significant differences among the treatments. Values with different letters are significantly different at $P < 0.05$.

2 Results

2.1 Mycorrhizal colonization and infection types

Mycorrhizal colonization was not detected in the roots of the non-mycorrhizal plants due to soil sterilization. In the mycorrhizal plants, however, roots were well colonized by AMF (Fig. 1). Notably, elevated O_3 slightly affected overall colonization rate and arbuscular colonization ($P > 0.05$), but significantly increased the proportional frequency of hyphae and decreased the proportional frequencies of spores and vesicles ($P < 0.05$), indicating that elevated O_3 affected mycorrhizal structure and altered the infection types of AMF.

2.2 Plant growth

The dry mass of shoot, root and whole-plant was significantly decreased by elevated O_3, whereas they were significantly increased by inoculation with AMF (Fig. 2). Significant interactive effects of O_3 and AMF were observed in dry mass of shoot and whole-plant, respectively, due to the greater enhancement by AMF in CFO1 (32.0% and 37.2%, respectively) relative to CF (20.1% and 23.7%, respectively) and CFO2 (27.4% and 33.8%, respectively).

2.3 Seed yield

Seed yield was significantly reduced after O_3 exposure, while it was increased by the inoculation with AMF (Fig. 3). Significant interaction was observed, as shown that CFO1 induced similar decreases in mycorrhizal or in non-mycorrizal plants, whereas CFO2 induced much larger seed yield loss in non-mycorrhizal plants (53.6%) than in mycorrhizal plants (29.6%).

2.4 Nutrient concentration

Elevated O_3 significantly increased the concentrations of N, P, Ca, and Mg, in both shoot (Table 1) and root (Table 2), and K concentration in root ($P < 0.05$). Inoculation with AMF significantly increased the concentrations of N, P and Mg in shoot and N, P, Cu and Mg in root. Significant interaction was found in the concentrations of N, P, and Mg in shoot and in root. In the shoot of non-

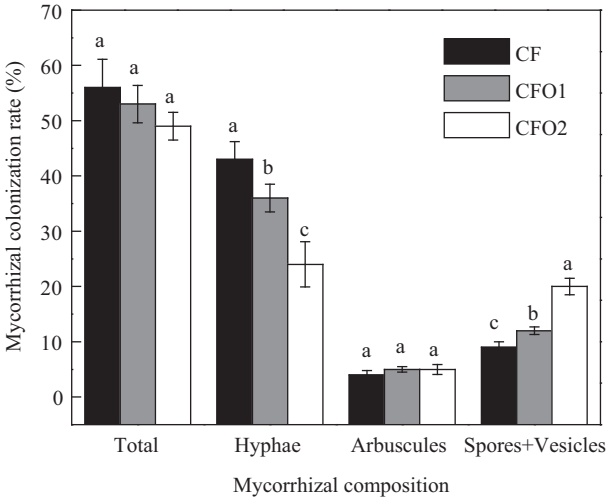

Fig. 1 Effect of elevated O_3 (charcoal filtered air, CF (20 ng/L) and elevated O_3 (CFO1, 80 nL/L and CFO2, 120 nL/L)) on mycorrhizal colonization rate (mean ± SD, $n = 3$). For each variable, bars with different letters are significantly different among treatments at $P < 0.05$.

