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Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seedling growth of five hardwood species growing in China

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Abstract

Seeds and seedlings of five hardwood species were subjected to a simulated acid rain adjusted to pH values of 2.0, 3.5, 5.0, 6.0, and to distilled water (the control). Seed germination was remarkably inhibited by pH 2.0 treatment for three species. Significant foliar damage, decline in chlorophyll contents, and retardation of growth for the seedlings of all the species, were observed at pH 2.0; while seedling growth was stimulated at pH levels between 3.5 and 5.0. The pH 2.0 treatment seemed to be a threshold level for inhibition of seed germination and seedling growth for all the treated species. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Simulated acid rain; Hardwood species; Foliar damage; Chlorophyll; Seedling growth

1. Introduction

Acid rain emerged as an environmental issue of increasing concern in China in the late 1970s. The nationwide monitoring data gathered so far demonstrate that widespread occurrence of acid rain is concentrated in southern part of the Yangtze River, especially in southwestern region, where precipitation in the late 1980s was found to have average pH's between 3.5 and 4.8 (Yang, 1989; Xu and Hao, 1990) Owing to the increasing seriousness of acid rain in southwest China, a series of research programs have been set up by the National Environmental Protection Agency (NEPA) since 1982 (Feng, 1993). Among them a project entitled 'A Study on the Acid Rain in China' was listed as one of the key projects of the State's seventh five-year plan (1985–1990). The results concluded that local pollution was the major reason for acidic deposition, whereas long range transport played a relatively minor role. However, in the past decade the acid rain has risen over an extensive area in China along with the increasing emissions of SO_2 and NO_x. Following Europe and North America, South China has become the third most severely affected region by acid rain in the world (Wang and Ding, 1997).

There are growing indications that local dieback and regional decline of forests in central and south-

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western China are caused by air pollutants. Since the beginning of the 1980s, about 2000 hectares of Masson's pine (*Pinus massoniana*) forests in Nanshan, the southern suburb of Chongqing City, Sichuan Province, have exhibited serious decline symptoms, including tip necrosis of needles, thin crown, reduced needle length, premature abscission, branch dieback, and reduced radial growth, which was supposed to be caused by a combination of SO_2 and fluoride pollution (Yu et al., 1990; Bian and Yu, 1992). The total forest areas damaged by acid deposition in seven provinces of South China were estimated to be 1.2821×10^6 hectares, of which *P. massoniana* stands were 7.908×10^5 hectares, and *Cunninghamia lanceolata* stands 4.913×10^5 hectares(Feng and Tao, 1998).

Based on field investigations and experimental results with simulated acid rain, the potential effects of acid rain on plants include: injury to foliage (Leith et al., 1989; Back and Huttunen, 1992); interference with normal metabolism (Pell, 1988; Magel et al., 1990); accelerated leaching of nutrients from plant foliage and soil (Turner and Tingey, 1990; Reddy et al., 1991); effects of increasing Al^{3+} in soil solution on the fine roots (Schaedle et al., 1989); influences on seedling emergence and growth (Lee and Weber, 1979; McColl and Johnson, 1983; Haines and Carson, 1989); alterations of symbiotic associations and host-parasite interactions (Shriner, 1976; Walker and McLaughlin, 1991); and increased susceptibility to some environmental stress factors (Tomlinson, 1983; Johnson and Siccama, 1983). Hence, acid precipitation has the potential to affect forests in many ways.

Unfortunately, the progress of acid rain research, especially on its ecological consequences, lags behind the development of acid rain in China. The data available to support the hypothesis that acid precipitation is primarily responsible for forest decline are not convincing. The purpose of this paper is to study the effects of simulated acid rain on seed germination and seedling growth of five common hardwood species growing in South China.

2. Materials and methods

2.1. Germination test

The five tree species used in the test were *Cinnamomum camphora* L., *Ligustrum lucidum* Ait., *Castanopsis fissa* Rehd. et Wils., *Melia azedarach* L., and *Koelreuteria bipinnata* Franch. Among them *C. camphora* and *L. lucidum* are native evergreen hardwood species in South China, and commonly used in garden, in ornamental beautification planting, or as shade trees in urban areas where air pollution is generally concentrated. The other three species were considered to have different responses to air pollution, with *K. bipinnata* showing relatively high sensitivity, *C. fissa* intermediate, and *M. azedarach* low (Feng, 1993).

