Physiological Responses of Three Kinds of Street Trees to Acute Stress of Ozone

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Abstract: The stress impacts of ozone pollution on the common street trees in tropical cities were investigated in this study. Three kind of common trees(*C.camphora*, *M.indica* and *F.altissima*) in Xiamen were artificial ozone fumigation stressed for 8h, with the ozone concentration of 0.43 mg/m^3 and 0.86 mg/m^3 respectively, and their physiological index responses were also analyzed. The results showed that the cell membrane permeability and malondialdehyde (MDA) content of leaves increased but chlorophyll content decreased, the contents of proline and soluble sugar content to regulate osmotic potential, while the activity of superoxide dismutase (SOD) was inhibited when 3 kinds of street trees were subjected to acute ozone stress. In addition, the change amplitude of 6 indexes increased with the increment of ozone stress concentration, which can be used as reference indexes for plant ozone resistance. Based on the performance of plants after stressed, the resistance intensity of 3 species of street trees to ozone stress was: *M.indica>C. camphora> F.altissima.* The results could provide the reference for screening of ozone resistance trees in the tropics.

1. Introduction

Surface ozone (O_3) concentrations have more than doubled since the Industrial Revolution[1]. Among air pollutants, ozone is classified as a secondary pollutant which has a strong oxidation and has gradually become a public enemy of air pollution. According to the report of the Ministry of Environmental Protection, the major air pollutants are ozone in large and medium-sized cities in China, followed by PM2.5[2]. The harm of ozone to human health is closely related to its concentration and exposure time in the environment[3]. The plant photosynthesis would be inhibited and hence lead to reduce crop yield under the stress of ozone[4-9]. However, few studies were focused on urban landscaping plants of ozone pollution[10-12].

Xiamen city, based on its characteristics of the geographical location and climate in the southeastern of China, is selected as the studing zone in this work, *Cinnamomum camphora*, *Mangifera indica* and *Ficus altissima* representative for street trees in tropical city are selected and theirs physiological indexes response to ozone stress under different concentration were investigated[13]. The resistance of 3 kinds of street trees to ozone pollution in surface layer ground is evaluated by the monitored physiological indexes, which provide an important reference to select street trees species for the greening construction of urban road.

2. Materials and methods

2.1 Experimental materials

Three kinds of street trees: The potted plants of *Mangifera indica*, *Cinnamomum camphora* and *Ficus altissima* were bought from the local gardening market, about 1 year old and 0.5 m height respectively.

2.2 Experimental methods

3 kinds of plants were first placed in the semi-shading state to adaptive cultivate for 1 weeks, and then 3 plants with good growth and uniform height were selected as experimental subjects from each kinds plant. The laboratory hood was utilized to model the open top stress device for acute ozone stress.

Specific operations: The potted plants were placed inside the laboratory hood (volume: 120 cm × 120 cm × 200 cm), and the ventilator and ozone generator (ZA-D5G) were turn on for the formation of partially enclosed convection space in the laboratory hood. The humidity, temperature and light intensity in the laboratory hood are 70%-80%, room temperature and 24000 lx, respectively. The ozone stress with a low concentration of 0.43 mg/m^3, a high concentration of 0.86 mg/m^3 and non-ozone stress were carried out, and the ozone concentrations inside laboratory were online monitored by an ozone detector (Z-1200, USA ESC). Error control is within ±0.05 mg/m^3. The actue stress was treated for 8 h from 8:00 to 16:00. The 3-4 round mature leaves of the plants were selected in the next day after stressed, and theirs physiological and biochemical indexes, including cell membrane permeability, malondialdehyde（MDA）, proline, soluble sugar, cholrophyll and superoxide dismutase（SOD）were determined. The specific methods of determination can refer in the book of Experiment Guidance on Plant Physiology edited in chief by Zhiliang Zhang, et al. All experiments were repeated three times. The experiment datas were analysed using the software of SPSS 16.0 and Excell 2010, and theirs average values were determined as the final results. Meanwhile, these 3 kinds of plants were calculated again for 2 weeks and theirs appearance changes were observed during the calculation period.

