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Modeled Dosage–Response Relationship on the Net Photosynthetic Rate for the Sensitivity to Acid Rain of 21 Plant Species

Shihuai Deng · Shuzhen Gou · Baiye Sun · Wenlin Lv · Yuanwei Li · Hong Peng · Hong Xiao · Gang Yang · Yingjun Wang

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Abstract This study investigated the sensitivity of plant species to acid rain based on the modeled dosage–response relationship on the net photosynthetic rate (P_N) of 21 types of plant species, subjected to the exposure of simulated acid rain (SAR) for 5 times during a period of 50 days. Variable responses of P_N to SAR occurred depending on the type of plant. A majority (13 species) of the dosage–response relationship could be described by an S-shaped curve and be fitted with the Boltzmann model. Model fitting allowed quantitative evaluation of the dosage–response relationship and an accurate estimation of the EC_{10}, termed as the pH of the acid rain resulting in a P_N 10 % lower than the reference value. The top 9 species (*Camellia sasanqua*, *Cinnamomum camphora*, etc. $EC_{10} \leq 3.0$) are highly endurable to very acid rain. The rare, relict plant *Metasequoia glyptostroboides* was the most sensitive species ($EC_{10} = 5.1$) recommended for protection.

Shihuai Deng, Shuzhen Gou, and Baiye Sun are co-first authors and have equally contributed to this paper.

S. Deng (✉) · S. Gou · B. Sun · W. Lv · Y. Li · H. Peng · H. Xiao · G. Yang · Y. Wang
Provincial Key Laboratory of Agro-Environmental Engineering, College of Resources and Environment, Sichuan Agricultural University, Chengdu 611130 Sichuan, People's Republic of China
e-mail: shuzhen0827@163.com; shdeng8888@163.com

S. Gou
e-mail: shuzhen0827@163.com

B. Sun
e-mail: bys298@yahoo.com.cn

B. Sun
Department of Agricultural Engineering, Weifang Vocational College, East Dongfeng Street, Weifang 261061 Shandong, People's Republic of China

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Acid precipitation in the East Asia resulting from increasing emission of SO_2 and NO_x into atmosphere, a consequence of economy development, has arisen great concerns recently (Xu and Carmichael 1999; Rodhe et al. 2002). Monitoring data for the year 1993 to the year 2004 from 17 monitoring stations in the Southwest, one of the regions seeing most rapid economic growth in China, shows that 23.53 % of the monitoring stations had a recorded rainwater pH < 4.0, 17.65 % had a rainwater pH < 4.5, and 47.06 % had a rainwater pH < 5.6. About 41.18 % of the stations had a frequency of acid rain beyond 50 % or even 90 % (in certain areas). This reflects that the acid rain in the entire Southwest region is of high frequency, high acidity, and being heavily polluted (Tang et al. 2009).

Acid precipitation may not only generate visible damage such as chlorotic spot and necrotic spot in leaves, but also have other negative impact such as inhibiting photosynthesis, flushing off nutritive elements, breaking water balance, and reducing activity of enzyme (Wood and Bormann 1977; Westman and Temple 1989; Fan and Wang 2000; Jagels et al. 2002; Singh et al. 2004). Consequently, acid precipitation may result in degradation of forest vegetation coverage (Ouimet et al. 2001; Driscoll et al. 2003). As a matter of fact, a vegetation coverage of about two hundred and seventy-five point six thousand square kilometres, accounting for 31.9 % of the total forest area in the Southwest and Sichuan Basin of China, is being jeopardized by acid rain (Ren 1997). The declining growth rate of the forest as a consequence of acid rain is responsible for an annual economic loss of RMB 0.14 billion solely in timber industry in Sichuan Basin (Ren 1997).