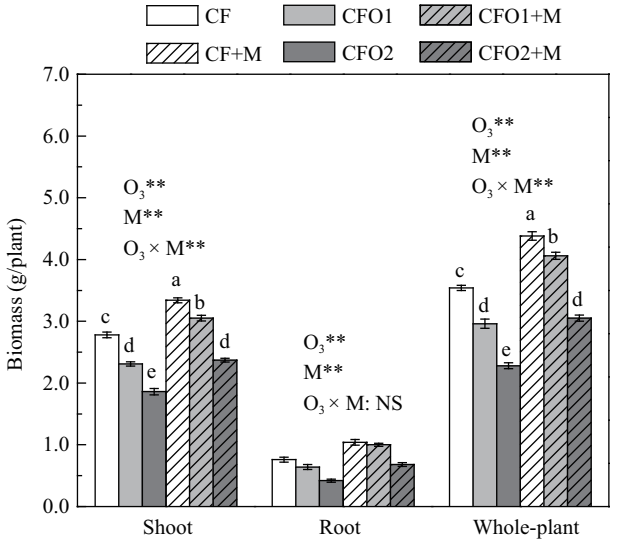

Fig. 2 Effects of O_3 (charcoal filtered air, CF (20 nL/L) and elevated O_3 (CFO1, 80 nL/L and CFO2, 120 nL/L)) and the combination of O_3 and arbuscular mycorrhizal fungi (CF+M, CFO1+M, CFO2+M) on dry mass of plant organs (mean ± SD, $n = 3$). For each variable, bars with different letters are significantly different among treatments at $P < 0.05$. For ANOVA results, ** indicate significant difference among treatments at $P < 0.01$. NS: not significant.

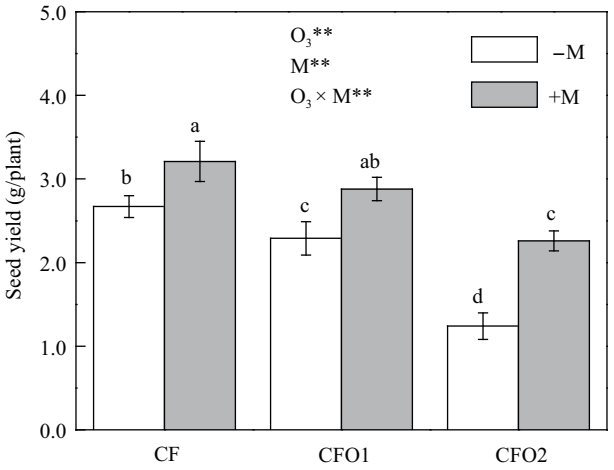

Fig. 3 Effects of O_3 (charcoal filtered air, CF (20 nL/L) and elevated O_3 (CFO1, 80 nL/L and CFO2, 120 nL/L)) and/or arbuscular mycorrhizal fungi (mycorrhizal: +M, non-mycorrhizal: –M) on seed yield (mean ± SD, n = 3). Bars with different letters are significantly different among treatments at $P < 0.05$. For ANOVA results, ** indicates significant difference at $P < 0.01$.

mycorrhizal plants, the concentrations of N, P and Mg were significantly increased only in CFO2 treatment, but in the mycorrhizal plant, they were markedly increased by the CFO1 and CFO2 treatments (Table 1). The concentrations of P and Mg in root showed similar trends as those in shoot (Table 2). AMF did not affect N concentration in root in both CFO1 and CFO2 treatments, but significantly increased it in CF treatments.

2.5 Nutrient content

The contents of the five investigated elements in shoot and in root were significantly decreased by elevated O_3 (especially CFO2), but were increased in AMF (Tables 3 and 4). Significant interaction was found in P, Ca and Mg in both shoot and root, and N in shoot ($P< 0.01$). In the shoot, the contents of N, P, Ca, and Mg in the non-mycorrhizal plants were significantly reduced in the CFO1 and CFO2 treatments relative to the CF treatment, but in the mycorrhizal plants the significantly reduction appeared only in CFO2. In the root, the contents of P, Ca, and Mg in non-mycorrhizal plants were significantly reduced only in CFO2 relative to CF. However, in mycorrhizal plant, P and Mg contents were significantly increased by CFO1, and P content was not affected by CFO2.