3. Results and discussion

3.1 Effects of ozone stress on the cell membrane permeability of the plant

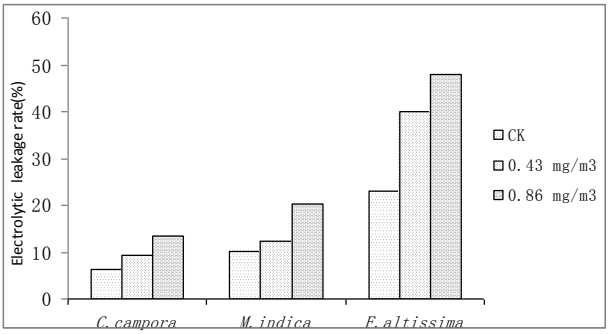

Figure 1. The electrolytic leakage rate in three tree species

When plant were placed in a stress environment, its cell membrane permeability usually becomes large which leads to the partial electrolyte exudation from cells and increases the conductivity of external liquid. Therefore, the more of the conductivity of external liquid indicates the more serious damage of cell membrane as well as the lower resistance of plant but higher resistance on the contrary side. As shown in Fig.1, the cell membrane permeability of 3 kinds of plants became large after actue ozone stressed and increased with the increment of ozone concentrations. Compared to conventional state, no significant difference in the relative extravasation rate of cell membranes for *M.indica* and *C.camphora* ,whlie more significant difference for *F.altissima* were observed under low ozone concentration actue stress, which indicates that the low ozone concentration has a low damage on *M.indica* and *C.camphora*. However, significant differences in the relative extravasation rate of cell membranes for 3 kinds of plants were observed under high ozone concentration, which are 2.16, 1.84 and 2.08 times higher than that of the control one, respectively. This result indicates that the cell membranes are damaged in varying degrees under high concentration of ozone acute stress. In

summary, 3 tree species are resistant to low concentrations of ozone, and in high concentration, *M.indica* and *C.camphora* resistance are stronger relatively.

3.2 Effects of ozone stress on the MDA content of the plant

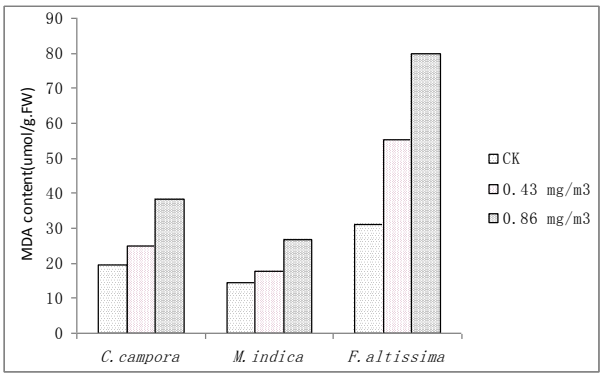

Figure 2. The malondialdehyde (MDA) content in three tree species

The content of MDA, an important indicator of the degree of leaf senescence, indicates the peroxidation degree of membrane lipid and the response intensity of plant to adverse conditions. As shown in Fig.2, the MDA content of the leaves for 3 species of plants increased compared to the control group under ozone stress. Among them, *F.altissima* has an obvious increment magnitude with 1.8 and 2.6 times to control under low and high ozone concentration stress, respectively. Under the same conditions, MDA in both *M.indica* and *C.camphora* have a low increment magnitude in the content respective with 1.29/1.96 and 1.22/1.84 times to the control group. In summary, *F.altissima* is more sensitive to ozone stress, with free radical accumulation and damage appeared in cell under low ozone concentration stress obviously.