Use of acid rain endurable plants for ecological restoration may not only alleviate vegetation degradation but also increase the biodiversity and productivity of the ecosystem in the acid rain region (Bradshaw 1983; Jordan et al. 1987; Naveh 1994; Cairns and Heckman 1996). It is expected that species with a high ecological value or those widespread in the polluted environment must have due capacity to stand with the pollutants specific to that environment. In this principle, Lee et al. (2004) chose *Quercus serrata* and *Alnus firma* as endurable species to SO_2 and Al^{3+} respectively. In the same sense, recognizing and sifting out plant species against acid rain for acid rain region are very important (Bell et al. 1993).

However, the complex two-sided effect of acid rain on plant poses big challenge to estimating the acid rain toxicity threshold (toxicity threshold) of the plant. Acid rain as a mixture of a variety of substances is a complex pollutant comprised of a wide variety of ions including H^+, $SO_4{}^{2-}$, $SO_3{}^{2-}$, $NO_3{}^-$, Cl^-, $NH_4{}^+$, K^+, Ca^{2+} and many others. Among them ions like H^+, $SO_4{}^{2-}$, and $SO_3{}^{2-}$ may have toxic effects on plant while ions like $NO_3{}^-$, K^+ and Ca^{2+} may be fertilizer for plants in most cases (Hindawi et al. 1980; Scherbatskoy and Klein 1983). Acid rain may thusly have both promotional and inhibitory effects on plants (Wood and Bormann 1974; Lee and Weber 1979). Estimation of the toxicity threshold is difficult, as the dosage–response relationship, showing the inhibitory effect of acid rain on plant growth, is a manifestation of the coupled effect of a number of complex inhibitory types, rather than a single type, and shows nonconformity to the traditional dosage–response relationship described by classic toxicology. Campos da Silva et al.(2005) carried out a simulation study on the influence of acid rain on the growth of the plants, and estimated and graded the toxicity threshold values according to the ratio of visible chlorotic spot and necrotic spot in the leaf surface (<5 %, 5 %–30 %, 30 %–50 %). The authors noted that, among the five young plant species from Brazil, *Joannesia princeps* shows highest sensitivity to acid rain. Haines et al. (1980) by measuring the diameter of leaf injury spot suggested a toxicity threshold of pH 0.5 to pH 1.0 for *Pinus strobus* and of pH 2.0 to pH 2.5 for the remaining seven plant species. Munzuroglu et al. (2003) by measuring the pollen germination rate and the length of pollen tube growth determined acid rain threshold proportion value to be around pH 3.3 and pH 3.4 for apple pollen germination and pollen tube elongation, respectively. In spite of the intensive investigations on the toxicity threshold value of acid rain, most of them estimating the value just from the designed experimental pH (inhibitory dosage) while more accurate estimation of the threshold value based on a full-scale dosage–response curve remains unachieved. Since the response of plants to the sustained change in acidity is also a continuous process, estimating the toxicity threshold value from the designed pH values may inevitably incur certain amount of artificial errors from the experimental gradient design, resulting in a low accuracy in estimation. This study investigated the P_N inhibitory rate of simulated acid rain (SAR) of 21 plant species (including herbaceous plant, bush, and arbour) based on the complete dosage–response relationship. Model fitting of the dosage–response curve allowed the toxicity threshold value of the acid rain to be estimated. Moreover, a classification of the plant species were carried out according to their sensitivity to acid rain, which may provide a basis for sifting out appropriate plant species for greening, beautifying, and ecological restoration of the urban environment polluted by acid rain in Southwest of China.

