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Selection of Landscape Tree Species of Tolerant to Sulfur Dioxide Pollution in Subtropical China*

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Sulfur dioxide (SO_2) is a major air pollutant, especially in developing countries. Many trees are seriously impaired by SO_2, while other species can mitigate air pollution by absorbing this gas. Planting appropriate tree species near industrial complexes is critical for aesthetic value and pollution mitigation. In this study, six landscape tree species typical of a subtropical area were investigated for their tolerance of SO_2: *Cinnamomum camphora* (L.) J. Presl., *Ilex rotunda* Thunb., *Lysidice rhodostegia* Hance, *Ceiba insignis* (Kunth) P. E. Gibbs & Semir, *Cassia surattensis* Burm. f., and *Michelia chapensis* Dandy. We measured net photosynthesis rate, stomatal conductance, leaf sulfur content, relative water content, relative proline content, and other parameters under 1.31 $mg \cdot m^{-3}$ SO_2 fumigation for eight days. The results revealed that the six species differed in their biochemical characteristics under SO_2 stress. Based on these data, the most appropriate species for planting in SO_2 polluted areas was *I. rotunda*, because it grew normally under SO_2 stress and could absorb SO_2.

Keywords: Sulfur Dioxide; Fumigation; Landscape Trees; Air Pollutant Tolerance; Sulfur Content; Net Photosynthesis Rate

Introduction

As one of the six major atmospheric pollutants, sulfur dioxide (SO_2) levels are currently a health concern. Sulfur dioxide can cause asthma and other respiratory health problems in people, forming acid rain that can damage forests and crops, and erode buildings. Because of industrialization and urbanization, developing countries, especially China, suffer from increasing concentrations of SO_2 in the air. Since 1990, SO_2 generated in China has been responsible for about one-fourth of the global emissions and more than 90% of the East Asian emissions. From 21.7 Tg (1 Tg = 10^{12} g) in 2000, SO_2 emissions increased by 53% to 33.2 Tg in 2006, at an annual growth rate of 7.3%. In 2007, Guangdong, a province in the Pearl River Delta Industrial district, emitted a total of 1177 Gg of SO_2, about 97% of which was emitted by power plants and industries (Lu, 2010).

Damage to plants is an important consequence of atmospheric SO_2. Gaseous pollutants, particularly SO_2, enter plants through the stomata by the process of photosynthesis and respiration. Nitrogen dioxide (NO_2) and SO_2 react with water on the cell walls inside leaves; by transfer and assimilation, the resulting sulfurous, sulfuric, nitrous, and nitric acids, react with other compounds and are transported to various parts of plants. If plants are exposed to air pollutants for a long time or the pollutant concentrations exceed a critical threshold, plants may be injured (Jim, 2007). Plant injury is usually cumulative in nature, reducing growth and yield and accelerating senescence. The injury often has no overt visible symptoms aside from some degree of chlorosis (WHO, 2000). Because of the harmful effects of SO_2, plants cannot grow robustly and some also die in severely polluted industrial districts, creating "dead zones" without greenery in these areas. Many studies have investigated various aspects of the damage caused by SO_2 to plants, including photosynthesis (Swanepoel, 2007), stomatal density and function (Haworth, 2012), and carbon fixation efficiency (Chung, 2010).

Each plant is a living entity, and individuals vary in their adaptations to the environment and abilities to absorb pollutants. Suitable plants must be carefully selected for cultivation; otherwise they may not thrive or may die in adverse conditions of environmental pollution (Chung, 2010). In 2000, about 42.62 Mg of SO_2 was removed from the atmosphere by urban trees in Guangzhou. Because it costs less to remove SO_2 in the air in China compared to other developed countries, the monetary value of this service is low (Jim, 2007). Some studies have not only investigated the effects of air pollutants on plants, but also evaluated suitable air pollutant-tolerant plants, for example near a lignite-based thermal power station (Govindaraju, 2011), industrial complexes (Lee, 2004), and a coal-fired power plant (Sharma, 2008). The other studies assessed SO_2-tolerant plants, e.g., among wetland plants (Sha, 2010). Nevertheless, few investigations have continuously observed the responses of landscape tree species under high SO_2 concentrations in the subtropical areas of southern China. This study aims to understand how trees adapt to the stress of SO_2 and to facilitate the identi-

*Selection of high SO_2 tolerance species.

fication of species that can assimilate atmospheric SO_2 while growing normally in this area.