Table 1 Effects of O_3 and/or AMF on concentrations of N, P, K, Ca and Mg in shoot of snap bean

Treatment		N (mg/g dw)	P (mg/g dw)	K (mg/g dw)	Ca (mg/g dw)	Mg (mg/g dw)
O_3	AMF					
CF	–M	14.82 e	2.47 d	8.23	8.87	3.08 c
CFO1	–M	15.20 de	2.54 cd	8.33	9.03	3.21 c
CFO2	–M	17.43 b	2.84 b	8.56	9.33	3.45 b
CF	+M	15.45 d	2.71 bc	8.09	8.53	3.16 c
CFO1	+M	16.78 c	3.13 a	8.18	8.80	3.49 b
CFO2	+M	18.69 a	3.23 a	8.38 ab	9.05	3.85 a
Average effect	–M	15.81 B	2.62 B	8.37 A	9.07 A	3.21 B
	+M	16.97 A	3.02 A	8.22 A	8.79 B	3.50 A
	CF	15.14 C	2.59 C	8.16 A	8.70 B	3.12 C
	CFO1	15.99 B	2.84 B	8.26 A	8.91 B	3.35 B
	CFO2	18.06 A	3.04 A	8.47 A	9.19 A	3.59 A
ANOVA	O_3	**	**	NS	**	**
	M	**	**	NS	*	**
	O_3×M	*	*	NS	NS	*

dw: dry weight. Values with different lowercase letters within the same column are significantly different among treatments at $P < 0.05$. Average values with different capital letters in the same column are significantly different in either group (O_3 or AMF) at $P < 0.05$. *, ** indicate significant difference among treatments at $P < 0.05$ and $P < 0.01$, respectively. NS: not significant.

Table 2 Effects of O_3 and/or AMF on concentrations of N, P, K, Ca and Mg in root of snap bean

Treatment		N (mg/g dw)	P (mg/g dw)	K (mg/g dw)	Ca (mg/g dw)	Mg (mg/g dw)
O_3	AMF					
CF	–M	9.11 d	1.21 e	3.85	3.13	1.48 c
CFO1	–M	10.07 b	1.32 de	4.03	3.22	1.54 c
CFO2	–M	11.92 a	1.64 c	4.04	3.42	1.69 b
CF	+M	9.61 c	1.44 cd	3.74	3.31	1.51 c
CFO1	+M	10.28 b	1.91 b	3.77	3.55	1.81 ab
CFO2	+M	11.84 a	2.11 a	3.98	3.81	1.87 a
Average effect	–M	10.37 B	1.42 B	3.97 A	3.26 B	1.57 B
	+M	10.58 A	1.82 A	3.84 B	3.55 A	1.73 A
	CF	9.36 C	1.33 C	3.80 B	3.22 B	1.50 C
	CFO1	10.18 B	1.66 B	3.92 AB	3.39 B	1.68 B
	CFO2	11.88 A	1.88 A	4.01 A	3.61 A	1.78 A
ANOVA	O_3	**	**	*	**	**
	M	*	**	*	**	**
	O_3×M	*	*	NS	NS	*

Values with different lowercase letters in the same column are significantly different among treatments at $P< 0.05$. Average values with different capital letters in the same column are significantly different in either group (O_3 or AMF) at $P< 0.05$. *, ** indicate significant difference among treatments at $P< 0.05$ and $P< 0.01$, respectively. NS: not significant.

Table 3 Effects of O_3 and/or AMF on contents of N, P, K, Ca and Mg in shoot of snap bean

Treatment		N (mg/plant)	P (mg/plant)	K (mg/plant)	Ca (mg/plant)	Mg (mg/plant)
O_3	AMF					
CF	–M	41.25 c	6.89 c	22.90	24.68 c	8.57 b
CFO1	–M	35.20 d	5.88 d	19.30	20.91 d	7.44 c
CFO2	–M	32.48 e	5.30 e	15.96	17.39 e	6.42 d
CF	+M	51.57 a	9.03 a	27.00	28.45 a	10.54 a
CFO1	+M	51.24 a	9.57 a	24.99	26.87 ab	10.66 a
CFO2	+M	44.35 b	7.66 b	19.88	21.47 d	9.14 b
Average effect	–M	36.31 B	6.02 B	19.39 B	21.00 B	7.41 B
	+M	49.06 A	8.75 A	23.96 A	25.60 A	10.11 A
	CF	46.42 A	7.96 A	24.95 A	26.57 A	9.56 A
	CFO1	43.22 B	7.72 A	22.15 B	23.89 B	9.05 B
	CFO2	38.42 C	6.48 B	17.92 C	19.43 C	7.67 C
ANOVA	O_3	**	**	**	**	**
	M	**	**	**	**	**
	O_3×M	**	**	NS	*	*