Seeds of five hardwood species were placed in culture dishes with filter sheets to germinate in a culture container kept at a constant temperature of 25°C. Before experiment, the dishes were pasteurized at 100°C for 1 h in oven, and seeds with 0.5% formalin solution for 20 min. For each species, 25 dishes were randomly divided into five groups. Totally 125 dishes were used, and each dish received 50 seeds. The filter sheets of dishes in each group were maintained in wet condition by spraying one simulated acid solution adjusted to one of the following pH's: 2.0, 3.5, 5.0, 6.0, and distilled water (the control). The simulated acid solution was prepared with solution of 1 N H_2SO_4 and 1 N HNO_3 in the ratio of 10 to 1 by chemical equivalents, with reference to the general anion composition of rainfall in South China (Fan, 1993). During the treatment, the numbers of germinated seeds were recorded each day.

2.2. Foliar application test

In December 1994, 125 pots with each containing 7.5 kg of red earth obtained from the Experimental Forest of Fujian Forestry College, Fujian, were randomly divided into five subsets, and placed in a greenhouse. The soil used in the experiment is one of the typical soil types supporting evergreen broad-leaved forests in the sub-tropical region in South China. It was a moderately well-drained sandy loam, had a pH of 4.32 and cation exchange capacity of 4.98 meq/100 g. In each subset of 25 pots, 5 pots each were planted with 50 seeds of one species (ten seeds in each pot). The five species used were all the same as in germination test. The soils were irrigated with distilled water before treatment. About three weeks after seedling emergence, each pot was thinned to three seedlings.

Beginning on 10 May 1995 and continuing for the next six months, each subset was subjected to one pH-level simulated acid rain (prepared by the same method as in germination test). The acid rains and distilled water were sprayed over the seedlings via sprinklers capable of delivering droplet sizes in the range of 0.4–0.6 mm diameter. The spraying was applied in the late afternoon near sunset, once a week during the first six weeks, afterwards twice a week, on an average of 40 mm of solution per week. The light intensity during simulated rain events was only 25–45% of that at noon. The temperature in the greenhouse was generally 1–3°C higher than the outside.

On 5 September 1995, fresh leaves were sampled from each treatment, and their chlorophyll a and b were extracted with 95% alcohol and then measured by 721-spectrophotometer (Xue, 1986). At the end of treatment (on 5 November 1995), the numbers of the total leaves and the leaves with visible necrosis(with more than 20% necrotic lesion areas) for each seedling were recorded separately. Percentage of damaged leaves to the total was used as an indicator for degree of foliar damage. The height and diameter at foot for each seedling were also measured. After that, the seedlings were harvested, and the leaves, shoots and roots were separated, ovendried, and weighed.

Analyses for chlorophyll contents and dry weight were in quintuplicate and statistical analysis of all the data was carried out using the computer package EXCEL 97.

3. Results

3.1. Effects on germination

Table 1 gives the germination of each species by treatments. Based on F-test, significant treatment effects were observed for three species, whose germination was remarkably inhibited by pH 2.0 acid solution. Compared with the controls, pH 2.0 caused germination reduction by 51.09%, 76.61%, and 56.32% for *C. camphora*, *C. fissa*, and *K. bipinnata*, respectively. But the other treatments produced no statistically significant impacts on seed germination except for *C. fissa*, which showed slight inhibition at pH 5.0. For all the species, however, continuous exposure to pH 2.0 solution after the seeds germinated caused the primary shoots and roots of the tender seedlings to rot away gradually, leading to very low seedling emergence.

3.2. Effects on seedlings

Simulated acid rain produced severe damage to the leaves of all five species. The percentages of the