Materials and Methods

Tree Seedlings and Growing Conditions

Six popular landscape tree species in Southern China were selected for this experiment: *Cinnamomum camphora* (L.) J. Presl, *Ilex rotunda* Thunb., *Lysidice rhodostegia* Hance, *Ceiba insignis* (Kunth) P. E. Gibbs & Semir, *Cassia surattensis* Burm. f. and *Michelia chapensis* Dandy. For each species, eighteen healthy 1-year-old seedlings of approximately the same size were potted into 2 kg bags (height: 12 cm, radius: 4 cm) with loess containing 0.302 $g \cdot kg^{-1}$ N, 0.3 $g \cdot kg^{-1}$ P, 9.761 $g \cdot kg^{-1}$ K, 21.21 $mg \cdot kg^{-1}$ hydrolysable N, 4.3 $mg \cdot kg^{-1}$ rapidly available P, and 28.47 $mg \cdot kg^{-1}$ rapidly available K.

The average tree height, root collar diameter, and canopy of the trees are listed in **Table 1**. These seedlings were grown under natural conditions for 1 month with regular watering to allow their physiology to stabilize before the experiment began.

Controlled Environmental Conditions

In the experiment, three seedlings of each species (18 seedlings total) were placed under natural conditions with daily watering as a control group for normal growth without treatment. The other 15 samples of each species were placed together as an experimental group in a 2.0 m × 1.2 m × 1.8 m phytotron at Guangdong Academy of Forestry, China. From January 28 to February 5, 2013, these seedlings experienced 8 days of fumigation with 1.31 $mg \cdot m^{-3}$ (= 0.5 ppm) SO_2 (MIC-SO_2) with the following conditions: temperature, 15˚C - 25˚C; relative humidity (RH), 50% - 60%; concentration of carbon dioxide, 380 - 400 ppm; and light intensity, 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. According to the result of some studies, SO_2 can impact the growth and yield of plants while reducing its foliar starch and protein contents, pigmentation, and WUE at concentrations as low as 0.06 - 0.15 ppm (Swanepoel, 2007). We used an unnaturally high concentration of SO_2 (1.31 $mg \cdot m^{-3}$) to determine the relative sensitivities of species for which this information was almost unknown. According to the Pearl River Delta regional air quality monitoring reports (2006, 2007, 2008, 2009, 2010), the average of the monthly maxima of hourly averages of SO_2 in Huijingcheng (Foshan), one of the most severely polluted areas, is 0.394 $mg \cdot m^{-3}$. The SO_2 concentration in this study was about three times that value. During the fumigation, plants were watered daily.

Measurements of Biochemical Characteristics

Leaf parameters were measured at regular time intervals during the SO_2 fumigation treatment. To observe changes in different parts of the seedlings, the first round of tests were conducted the day before the SO_2 treatment to serve as a baseline. Then, every 2 days (on Jan 30, Feb 1, Feb 3, Feb 5), three seedlings (replicates) of each species were removed from the phytotron and three to four of their leaves were picked off to measure relative water content, relative electrolytic leakage and proline content. Five rounds of tests were done.

Relative chlorophyll content was measured with a portable chlorophyll content meter (CCM-200 plus, OptiSciences, Hudson, NH, USA) on six young fully expanded leaves for each seedling. Relative water content was determined by the following equation:

$$R_{WC} = \left(W_f - W_d\right) / \left(W_s - W_d\right) \times 100\% \qquad (1)$$

The fresh weight $\left(W_f\right)$, saturated fresh weight $\left(W_s\right)$, which was the weight after soaking the leaves in distilled water for 24 hours, and dry weight $\left(W_d\right)$,which was got by drying the fresh leaves in an oven of 80˚C overnight, were measured by a electronic scale of 0.01 g (JJ500, G & G GmbH, Neuss, Germany). Relative electrolytic leakage, *P*, was evaluated using a conductivity meter (DSSJ-308A, China) and calculated by the following equation:

$$P = \left(\left(C_1 - C_0\right) / \left(C_2 - C_0\right)\right) \times 100\% \qquad (2)$$

where the conductivity of distilled water $\left(C_0\right)$ and of the sample solute before boiling $\left(C_1\right)$ and after boiling $\left(C_2\right)$ were known. Proline content in the leaves was determined as described by Chen & Wang (2006). To measure photosynthesis, a portable photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA) was used to test three healthy leaves near the top of each seedling. To ensure the consistency of incident light intensity and leaf surface temperature, we tested the following parameters in both the control and treatment groups from 9:00 - 11:00 A.M. for 2 days after fumigation (on Feb 6 and 7): net photo synthetic rate (P_n, $\mu mol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (G_s, $mol \cdot m^{-2} \cdot s^{-1}$), transpiration rate ($T_r$, $mmol \cdot m^{-2} \cdot s^{-1}$), intercellular carbon dioxide concentration (C_i, $\mu mol \cdot mol^{-1}$), photosynthetic

Table 1.
Growth status of the plants before sulfur dioxide fumigation.