3.3 Effects of ozone stress on the proline content of the plant

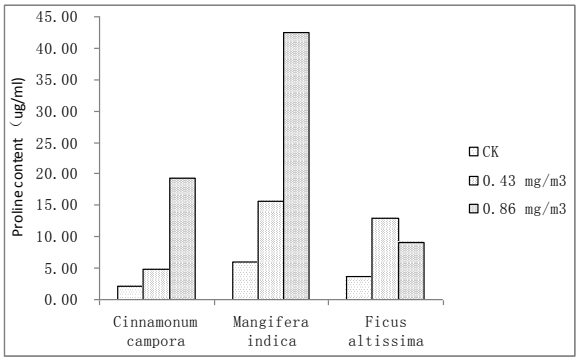

Figure 3. The proline content in three tree species

Proline is one of the important components of plant protein and usually exists with a free state in the plant. When the plant is under adversity stress, the body tends to accumulate proline and increasing significantly, which is beneficial for plant to resist to external stress. Therefore, the plant that its proline contents can increase after stressed has a relative strong resistance. As shown in Fig.3, the proline contents in the cells of 3 kinds of street trees exhibited an increased in various degree, which indicates all the plants has a certain resistance to ozone stress. Under low ozone concentration stress, no significant differences were observed on the relative accumulation rates of proline between 3 kinds of plants and theirs value were 2.26, 2.64 and 3.44, respectively. However, the relative accumulation rates of proline for *M.indica* and *C.camphora* can respectively reach to 9.1 and 9.2 under high ozone concentration stress, which indicate that they have a good emergency response to ozone stress. On the contrary, the proline concent of *F.altissima* is obvious lower than former two which indicates an increased damage on *F.altissima* under high ozone concentration stress.

3.4 Effects of ozone stress on the soluble sugar content of the plant

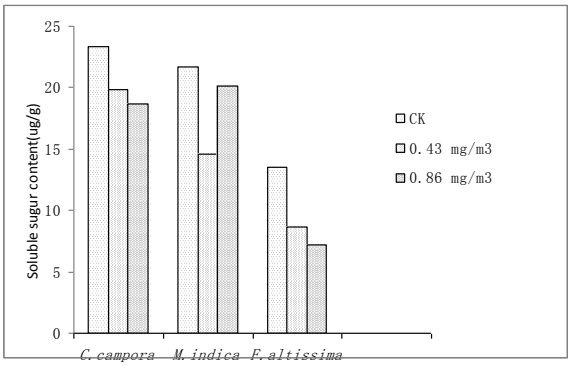

Figure 4. The soluble sugar content in three tree species

The content of soluble sugar is closely related to the resistance of plants and the solube sugar as an osmotic regulator and an anti-dehydrating agent plays a role in the plant growth. As shown in Fig.4, a common reduction tendency existed in the accumulation of soluble sugar contents in the body of 3 kinds of plants. Among them, the change magnitudes for *C.camphora* were close between low and high ozone concentration stress, and respectively reduced by 15% and 22% compared to the control one. The no obvious difference indicates the *C.camphora* had a strong resistance to ozone stress. The change tendency of *F.altissima* was similar to that of *C.camphora*, however, the change magnitudes of *F.altissima* was significant when compared to that of the control one and can reach to 35% and 46%, respectively. The no obvious difference between the low and high concentration experiment indicated the limitation of emergency response capability of *F.altissima* under low ozone concentration as well as the relative weak resistance of F.altissima. The soluble sugar content of *M.indica* exhibited an obvious decline with a rate of -33% under low ozone concentration stress while recoveried to the level of that of the control one with a rate of -8% under high ozone concentration stress, which may be attributed to the formation of adaptability to the external stress and the start-up of resistance mechanism of *M.indica* and hence induced the content of soluble sugar to recovery to the level before stressed.

3.5 Effects of ozone stress on the chlorophyll content of the plant

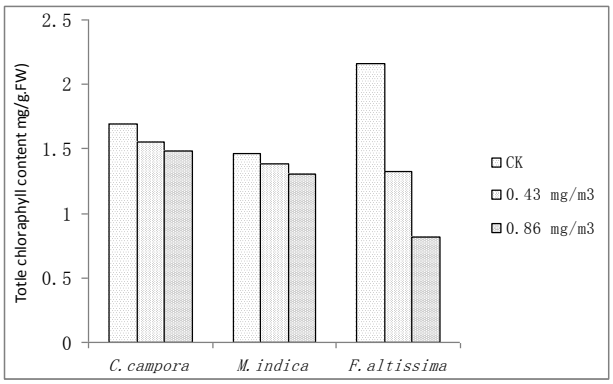

Figure 5. The total chlorophyll content in three tree species

The structures of chloroplast organelles will be subjected to damage after stressed, and the more decline of chlorophyll content implies the more damage from stress, the more sensitive and the more weak resistance of the plant and vice versa. As shown in Fig. 5, the chlorophyll content of 3 kinds of plants generally exhibited a decline tendency. Compared to control, the decline magnitudes for *C.camphora* and *M.indica* were minuscule under low ozone concentration stress, while relative high decline magnitudes with a respective rate of 12% and 11% for *C.camphora* and *M.indica* were observed under high ozone concentration stress which indicated that the chloroplast was damaged slightly under high ozone concentration. Furthermore, significant difference were observed between

the ozone-stressed and the control group on *F.altissima*, and the decline magnitudes were respectively up to 39% and 62% under high and low ozone concentration stress. This result indicated the damage of chloroplast for F.altissima was more serious than that of the former two.