Table 1
Germination percentage of the seeds treated with simulated acid solution

Species	Statistic	Treatments					$LSD_{0.05}$	$LSD_{0.01}$	*P*-value
		pH 2.0	pH 3.5	pH 5.0	pH 6.0	Control			
C. camphora	Mean	45.0[b]	90.0	88.0	93.0	92.0	11.3414	15.4680	<0.001
	SE	2.0976	4.8990	5.9749	1.5166	2.7386			
L. lucidum	Mean	21.0	19.0	24.0	26.0	24.0	11.6890	15.9422	0.742
	SE	2.7568	4.6152	6.3008	2.0977	2.3452			
C. fissa	Mean	8.0[b]	29.0	22.4[a]	30.4	34.2	11.3368	15.4617	0.001
	SE	1.4142	2.0396	3.4871	6.6151	3.4293			
M. azedarach	Mean	12.0	14.0	10.0	8.0	10.0	8.9246	12.1718	0.704
	SE	3.1241	5.5777	3.4641	3.0332	1.4142			
K. bipinnata	Mean	38.0[b]	85.0	88.0	76.0	87.0	11.7781	16.0635	<0.001
	SE	6.0910	3.3615	3.1623	4.0000	2.3022			

[a] indicates that the treatment differences from the control are significant at $P < 0.05$ based on least significant difference (LSD) test.
[b] indicates that the treatment differences from the control are significant at $P < 0.01$ based on least significant difference (LSD) test.

Table 2
Damage of simulated acid rain to seedling leaves, expressed by percentage of the number of leaves damaged with visible necrosis

Species	Statistic	Treatments					$LSD_{0.05}$	$LSD_{0.01}$	*P*-value
		pH 2.0	pH 3.5	pH 5.0	pH 6.0	Control			
C. camphora	Mean	51.7[b]	14.2[b]	6.3	6.4	1.8	7.0490	9.3512	<0.001
	SE	5.0530	1.7886	1.0769	0.9209	0.5695			
L. lucidum	Mean	80.1[b]	3.6	14.7[a]	1.2	0	12.6852	16.8282	<0.001
	SE	4.2794	1.0803	9.9687	0.8274	0			
C. fissa	Mean	94.9[b]	16.6	26.8[b]	26.3[b]	11.6	6.7934	9.0121	<0.001
	SE	1.6606	2.5436	2.4520	2.9440	2.2238			
M. azedarach	Mean	35.5[b]	6.9	13.3	0	0	18.8021	25.2338	0.002
	SE	10.9332	4.9112	9.0851	0	0			
K. bipinnata	Mean	99.4[b]	18.9	18.6	12.3	13.8	7.4160	9.8380	<0.001
	SE	0.6067	3.0745	3.0431	2.6791	2.8503			

[a] indicates that the treatment differences from the control are significant at $P < 0.05$ based on least significant difference (LSD) test.
[b] indicates that the treatment differences from the control are significant at $P < 0.01$ based on least significant difference (LSD) test.

Table 3
Effects of simulated acid rain on chlorophyll contents of seedlings (mg/g)

Species	Chlorophyll	Statistic	Treatments					$LSD_{0.05}$	$LSD_{0.01}$	*P*-value
			pH 2.0	pH 3.5	pH 5.0	pH 6.0	Control			
C. camphora	a+b	Mean	0.729[b]	0.986[b]	1.134[a]	1.311	1.292	0.1281	0.2010	<0.001
		SE	0.0455	0.0315	0.0055	0.0435	0.0350			
	a/b	Mean	1.21	1.13[a]	1.14[a]	1.23	1.25	0.0776	0.1216	0.025
		SE	0.0101	0.0050	0.0300	0.2500	0.2500			
L. lucidum	a+b	Mean	0.736[b]	1.286	1.486	1.247	1.302	0.2324	0.3644	0.003
		SE	0.0585	0.0510	0.0415	0.1070	0.0350			
	a/b	Mean	1.10[a]	1.21	1.13	1.16	1.23	0.1189	0.1865	0.157
		SE	0.0100	0.0100	0.0350	0.0550	0.0300			
C. fissa	a+b	Mean	0.675[b]	0.814	0.942	1.003	0.904	0.1208	0.1895	0.004
		SE	0.0260	0.0325	0.0225	0.0160	0.0551			
	a/b	Mean	1.16	1.10	1.15	1.17	1.22	0.2909	0.4562	0.597
		SE	0.0300	0.0050	0.1130	0.0600	0.1200			
M. azedarach	a+b	Mean	0.767[b]	1.386	1.462[a]	1.046	1.238	0.2004	0.3143	0.002
		SE	0.0455	0.0395	0.4675	0.0265	0.0990			
	a/b	Mean	1.13	1.17	1.19	1.13	1.16	0.0801	0.1256	0.287
		SE	0.0100	0.0100	0.0100	0.0100	0.0450			
K. bipinnata	a+b	Mean	0.666[b]	1.179[a]	1.321[b]	1.419[b]	1.027	0.1365	0.2141	<0.001
		SE	0.0370	0.0520	0.0160	0.0255	0.0455			
	a/b	Mean	1.24	1.24	1.21	1.17	1.17	0.2197	0.3445	0.420
		SE	0.0950	0.0100	0.0071	0	0.0950			

