# DIRECT FOLIAR EFFECTS OF SIMULATED ACID RAIN

# I. DAMAGE, GROWTH AND GAS EXCHANGE

## BY H. S. NEUFELD<sup>1</sup>, J. A. JERNSTEDT<sup>2</sup> AND B. L. HAINES<sup>2\*</sup>

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<sup>1</sup>Biology Department, New Mexico State University, Las Cruces, New Mexico, 88003, USA, and <sup>2</sup>Botany Department, University of Georgia, Athens, Georgia, 30602, USA

#### SUMMARY

The effects of foliar applications of simulated acid rain (pH 5·6, 4·0, 3·0 and 2·0) were observed for seedlings of four deciduous tree species native to the eastern United States, Liriodendron tulipifera L., Platanus occidentalis L., Liquidambar styraciflua L., and Robinia pseudo-acacia L. Damage occurred only at pH 2·0. Liriodendron was least damaged, Platanus the most. For Liriodendron and Platanus, older leaves showed more damage than younger ones, whereas for Robinia the opposite was true. No age effect was noted for Liquidambar. Height growth was reduced at pH 2·0 in Platanus and Robinia, and less so in Liquidambar. Total biomass was significantly reduced at pH 2·0 for Platanus and Liquidambar, resulting mainly from reduction in stem and root biomass. Leaf biomass was not greatly affected in any of the species. Photosynthetic rates in Platanus were reduced after exposure to rain of pH 2·0, and were the result of changes in mesophyll conductance to  $CO_2$ , as stomatal conductances were not affected. In Liquidambar, stomatal conductances to water vapour were lowered after exposure to rain of pH 2·0. Results show that under greenhouse conditions only rain of extremely low pH causes foliar damage and growth reductions. Lowered rates of photosynthesis in Platanus may have contributed to the observed reductions in growth.

Key words: Acid rain, gas exchange, photosynthesis, growth, leaf conductance.

#### INTRODUCTION

Acid rain occurs over much of the eastern United States (Brezonik, Edgerton & Hendry, 1980) from Maine to Florida (Haines, 1979; Likens & Butler, 1981) and may have deleterious impacts on the environment. Effects of acid rain on plant growth are often species-specific; some plants show enhanced growth (Wood & Bormann, 1977; Evans & Lewin, 1981; Lee *et al.*, 1981; Raynal, Roman & Eichenlaub, 1982; Troiano, Heller & Jacobson, 1982), some no effect (Lee *et al.*, 1981) and others reduced growth (Lee *et al.*, 1981; Evans, Curry & Lewin, 1981; Raynal *et al.*, 1982; Johnston *et al.*, 1982). These growth responses have been attributed mainly to direct effects of acid rain on foliage and not to indirect effects resulting from alterations of soil chemistry (Lee *et al.*, 1981; Johnston *et al.*, 1982; Raynal *et al.*, 1982). The mechanisms responsible for the growth alterations caused by acid rain are not yet understood. Changes in photosynthesis, foliar nutrient levels, and/or translocation rates have been suggested to account for these patterns,

<sup>\*</sup> To whom correspondence should be addressed.

but no clear understanding of the actual mechanisms involved has emerged (Troiano et al., 1982).

When visible foliar damage occurs as a result of acid rain, photosynthesis may be reduced simply because of the loss of viable photosynthetically active tissue. Raynal *et al.* (1982) found, however, that sugar maple seedlings subjected to moderate levels of acid rain grew more than seedlings receiving normal rain (pH of 5.6) even though the former had visible signs of leaf necrosis. This suggests a possible enhancement of photosynthesis after exposure to acid rain. In other instances growth and crop yields have been reduced without visible signs of foliar damage (Evans *et al.*, 1981; Hindawi, Rea & Griffis, 1980; Lee *et al.*, 1981). This strongly suggests inhibition of photosynthesis and/or stimulation of respiration.

Few studies have addressed the question of whether acid rain may affect the photosynthetic rates of plants. Ferenbaugh (1976) measured  $O_2$  release from excised bean tissue exposed to acid rain and found stimulation at low pH. Higher rates of gross photosynthesis were also found in soybean plants subjected to acid rain (Irving & Miller, 1980). In contrast, Jaakkola *et al.* (1980) found that acid misting reduced net photosynthesis in seedlings of Scots pine. Sheridan & Rosenstreter (1973) found that acid rain reduced photosynthesis in the moss *Tortula ruralis*, while Lechowicz (1982) reported that the maximum rate of gross photosynthesis was depressed, and the time needed to reach that rate increased, when caribou lichen was wetted with acid rain solutions.

These studies are the only known reports concerning the effects of acid rain on photosynthesis and, except for the study of Scots pine, no data are available on the effects of acid rain on *net* photosynthesis. The only study of the effects on transpiration is by Evans *et al.* (1981) which showed that stomatal conductance to water vapour increased after exposure of plants to acid rain. Clearly there is a need to assess the response of net photosynthesis and transpiration to the effects of acid rain. This will allow determination of whether any changes are the result of alterations in stomatal functioning or mesophyll metabolism, providing a better understanding of the mechanisms responsible for growth changes after exposure to acid rain.