Materials and Methods

The 21 plant species (Heshengyuan Greening Service Department, Ya'an City, Sichuan Province, China) used for the experiments were the carefully selected 2 year-old trees, in a good growing situation, cultured in pot (27 cm height and 25 cm diameter), and placed in a big arch shelter of a greenhouse. The experiment had four designed pH levels, respectively pH 5.6 (control), pH 4.0, pH 3.0 and pH 2.0, and was carried out in quadruplicate (4 trees for each species). The trees were cultured in isolated compartments to avoid cross-contamination during acid rain spraying. A stock solution of pH 1.0 was first made with an ionic mixing concentration of $SO_4{}^{2-}:NO_3{}^-:Cl^- = 5:1:0.36$. This was followed by adding 1.02 mg L^{-1} $CaCl_2$ and 5.35 mg L^{-1} $(NH_4)_2SO_4$ into the stock solution (Wei et al. 2001). Finally, pH was buffered to pH 5.6, pH 4.0, pH 3.0, and pH 2.0 respectively. To guarantee the experimental accuracy, the pH of the SAR shall be verified with a pH meter each time before spraying. In case of any discrepancy between the designed and the measured pH values, a new solution shall be made for use. Each acid rain spraying operation was sustained, using a hand knapsack sprayer (3WBS-16f; Taizhou Luqiao Lamsin Industrial and Trading Co., Ltd., Taizhou, Zhejiang, China), until formation of droplets on the leaves. Spraying was executed for five times over a period of 50 days, from July 13, 2009 to September 1, 2009, at a frequency of once per 10 days. Normal water and pest management was implemented during the culturing period. P_N of the tested plant was recorded by a portable photosynthesis system (LI 6,400; LI-COR, Inc., Lincoln, NE, USA) between 9:00 a.m and 11:00 a.m on September 1, 2009. For each plant, three leaves were selected, respectively from comparable positions to those in the other plants, and five readings were taken for each leaf.

The P_N inhibitory rate was calculated using the method described in Sun et al. (2010). The dosage–response relationship curve was plotted in the coordinate system using 7-pH as the horizontal axis and inhibitory rate as the longitudinal axis. Wherever the dosage–response curve exhibited an S-shape, EC_{10} was estimated by curve fitting using the Boltzmann model (Sun et al. 2010); otherwise, EC_{10} was calculated by linear interpolation using the two points located close to the inhibitory rate of 10 % using Eq (1):

$$I = a \cdot (7 - pH) + b. \quad (1)$$

where I is the P_N inhibitory rate and a and b are constants.

All results were expressed as mean ± standard deviation (SD). The significance of differences between mean values was determined by least significant difference (LSD) technique, with $p > 0.05$, $0.01 < p \leq 0.05$, and $p \leq 0.01$ suggesting non-significance, significance, and extremely high significance, respectively. One-way ANOVA was performed using SPSS11.5 (SPSS Inc., Chicago, IL, USA). Origin 8 (OriginLab Corporation, Northampton, MA, USA) was employed to fit the dosage–response curve.

Results and Discussion

Following the 50 day SAR spraying exercise, four influence types of the acid rain on P_N were observed: (a) promotional; (b) non-affected; (c) promotional at low acidity but inhibitory at high acidity (hormesis); and (d) inhibitory.

(a) *Promotional type* The P_N of the three plant species, *C. sasanqua*, *C. camphora*, and *B. papyifera*, after being processed by the SAR, became significantly higher ($p \leq 0.01$) than or comparable to the reference value at pH 5.6 (5.557, 4.521 and 6.278 μmol m^{-2} s^{-1}, respectively), suggesting that the promotional effect of the nutrient element of the SAR predominated over its toxic effect over the range of the pH investigated (pH ≥ 2.0). Therefore, SAR promoted rather than inhibited the P_N (Fig. 1).

(b) *Non-affected type* The P_N of the three plant species *V. odoratissimum*, *M. basjoo*, *P. orientalis*, and *G. montanum*, following the processing by SAR, showed no remarkable deviation from the reference value (10.960, 13.450, 4.250 and 6.335 μmol m^{-2} s^{-1}, respectively) (Fig. 1). suggesting that SAR had insignificant effect ($p > 0.05$) on the P_N over the experimental pH range (pH ≥ 2.0).