Values with different lowercase letters in the same column are significantly different among treatments at $P< 0.05$. Average values with different capital letters in the same column are significantly different in either group (O_3 or AMF) at $P< 0.05$. *, ** indicate significant difference among treatments at $P< 0.05$ and $P< 0.01$, respectively. NS: not significant.

Table 4 Effects of O_3 and/or AMF on contents of N, P, K, Ca and Mg in root of snap bean

Treatment		N (mg/plant)	P (mg/plant)	K (mg/plant)	Ca (mg/plant)	Mg (mg/plant)
O_3	AMF					
CF	–M	6.92	0.92 c	2.93	2.38 b	1.12 cd
CFO1	–M	6.45	0.85 cd	2.58	2.06 bc	0.99 d
CFO2	–M	5.04	0.69 e	1.71	1.45 d	0.72 e
CF	+M	10.02	1.50 b	3.90	3.45 a	1.58 b
CFO1	+M	10.31	1.91 a	3.78	3.56 a	1.81 a
CFO2	+M	8.05	1.44 b	2.71	2.59 b	1.27 c
Average effect	–M	6.14 B	0.84 B	2.40 B	1.96 B	0.94 B
	+M	9.46 A	1.62 A	3.46 A	3.20 A	1.56 A
	CF	8.47 A	1.21 B	3.41 A	2.92 A	1.35 A
	CFO1	8.38 A	1.41 A	3.17 B	2.81 A	1.40 A
	CFO2	6.55 B	1.07 C	2.21 C	2.01 B	1.00 B
ANOVA	O_3	**	**	**	**	**
	M	**	**	**	**	**
	O_3×M	NS	*	NS	*	**

Values with different lowercase letters in the same column are significantly different among treatments at $P< 0.05$. Average values with different capital letters in the same column are significantly different in either group (O_3 or AMF) at $P< 0.05$. *, ** indicate significant difference among treatments at $P< 0.05$ and $P< 0.01$, respectively. NS: not significant.

3 Discussion

3.1 Effect of elevated O_3 on mycorrhizal colonization

Although overall mycorrhizal colonization was slightly affected by elevated O_3, the proportional frequency of hypha, spores and vesicles in colonization rate changed markedly (Fig. 1), suggesting elevated O_3 had significant effects on AMF activity and mycorrhizal colonization. This result is consistent with the result of Duckmanton and Widden (1994), who found that O_3 had no effect on overall levels of AM colonization, but reduced the proportional frequency of arbuscules compared with vesicles, hyphal coils and internal hypha. In root, hypha growth completely depends on the carbohydrates produced by photosynthesis, however, both photosynthesis and the transportation of carbohydrates to roots were decreased when plants were exposed to O_3 (McCrady and Andersen, 2000; Andersen, 2003; Morgan et al., 2003; Feng et al., 2008). Thus, it can be inferred that hyphal growth was reduced due to carbon shortage. In soil, the hypha from germinating spores produce a fine and highly branched mycelium (Buée et al., 2000) which was related to root exudates, such as strigolactones (Besserer et al., 2006). Root exudates were generally decreased after O_3 fumigation due to less transportation of carbohydrates to roots (Sild et al., 2002). Therefore, decreased hyphal colonization in this study possibly resulted from reduced hyphal growth in root and soil. However, present results were conflicted with the findings of McCool and Menge (1983), in which ozone can reduce overall AM infection of tomato. The differences among studies may be related to O_3-sensitivity of plant, fumigation time and regime, and O_3 concentration. The detailed mechanism would require additional research.