[a] indicates that the treatment differences from the control are significant at $P < 0.05$ based on least significant difference (LSD) test.
[b] indicates that the treatment differences from the control are significant at $P < 0.01$ based on least significant difference (LSD) test.

damaged leaves were significantly higher at pH 2.0 than at all other levels (Table 2). The initial symptom was the formation of marginal and interveinal necrotic spots with yellow-brown color. Eventually these necrotic zones extended through the leaf and were visible on upper surfaces. Some damaged leaves curled as the necrotic areas dried. The degree of damage varied with the tree species, with *C. fissa* and *K. bipinnata* being the most, and *M. azedarach* the least affected.

Simulated acid rain at lower pH levels (between 2.0 and 3.5) decreased chlorophyll contents of the seedlings, with the minimum at pH 2.0 for all the species, while maximum chlorophyll contents occurred at pH levels between 5.0 and 6.0 (Table 3). However, the ratios of chlorophyll a to b were not significantly affected by the treatment for four species (except for *C. camphora*), suggesting that the extent to which both chlorophyll a and b were influenced were approximately the same.

Growth in height and diameter was both significantly affected by the treatment for *C. fissa* and *M. azedarach*, but adverse effects were confined to only one measured parameter for the other three species (Fig. 1). As compared to the controls, pH 3.5 stimulated height and diameter growth of *C. camphora*, *M. azedarach*, *K. bipinnata*, and height growth of *L. lucidum*, but except for *C. fissa*, whose growth was greatest when treated with distilled water.

The differences in average dry weight of the leaves, shoots and roots of each species by treatment can be seen in Fig. 2. All the species showed a significant

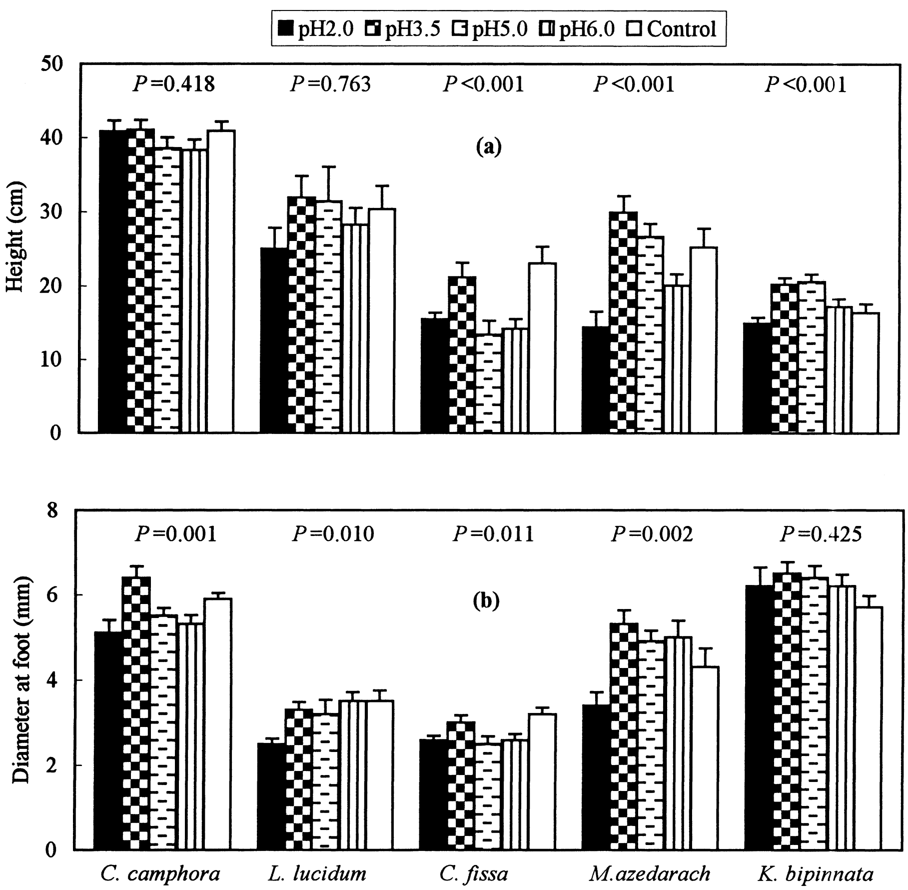

Fig. 1. Effects of simulated acid rain on seedling growth in (a) height, and (b) diameter at foot. Vertical bars represent standard errors of the means.