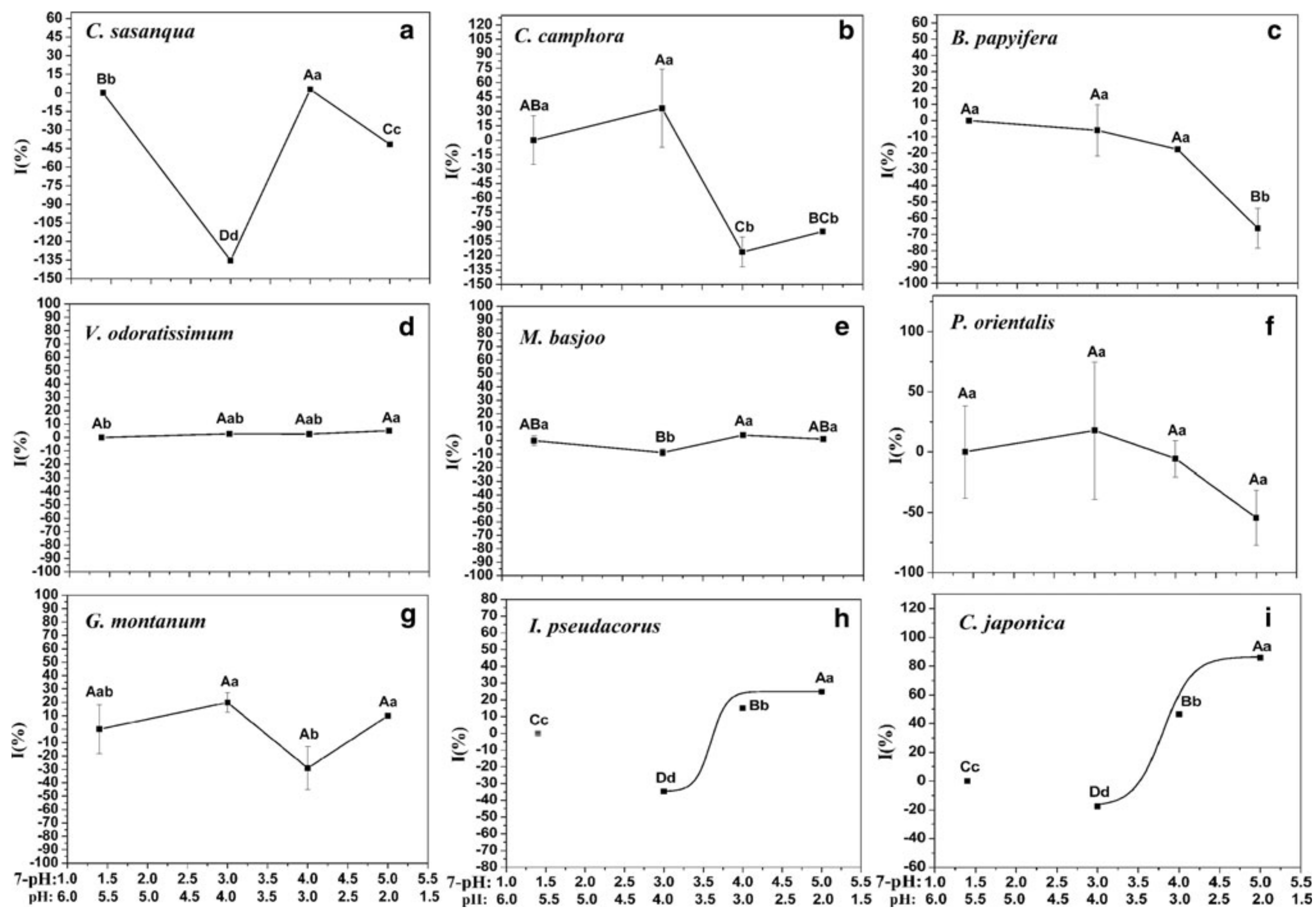

Fig. 1 Dosage–response relationship showing promotional effect (**a–c**), non-affection (**d–g**), and promotional effect at low acidity but inhibitory at high acidity of acid rain on P_N (**h**, **i**). Discrete points are the measured data and the smooth curves are simulated by the Boltzmann equation for fitting the experimental data