Species	Code	Growth parameters			Family
		Height (cm)	Root collar diameter (cm)	Canopy (cm^2)	
Cinnamomum camphora	A	51.20 ± 0.70	0.47 ± 0.01	242.20 ± 17.16	Lauraceae
Ilex rotunda	B	67.13 ± 0.80	0.66 ± 0.03	139.07 ± 11.78	Aquifoliaceae
Lysidice rhodostegia	C	54.47 ± 1.06	0.59 ± 0.02	377.60 ± 46.51	Caesalpinioideae
Ceiba insignis	D	87.53 ± 1.37	1.60 ± 0.05	977.60 ± 88.73	Malvaceae
Cassia surattensis	E	46.73 ± 0.76	0.55 ± 0.02	225.53 ± 12.74	Caesalpinioideae
Michelia chapensis	F	64.93 ± 1.07	0.70 ± 0.01	281.80 ± 24.36	Magnoliaceae

cally active radiation (PAR, μmol·m^{-2}·s^{-1}), atmospheric carbon dioxide concentration (C_a, μmol·mol^{-1}), atmospheric temperature (T_a, ˚C), leaf temperature (T_l, ˚C), RH (%) and water use efficiency (WUE, μmol·mmol^{-1}). WUE was calculated as:

$$WUE = P_n / T_r \quad (3)$$

The sulfur content was tested by barium sulfate turbidimetry with various parts of the leaves selected. The ratio of leaf injury was estimated based on the percentage of visible leaf damages.

Statistical Analysis

Statistical analyses were conducted with Microsoft Office Excel 2007 (Redmond, WA, USA) and SPSS 16.0 (IBM, Chicago, IL, USA). ANOVAs and multiple comparisons were used to analyze significant difference of the relative proline content, sulfur content in leaves and the net photosynthesis rate. The tests of homogeneity were checked before multiple comparisons. The change of P_n (R, %), which was calculated by the equation:

$$R = \frac{(P_n)_{\text{after}} - (P_n)_{\text{before}}}{(P_n)_{\text{before}}} \times 100\% \quad (4)$$

and the absolute differences between the value of sulfur content on 0 hour and 192 hour are calculated to compare the ability of species of tolerance to the SO_2 fumigation.

Results

In general, the sulfur content in the leaves of all six species increased significantly before and after the treatment ($P < 0.05$) *Cassia surattensis* had both the highest original sulfur content in the leaves (4.49 ± 1.035 μg·g^{-1}) and the greatest increase (to 9.345 ± 1.172 μg·g^{-1}), which demonstrated its strong ability to absorb SO_2. It was followed by *I. rotunda*. The sulfur content in leaves of *I. rotunda* was higher under SO_2 fumigation than in the control (5.155 ± 0.411 versus 2.273 ± 0.123 g·kg^{-1}). It showed higher ability to absorb SO_2 gas than *C. camphora*, *L. rhodostegia*, *C. insignis*, or *M. chapensis* (**Figure 1**). The sulfur content in the leaves of *C. camphora* remained low at around 0.795 ± 0.236 and only increased to 2.616 ± 0.385 g·kg^{-1} during the treatment. The sulfur content of *L. rhodostegia* was not initially high nor did it increase much during treatment. The low sulfur contents in *C. insignis* and *M. chapensis* showed their weak ability to absorb SO_2.

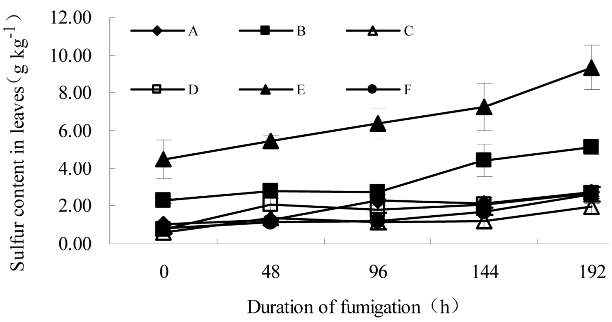

Figure 1.
Changes in the sulfur content of leaves during sulfur dioxide fumigation. A, *Cinnamomum camphora*; B, *Ilex rotunda*; C, *Lysidice rhodostegia*; D, *Ceiba insignis*; E, *Cassia surattensis*; F, *Michelia chapensis*. The error bar on each point indicated standard error.