3.6 Effects of ozone stress on the SOD activity of the plant

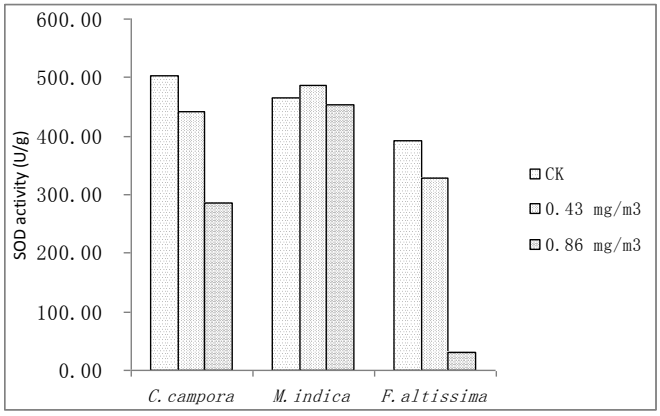

Figure 6. SOD activity in three tree species

SOD plays an important role in the antioxidant system and is a key enzyme for eliminating the reactive oxygen species produced by the stress of external ozone. The activity of SOD can reflect the adversity resistance of the plant. As shown in Figure 6, besides the slight increase of SOD activity for *M.indica* under low ozone concentration stress, all of SOD activity exhibited the decline tendency under ozone stressed. These results indicated that the activity of SOD was inhibited and the inhibition intensity became strong with the increasing of ozone concentration. The decline magnitudes of SOD activities for *C.camphora* and *F.altissima* were obvious when stressed under high ozone concentration and the change rates for them were respectively reach to 43% and 92% compared to the control group.

3.7 Apparent damage symptoms of plant after ozone stressed for 2 weeks

According to the literature, ozone can directly diffuse into the cell through the stomata of plant leaves and destroy the cell, and hence the apparent changes in leaves are the direct responses of plants to the physiological effects of ozone stress[14-15]. After 2 weeks of observation, the damage symptoms and recovery state as follows.

C.amphora and *M.ndica*: No obvious changes on leaves were observed when stressed with 0.43 mg/m^3 ozone concentration. After stressed with 0.86 mg/m^3 for 1 week, small gray spots appeared around the main vein of *C.camphora* leaves with an area ratio of 15%, and small brown spots with an area ratio of 10% appeared at the tip and edge of the leaves of *M.indica* leaves. Both leaves of *C.camphora* and *M.indica* did not shed during 2 weeks.

F.altissima: After stressed with 0.43 mg/m^3 ozone concentration for 1 week, a number of black punctate spots with an area ratio of 20% appeared in the leaves. Furthermore, the area of black spots was further enlarged to 45% after stressed with 0.86mg/m^3, and the tip of leaves curled with dehydration. Subsequently, the leaves fell off but the apical bud maintained the vitality. After that, new buds could grow again in the axils of deciduous leaves and plants recovered to the vitality.

4. Conclusions

Based on the analysis of the response of 3 kinds of trees to ozone stress, the following conclusions could be obtained. When trees were stressed with ozone, the indexes representative for the damaged degree of cell appeared different changes. Increased in the relative membrane permeability and the content of MDA, declined in the content of chlorophyll, inhibited to SOD activity, and increased proline and soluble sugar content to regulate osmotic potential. The index change regulation of each

was consistent with the observation feedback to damage after being stressed, which could be used as reference index for evaluating the resistance intensity to ozone of garden plants.

Comprehensive analysis showed that 3 kinds of street trees have relative strong resistance to ozone and the intensity in the following order: *M.indica>C.camphora>F.altissima*. *M.indica* and *F.altissima* are typical tropical evergreen plants and *C.camphora* is a subtropical broad-spectrum plant, which exhibited excellent anti-typhoon abilities during the period of typhoon "Meranti" in 2016 and have good ecological effects. Therefore, it is suggested to give priority to use these 3 kinds of plants for the planning of urban street trees in Xiamen city.

Acknowledgments
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