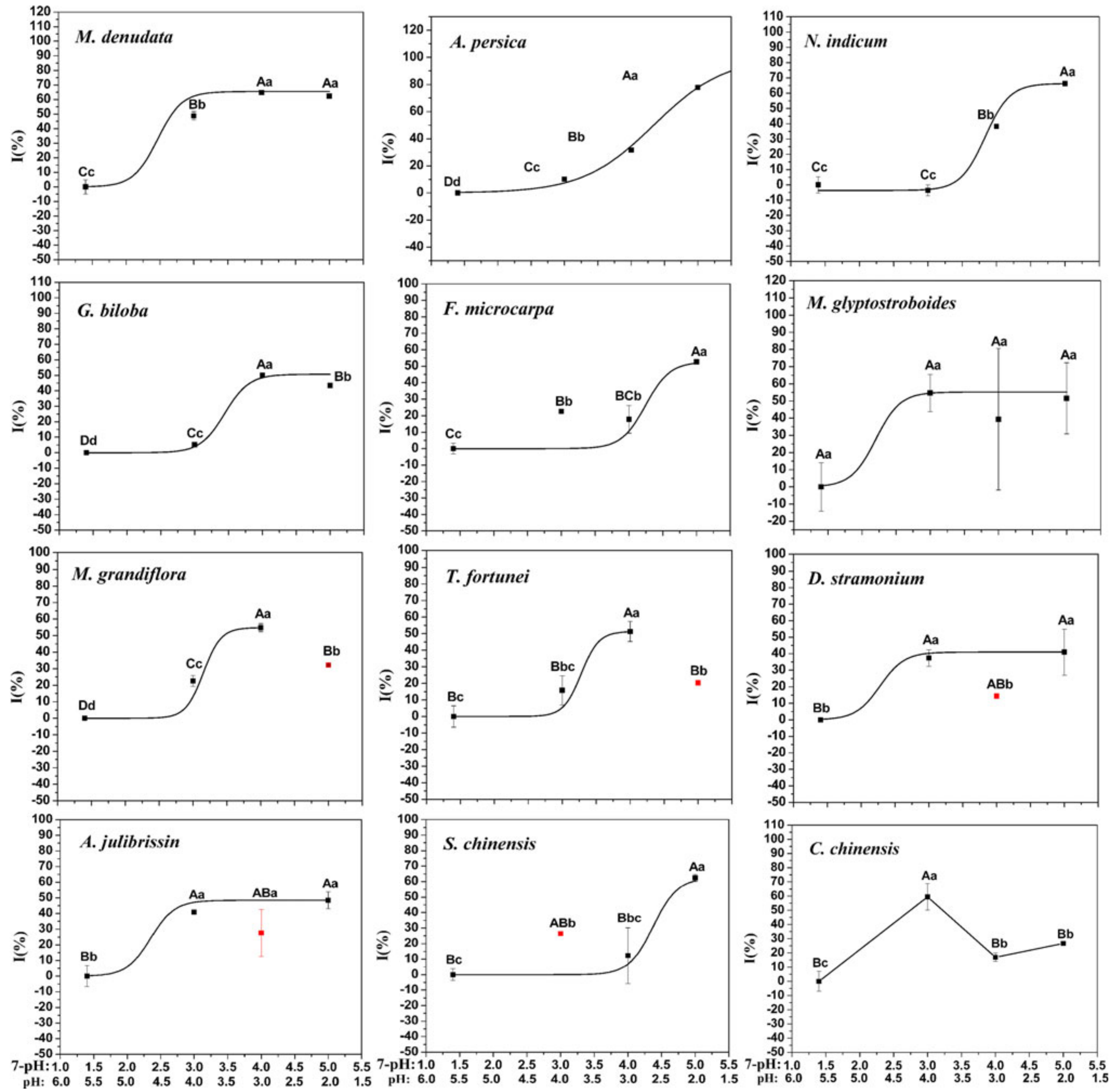

Fig. 2 Dosage–response relationship showing inhibitory effect of acid rain on P_N. Discrete points are the measured data and the smooth curves are simulated by the Boltzmann equation for fitting the experimental data