In this study, three species showed significantly decline in the P_n after fumigation: *L. rhodostegia*, *C. insignis*, *Cassia surattensis* ($P < 0.05$) (**Figure 2**). *Ceiba insignis* declined the greatest amount in P_n, from 5.77 ± 1.33 to 0.75 ± 0.08 μmol·m^{-1}·s^{-1}, in G_s, from 0.09 ± 0.03 mol·m^{-2}·s^{-1} to 0.02 ± 0.003 mol·m^{-2}·s^{-1}, and in T_r, from 1.23 ± 0.38 to 0.27 ± 0.06 mmol·m^{-2}·s^{-1}. In addition, WUE was reduced by fumigation from 4.91 ± 0.35 to 2.92 ± 0.47 μmol·mmol^{-1}. *Lysidice rhodostegia* had a relatively large decrease in P_n, from 3.28 ± 0.48 to 0.95 ± 0.09 μmol·m^{-1}·s^{-1}. Also, both G_s and T_r decreased during fumigation from 0.04 ± 0.006 to 0.02 ± 0.001 mol·m^{-2}·s^{-1} and from 0.64 ± 0.07 to 0.35 ± 0.02 mmol·m^{-2}·s^{-1}, respectively. WUE declined the most of the six species, from 5.13 ± 0.25 μmol·mmol^{-1} before fumigation to 2.71 ± 0.12 μmol·mmol^{-1} afterwards. C_i increased from 262.7 ± 4.4 to 337 ± 2.5 μmol·mol^{-1}. The P_n of *C. surattensis* declined from 4.22 ± 0.07 to 2.90 ± 0.29 μmol·m^{-1}·s^{-1}, while its C_i increased from 299.4 ± 3.9 to 338.5 ± 1.2 μmol·mol^{-1}, and its T_r reduced markedly from 1.24 ± 0.05 to 0.77 ± 0.04 mmol·m^{-2}·s^{-1}. Moreover, its electrolytic leakage increased distinctly from 12.54 ± 0.97 to 29.62% ± 4.94%, indicating that part of the plasma membrane was damaged by SO_2 in fumigation, thus affecting its normal growth.

The other species did not show significant changes in *Pn* before and after fumigation: *C. camphora*, *I. rotunda*, and *M. chapensis* ($P > 0.05$) (**Figure 2**). The P_n *of I. rotunda* remained between 3.66 ± 0.51 and 4.01 ± 0.39 μmol·m^{-1}·s^{-1}. *Cinnamomum camphora* changed little in P_n, which ranged between 2.75 ± 0.18 and 3.16 ± 0.09 μmol·m^{-1}·s^{-1}. No visible damage was observed on the surfaces of its leaves. However, there was a decrease in WUE, from 5.25 ± 0.59 to 4.39 ± 0.59 μmol·mmol^{-1}. Similarly, *M. chapensis* did not have a significant change in P_n, which was 3.81 ± 0.25 before and 3.34 ± 0.73 μmol·m^{-1}·s^{-1} after fumigation.

The proline could prevent folded proteins from denaturing, interact with phospholipids to stabilize cell membranes, scavenge hydroxyl radicals, and function as an energy and nitrogen source (Claussen, 2004). An increase in proline content indicated that the plant was stressed (**Figure 3**). The proline was vital in adjusting osmotic pressure in *M. chapensis*, *C. insignis and C. surattensi*. The proline content of *M. chapensis* was the highest among the species during fumigation, with an initial value of 224.76 ± 50.84 μg·g^{-1} and the highest value of 350.46 ± 43.97 μg·g^{-1} during the process of fumigation. The proline content of *C. surattensis* was also high (207.25 ± 5.05 μg·g^{-1}) and increased during fumigation to 327.00 ± 21.82 μg·g^{-1}. The

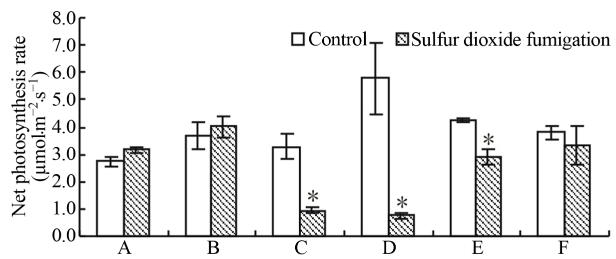

Figure 2.
Net photosynthesis rate before and after sulfur dioxide fumigation. A, *Cinnamomum camphora*; B, *Ilex rotunda*; C, *Lysidice rhodostegia*; D, *Ceiba insignis*; E, *Cassia surattensis*; F, *Michelia chapensis*. The error bar on each point indicated standard error. The asterisks indicated significant differences at 0.05.