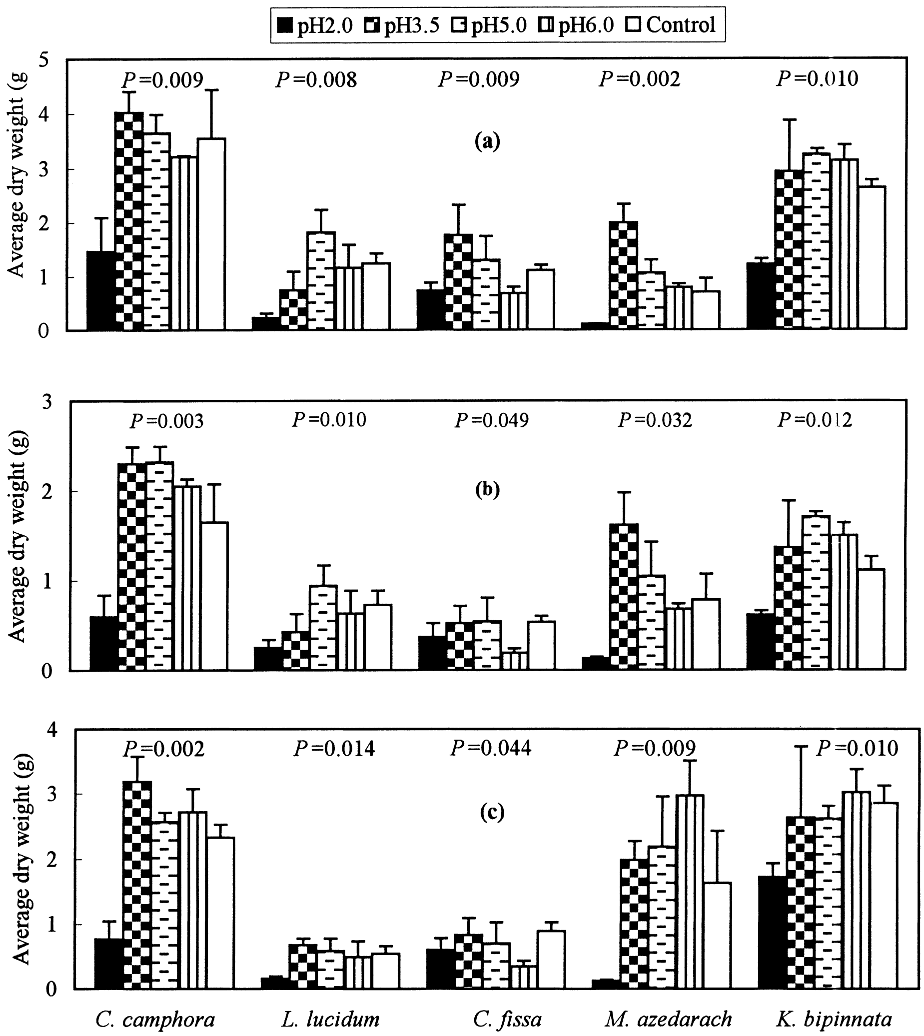

Fig. 2. Average dry weight of (a) leaves, (b) shoots, and (c) root of seedlings treated with simulated acid rain. Vertical bars represent standard errors of the means.

inhibition of top and root growth by pH 2.0. Compared with the controls, the reduction of the average dry weight by pH 2.0 treatment ranged between 34.39–83.83% for the leaves, 30.71–83.10% for the shoots, and 32.62–92.34% for the roots, depending on the species. But the total growth was generally greater at pH levels between 3.5 and 5.0.