3.2 Effect of O_3 and/or AMF on plant growth

In this study, elevated O_3 significantly reduced dry mass and yield in both non-mycorrhizal and mycorrhizal plants. However, the reduction in the mycorrhizal plants was lower than in the non-mycorrhizal plants, especially when O_3 concentration was high (CFO2), suggesting that AMF alleviates the negative effects of elevated O_3 on

plant growth. Many studies have indicated that AM enable the host plant to grow more efficiently under biotic and abiotic stresses including drought, acidity, salinity, heavy metal and organic pollution (Van der Heijden and Sanders, 2002). This was attributed to the fact that AMF increase the uptake of immobile nutrients such as P and other micronutrients through their microbial activity and their involvement in plant nutrient acquisition (Hodge et al., 2001). Also, AMF plays an important role in increasing photosynthetic and transpiration rates (Rillig, 2004) and modifying root architecture (Berta et al., 1995) to promote plant growth.

3.3 Effect of O_3 and/or AMF on plant nutrition status

In general, elevated O_3 tends to increase the concentrations of mineral elements in shoot and root due to the limitation of plant growth. In this study, a similar phenomenon was also observed in both non-mycorrhizal and mycorrhizal plants (Tables 1 and 2). This upwards trend was more evident in the mycorrhizal plants, as seen from the significant interaction between O_3 and AMF. For example, the concentrations of N, P and Mg in the non-mycorrhizal shoot and root were significantly increased only in CFO2, while in the mycorrhizal shoot and root they are increased in both CFO1 and CFO2. The improvement of AMF on host plant nutrition has been confirmed in many previous studies (Hodge et al., 2001; Van der Heijden and Sanders, 2002). In the non-mycorrhizal plants, O_3-induced increase of nutrient concentration partially attributed to the nutrient allocation from grains to other organs (Pleijel et al., 2006) and nutrient accumulation because of low dry mass (Fig. 2).

The concentration of K in shoot and root is slightly affected by AMF or O_3 (Tables 2 and 3), possibly because K is highly mobile and easy to move among plant organs. Poss et al. (1985) and Heagle et al. (1998) also found similar results. The response of Mg in shoot to elevated O_3 in this study was opposite to the result of Fangmeier et al. (2002). Elevated O_3 increased the concentration of Ca in shoot, which was consistent with the results of Heagle et al. (1998) but different from that of Fangmeier et al. (2002) who found Ca concentration in leaves slightly increased. These differences suggest that the response of nutrient status in plant organs to elevated O_3 may be affected by plant species, development stages, O_3 concentration and regime, and soil fertility.

Although nutrient concentrations in both non-mycorrhizal and mycorrhizal plants showed upward trends with elevated O_3, their nutrient contents were decreased significantly, which largely attributed to high reduction in plant biomass induced by elevated O_3. Additionally, low microbial activity in O_3-treated plants negatively affected nutrient uptake of the root (Andersen, 2003; Chen et al., 2009). However, in the mycorrhizal plants, the O_3-induced reductions in N, P, Ca and Mg contents in shoot and P, Ca and Mg in root were significantly less than in non-mycorrhizal plants, especially in CFO1. This indicated that AMF alleviated negative effect of O_3 on most elements uptake. Notably, AMF also suffered from high O_3 concentration (e.g., CFO2), as seen from reduced growth and nutrition status of mycorrhizal plants in CFO2 when compared to CFO1.

4 Conclusions

Elevated O_3 did not significantly affect overall colonization rate, but significantly reduced the proportional frequency of hyphae and increase in the proportional frequency of spores and vesicles. Elevated O_3 significantly decreased plant biomass, yield and nutrition status in both mycorrhizal and non-mycorrhizal plants. However, the extent of the decrease in the above mentioned parameters in the mycorrhizal plants was much less than in the non-mycorrhizal plants. AMF can effectively alleviate the detrimental effects of increasing O_3 on host plants.

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