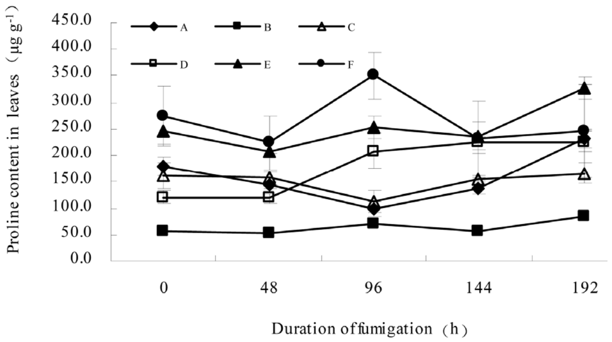

Figure 3.
Changes in proline content of leaves during sulfur dioxide fumigation. A, *Cinnamomum camphora*; B, *Ilex rotunda*; C, *Lysidice rhodostegia*; D, *Ceiba insignis*; E, *Cassia surattensis*; F, *Michelia chapensis*. The error bar on each point indicated standard error.

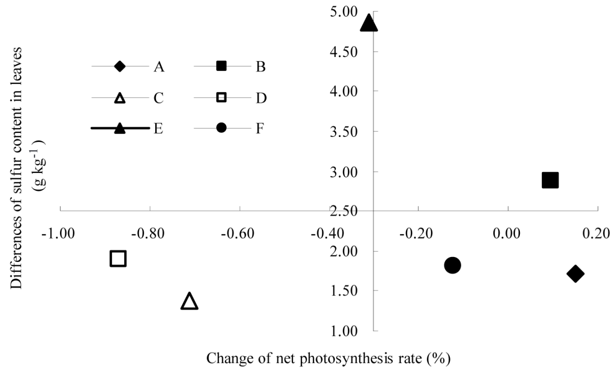

Figure 4.
Categorization of the six tree species based on their responses to SO_2 pollutant stress. A, *C. camphora*; B, *I. rotunda*; C, *L. rhodostegia*; D, *C. insignis*; E, *C. surattensis*; F, *M. chapensis.*

proline content of *C. insignis* ranged between 120.94 ± 12.49 and 226.16 ± 18.57 $\mu g \cdot g^{-1}$, presenting a significant increase ($P < 0.05$) under SO_2 stress, which corresponded with its remarkably decreased P_n. After fumigation, some leaves were dehydrated, yellowing, coiled and chlorotic, and many dehisced, and the ratio of leaf injury was 100%, which showed it was highly stressed. The proline content in the leaves of *I. rotunda* was relatively low, ranging from 51.44 ± 4.91 to 84.88 ± 7.97 $\mu g \cdot g^{-1}$. Its G_s increased after fumigation from 0.07 ± 0.012 to 0.10 ± 0.014 $mol \cdot m^{-2} \cdot s^{-1}$. Some leaves had rusty piebald patches or dehisced under SO_2. The ratio of leaf injury was 30%. The proline content of *L. rhodostegia* was not initially high nor did it increase much during treatment.

These six species could be divided into four types based on their responses to SO_2 fumigation, as shown in **Figure 4**. The horizontal axis is the change of P_n (R, %). The smaller the R value, the lesser the impact on P_n and the better the plant can adapt to the SO_2 environment. The vertical axis shows the absolute values of the differences in leaf sulfur content ($g \cdot kg^{-1}$) between 0 and 192 hours of fumigation; the larger this value, the greater the tree's capacity to absorb SO_2.

Discussion

The results could be used for environmental design, because they indicated that these six species have different tolerances and could be planted for different purposes, as summarized in **Figure 4**. *Ilex rotunda*, in the first quadrant, was the most appropriate of the six species to be planted near industry complexes and along roads, because it could absorb SO_2 while still growing robustly. To clean the air, *C. surattensis*, in the second quadrant, was a good choice for its strong SO_2 absorption ability. In contrast, if the goal was simply greenery near factories to improve aesthetics, the species in the fourth quadrant, *C. camphora* and *M. chapensis*, could be selected due to their healthy growing condition under high concentration of sulfur dioxide. The net photosynthesis of the plants in the third quadrant, *L. rhodostegia* and *C. insignis*, decreased a relatively large amount after fumigation, showing their poor growth condition under SO_2 stress. They absorbed almost no SO_2, so did not improve the environment. Thus, these two species were not highly tolerant to SO_2.