The direction and magnitude of growth effects varied with species and with treatment, but generally pH 2.0 seemed to be a threshold level for inhibition of growth, while pH levels between 3.5 and 5.0 stimulated the growth.

4. Discussion

The acid rain at pH 2.0 was found to have affected the seedlings adversely for all the five species, but inhibited seed germination for only three species. This is in accordance with the findings that germination of some American tree species was less affected by acid rain than seedling establishment (Lee and Weber, 1979; Percy, 1986; Scherbatskoy et al., 1987). For this phenomenon Eldhuset et al. (1994) proposed a possible explanation that germination starts by the passive process of water imbibition, swelling of existing tissue, and rupture of the seed coat, followed by cell division and tissue elongation in the embryo. But potential effects of the extremely low pH on germination were recorded for several species (McColl and Johnson, 1983; Moore and Gillette, 1988; Kim, 1987).

Woody plant studies that have examined acute foliar damage by acidic deposition are typically performed under controlled environmental conditions. Most data have been produced from experiments conducted in growth chambers or greenhouses. Close chambers, for example, typically have higher temperature and lower light than ambient conditions. In this experiment, more than 10% of leaf damage occurring in the control suggested that high temperature in the summer times may have partially contributed to the necrosis for *C. fissa* and *K. bipinnata*. The development trends in open-top chambers design may hopefully address this problem (Leith et al., 1989; Sasek et al., 1991; Qui et al., 1992).

The contents of chlorophyll a and b were significantly reduced by pH 2.0 treatment, while the ratio of these two pigments was not affected at any of the levels applied. Morrison (1984) pointed out that chlorophyll formation may be decreased by acid rain due to foliar leaching of nutrient elements, especially magnesium which is one of the major components of chlorophyll. However, in a study conducted with four deciduous tree species native to the eastern USA, no statistically significant effect of simulated acid rain on these pigments was found (Neufeld et al., 1985). Other studies showed that needle chlorophyll a content was not affected by acid treatments, while chlorophyll b level was reduced at lower pH values (Abouguendia and Bascak, 1987; Westman and Temple, 1989). Differences in sampling dates and heights at the seedlings may be partially responsible for these variations.

The average individual weight of the seedlings was significantly reduced at pH 2.0 for all the five species, resulting from reduction in shoot and root growth. Simulated acid rain at pH 2.0 was observed in other experiments to decrease seedling growth as a result of acceleration of respiratory rate, damage to fine roots and increased leaching of cations (Shan and Feng, 1989; Liao and Chen, 1992). However, stimulation of seedling growth by simulated acid rain at pH values between 2.3 and 4.0 was estimated to be caused by NO_3^- fertilization (Lee and Weber, 1979), but accelerated productivity was supposed to be a short-term phenomenon due to declining supplies of available cations (Wood and Bormann, 1976). Shelburne et al. (1993) also concluded that accelerated effects of acid rain on above-ground biomass and leaf area were due to an increasing concentration of soil N. Under natural environment the positive or negative effects observed in artificial controlled conditions may be modified or slowed down, but changes in soil biological processes and chemical properties by acid rain have profound consequences on forest productivity (Tamm et al., 1976). Red soil with high acidity and low cation exchange capacity may be expected to have low ability to buffer acidic inputs. Therefore, the direct effects of acid rain on soil chemistry can have long-term indirect effects on the plant growth.

The ratio of sulfate : nitrate in the simulated acid rain used in our study was 10 : 1. There are limited reports concerning both variation in acidity and anion content of simulated rain. Studies with red spruce indicated that the combination of high acidity (pH 3.5) and sulfate in simulated rain decreased volume of above-ground tissues of red spruce, while high acidity (pH 3.5) and nitrate increased volume (Jacobsen et al., 1990). Therefore, not only acidity itself, but also sulfate/nitrate ratio determines the extent to which acid precipitation influences forest trees. Higher sulfate/nitrate ratio in this study have probably exacerbated the negative effects of simulated rain on seedlings of the five hardwoods. But continued research may be needed to understand the complexity of different sulfate to nitrate ratios of simulated rain of differing pH levels on the growth of forest tree species.

The seedlings of five hardwood species were not extremely sensitive to simulated rain and as acidic as pH 3.5. This level of acidity does not occur consistently in the environment in South China, so impacts of precipitation less acidic than this probably have little effect on these species.

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