(c) *Type of promotion at low acidity but inhibition at high acidity* For *Iris pseudacorus* and *Camellia japonica*, the P_N at pH 4.0 was significantly higher ($p < 0.01$) than the reference value (9.704 and 12.500 μmol m^{-2} s^{-1}, respectively), corresponding to a negative inhibitory value, while the P_N inhibition rate at pH $\leq$ 3.0 was significantly higher than the reference value (Fig. 1). This suggests that low acidity may promote while high acidity may inhibit the photosynthesis of the two plant species. The dosage–response relationship occurring during the inhibitory phase exhibited an S-shape and could be described by the Boltzmann equation.

(d) *Inhibitory type* The P_N inhibition rate of the acid rain on the following eleven plant species was significantly higher ($p < 0.05$) than the reference value (10.810, 15.150, 18.280, 9.063, 13.041, 12.490, 9.074, 14.870, 10.696, 4.443 and 15.475 μmol m^{-2} s^{-1}, respectively): *Magnolia denudata*, *Amygdalus persica*, *Nerium indicum*, *Ginkgo biloba*, *F. microcarpa*, *Magnolia grandiflora*, *Trachycarpus fortunei*, *Darura stramonium*, *Albizia julibrissin*, *S. chinensis*, and *Cercis chinensis*, or higher ($p > 0.05$) than the reference value (9.563 μmol m^{-2} s^{-1}) for *M. glyptostroboides* (Fig. 2). Among them, the dosage–response relationship curve of the following six species displayed an S-shape and could be described by the Boltzmann equation: *M. denudata*, *A. persica*, *N. indicum*, *G. biloba*, *F. microcarpa*, *M. glyptostroboides*. For *M. grandiflora* and *T. fortunei*, inhibitory effect was observed at pH < 2.0 and the resulting S-shape in the dosage–response curve could be described by the Boltzmann equation; while at pH 2.0, the inhibitory effect vanished but the promotional effect became predominant.

Estimation of the toxicity threshold allows the difference in the plants' sensitivity to acid rain to be compared. This study used EC_{10}, the pH value of the acid rain resulting in a P_N 10 % lower than the reference value, as the toxicity threshold. The EC_{10} value of the 21 plants, subjected to a 50 day acid rain spraying exercise, was estimated on the basis of the dosage–response curve (Table 1) using the following procedure: for the promotional type (excluding *C. chinensis*) and the type of promotion at low acidity but inhibition at high acidity, EC_{10} was determined by fitting the S-shape in the dosage–response curve using the Boltzmann equation while for *C. chinensis* EC_{10} was determined by linear fitting using Eq. (1). For the seven plants, of promotional type and non-affected type, where no inhibitory effect occurred, EC_{10} was selected to be within the range of pH < 2.0.

Based on the classification of plant species according to their acid rain endurability (Shan 1994), this study used the acid rain endurability of the plants' P_N to classify plant species into three categories, which are respectively of low sensitivity to acid rain ($EC_{10} \leq 3.0$), of intermediate sensitivity to acid rain ($3.0 < EC_{10} \leq 4.0$), and of high sensitivity to acid rain ($EC_{10} > 4.0$). The investigated 21 plant species, including arbor, bush, and herbaceous plant, consist of 5 highly sensitive ones (23.81 % of the total) including *D. stramonium*, *C. chinensis*, *M. denudata*, *A. julibrissin*, and *M. glyptostroboides*, 7 intermediately sensitive ones (33.33 % of the total) including *I. pseudacorus*, *N. indicum*, *C. japonica*, *A. persica*, *G. biloba*, *T. fortunei*, and *M. grandiflora*, and 9 low sensitive plants (42.86 % of the total) including *M. basjoo*, *G. montanum*, *C. sasanqua*, *V. odoratissimum*, *B. papyifera*, *F. microcarpa*, *P. orientalis*, *S. chinensis*, and *C. camphora* (Table 1). The low sensitive species can be used for re-vegetation of the area impacted by acid rain while the species of high sensitivity shall be protected from the acid rain.