Many studies have investigated which pollutant-tolerant plants can be grown near areas with severe air pollution (Govindaraju, 2011; Lee, 2004; Sharma, 2008). However, since they chose the sampling sites and took the sample leaves to test different parameters, the variables, such as the concentration of pollutants and the existence of particles, could not be really controlled. The actual reason for a given species' biochemical characteristics could not be well explained, because they might have resulted from any number of factors. Also, climate factors, such as temperature, latitude, or humidity, might have affected the plants, so the results might not apply to other places or seasons. In this experiment, the environmental variables were all controlled, so that the impact of SO_2 on plants was isolated and repeatable.

Jim (2007) demonstrates that *C. camphora* tolerates SO_2, NO_x and particulates well. Nevertheless, according to our results, although *C. camphora* could survive in a severely polluted area, it could not extract atmospheric SO_2 to improve air quality. However, *C. camphora* did not change substantially in either P_n or sulfur content in the leaves, nor in the values of other measurements such as chlorophyll relative content, relative water content, and electrolytic leakage. Because this experiment focused only on the effects of SO_2, the tolerance of *C. camphora* to other air pollutants is still unknown. A previous study (Lyu, 2003) showed that *C. camphora* can absorb a small amount of SO_2, consistent with the results of this experiment. In addition, this species has a strong ability to extract hydrogen fluoride and is suitable for mildly polluted areas.

Wen et al. (2003) found that *L. rhodostegia* was highly sensitive to air pollutants. The authors concluded that the P_n of this species was reduced by pollutant stress, and the amount of decrease was much greater than the decrease in T_r. Thus, the plant suffered both weaker growth due to less photosynthesis and excessive water loss. That observation accorded with our data. Although Wen et al. investigated multiple air pollutants and we only studied the effects of SO_2, both studies concluded that *L. rhodostegia* can neither resist nor adapt to air pollution.

Surprisingly, the data for *I. rotunda* differed between our study and that of Wen et al. (2003). The latter showed that both P_n and G_s decreased nearly 40% under air pollution, while T_r declined by about 50%. In contrast, we found out that G_s increased and the values of the other measurements did not change significantly. These differences may result from the different environments of the two experiments; the other air pollutants in the study of Wen et al. might offset the effects of SO_2 and change the response of *I. rotunda*, leading to different results.

Because *I. rotunda* was the most suitable species for SO_2 polluted areas according to our results, it should receive more attention to fully elucidate the mechanisms by which it adapts to SO_2 stress. We believe that other species with similar behavioral responses can be found and planted to mitigate air pollution. In addition, more parameters of *C. camphora* can be tested in order to further understand the adaptation of it under sulfur dioxide. This species remains poorly understood because most of the data did not change significantly or show obvious trends in this experiment. Therefore, future experiments must be conducted to investigate the effects on plants of other air pollutants, individually and in combination, to permit the selection of the optimal species for severely polluted regions. To fully understand how plants change under pollution stress, more parameters should be evaluated, including stomatal density, chlorophyll fluorescence, and carbon fixation efficiency. To identify more air pollution tolerant plants, other common subtropical species, which were also mentioned in Wen et al. (2003), like *Ficus microcarpa*, *Camellia japonica* L., and *Tutcheria spectabilis* (Benth.) Dunn, can also be tested.

Conclusion

In this study, the performance of six common landscape tree species under SO_2 fumigation was studied to help select trees for greenbelts near industrial complexes in subtropical area, especially where SO_2 is the main emission. *Ilex rotunda*, which remained green and extracted a great amount of SO_2, is recommended as a key species for greenbelts. *Cassia surattensis* can be used to improve air quality in polluted areas. Both *C. camphora* and *M. chapensis* are also recommended for planting in severely polluted areas because of their high aesthetic values. From an economic and management perspective, *L. rhodostegia* and *C. insignis* are more suitable for cleaner, less-polluted environments. Integrating different tree species into a landscape can both contribute to greenery near factories and maintain biodiversity. As more studies are conducted on the appropriate species to grow in heavily industrial areas, the problem of air pollution can be effectively controlled.

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