Feng et al. (1999), based on a comparative study using the four parameters, namely the leaf toxicity threshold, the leaf injury spot ratio, and the dosage and time corresponding to the first symptoms, noted that *G. biloba*, *M. grandiflora*, and *A. persica* are intermediately sensitive plant species while *P. orientalis* is a low sensitive species. This is consistent with the finding of this study obtained by fitting of the dosage–response relationship curve on the P_N inhibitory rate. Liu et al. (2003), by evaluating the comprehensive growth ratio of stem length, basal diameter, and canopy breadth, noted that the plants of Moraceae and Theaceae are highly endurable to the typical and heavy pollution in atmosphere while plants of Magnoliaceae generally have low endurability. This finding is also confirmed by the finding of our study. Shan et al. (1994) investigated the threshold value of SAR, by evaluating the leaf injury spot ratio and the initial symptoms, and noted that: (a) among the Pinaceae, the Taxodiaceae, and the Cupressaceae, the Cupressaceae is most endurable while the Taxodiaceae is most sensitive; (b) the Theaceae and the Moraceae are the most endurable in the broad leaved trees; (c) the Apocynaceae and the Palmae of unifacially leaved trees have relatively high endurability. Our study results show good agreement with the finding of Shan et al. (1994).

Table 1 Classification of 21 types of plants according to their sensitivity to SAR

Category	Plant	EC10 (pH value)
Low sensitivity to acid rain ($EC_{10} \leq 3.0$)	*C. sasanqua*	<2.0
	C. camphora	<2.0
	B. papyifera	<2.0
	V. odoratissimum	<2.0
	M. basjoo	<2.0
	P. orientalis	<2.0
	G. montanum	<2.0
	S. chinensis	2.9
	F. microcarpa	3.0
Intermediate sensitivity to acid rain ($3.0 < EC_{10} \leq 4.0$)	*I. pseudacorus*	3.3
	C. japonica	3.4
	N. indicum	3.4
	A. persica	3.8
	G. biloba	3.8
	T. fortunei	3.9
	M. grandiflora	4.0
High sensitivity to acid rain ($EC_{10} > 4.0$)	*M. denudata*	4.8
	D. stramonium	4.9
	A. julibrissin	4.9
	M. glyptostroboides	5.1
	C. chinensis	5.3

In spite of the difference, in the experimental method, the investigated parameter, and the approach taken for data analysis, from previous studies, similar conclusions were drawn, suggesting that P_N may be used as a suitable experimental parameter, and that use of EC_{10} as a toxicity threshold and determining it by curve fitting of the S-shape of or linear fitting of the dosage (7-pH)–response (P_N inhibitory rate) relationship curve may be an appropriate approach. This approach no longer relies on a selection of toxicity threshold value between one or two arbitrarily set experimental pH values and thusly allows more accurate evaluation of the plants' sensitivity to acid rain.

Metasequoia glyptostroboides, with the nickname of 'living fossil' and as a valuable rare relict species in the world, shall be paid with high attention since it, although showing high sensitivity (vulnerability) to acid rain, originates from the southwest area of China being severally suffering from acid rain.

This study shows that the following 9 types of plants are highly endurable to very acid rain (i.e., pH < 2.0) and may be preferred for ecological restoration or greening work in the Southwest area of China where acid rain is prevalent: *C. sasanqua*, *C. camphora*, *B. papyifera*, *V. odoratissimum*, *M. basjoo*, *P. orientalis*, *G. montanum*, *S. chinensis*, and *F. microcarpa*.

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