The objectives of this study were to determine (1) if foliar application *only* of acid rain could affect growth of tree seedlings, (2) if it could alter the gas exchange characteristics of their leaves, and (3) if so, whether this resulted from changes in stomatal and/or mesophyll conductances.

#### MATERIALS AND METHODS

Seedlings of *Platanus occidentalis* L., *Liquidambar styraciflua* L., and *Robinia pseudo-acacia* L. were grown from seed in 4 l pots in a greenhouse. When seedlings were 3 to 6 cm tall they were thinned to one per pot for a total of 60 seedlings per species. Individuals of *Liriodendron tulipifera* L. were obtained from established seedlings growing in Athens, Georgia and transplanted into pots of the same size as those used for the other species. Seedlings were at the 1-2 leaf stage when collected. By the start of the experiments, most of the accumulated biomass had developed under greenhouse conditions. *Pinus rigida* Mill. and *Pinus virginiana* Mill. seedlings were also grown but not included in the final data analysis because of cultivation problems. A loamy sand mixture was used for the potting soil. Seedlings were kept well watered and fertilized weekly with 1/2 strength Hoagland's nutrient solution.

Prior to initiation of the acid rain treatments, 40 individuals of each species were selected to receive simulated acid rain. Individuals within a species were selected for uniform height and vigour and assigned to two randomized complete blocks of 80 trees each (20 rows/block, 4 species/row). Individuals were randomly assigned to rows and each species occurred once in each row. Placement within a row was at random. Four acid rain treatments were imposed (10 trees per species per treatment) and trees receiving a particular treatment occurred every fourth row, beginning with pH 5.6 in row 1, pH 4.0 in row 2, pH 3.0 in row 3, and pH 2.0 in row 4.

To prevent acid rain from entering the soil, non-toxic caulking (DAP, Dayton, Ohio) was placed around the base of the stem of each seedling (excluding the pines which were too small) and a white plastic bag with the bottom slit slipped over the seedling. The bag was then sealed to the caulking with a twist-tie. The caulking allowed for subsequent stem enlargement without the danger of girdling. Between rain applications, the bag was rolled up to allow for watering and aeration of the soil. Only the leaves and stem were therefore exposed to the simulated acid rains.

Simulated acid rain solutions consisted of deionized water plus a salt and acid component. The salt component contained average amounts of elements reported in rainfall at Coweeta, North Carolina (Swank & Henderson, 1976) in mg 1-1: Ca 0.23; Na 0.17; K 0.08; Mg 0.05; NH<sub>4</sub>-N 0.02; PO<sub>4</sub> 0.007. Rain solutions were amended with a 10:7:1 (mass ratio) mixture of  $SO_4$ :  $NO_3$ : Cl (Cogbill & Likens, 1974) using reagent grade acids to produce rains having pH values of 5.6 (0.0025 mequiv H+ l-1), 4.0 (0.1 mequiv H+ l-1), 3.0 (1.0 mequiv H+ l-1) and 2.0 (10.0 mequiv H<sup>+</sup> l<sup>-1</sup>). These pH values cover the range over which foliar damage has been reported to occur (Haines, Stefani & Hendrix, 1980). It proved to be impossible to make artificial rainwater with a pH of 5.6 without adding small amounts of NaOH to the solution. An accurate estimate of the amount added each time was not kept, and trees in this treatment therefore received slightly higher amounts of Na in the rainwater than trees in the other treatments. An Orion pH meter (Orion Research Corporation Inc., Cambridge, Massachusetts) equipped with a glass pH electrode (Corning, Corning, New York) was used to determine pH values. The meter was calibrated against reference buffers each time a solution was prepared.

Seedlings were moved to an adjacent bench to receive the rain treatments. Artificial acid rain was pumped by a variable speed pump (Flotec Inc., Norwalk, California, Model R2S1-110V with 316 stainless steel pumping head Model 834, viton seals with 3/8 IPT inlets and outlets) through a water filter (to remove particulates) to two stainless steel spray nozzles using a vinyl garden hose. Nozzles (TEEJET stainless 8004, Spraying Systems Co., Wheaton, Illinois) were attached to a boom which moved back and forth over the seedlings by means of an electric motor. The speed of the boom was adjusted so that seedlings received rain approx. every 15 s. Droplet size frequency distribution was determined by the flour method of Laws & Parsons (1943). Forty-nine percent  $(\pm sD \text{ of } 5\cdot 3, n = 3)$  of the mass was comprised of 0.59 to 1.0 mm diameter droplets while the remainder of the mass was comprised of 1.0 to 2.0 mm droplets. Seedlings were exposed to simulated acid rain every third day for 20 min a day (4.75 cm h<sup>-1</sup>) for a total of 16 exposures. All rainfall applications were done in the morning. After exposure, seedlings were moved to another area to dry off before being moved back to their regular growing locations. Care was taken that rain water from trees in one treatment did not drip on to trees in another treatment.

Damage estimates were made prior to the applications of rain, 2 d after the first rain, and approx. every 10 d thereafter. Percent necrotic leaf area for the adaxial surface was estimated visually for the youngest recognizable leaf and the fourth leaf from the apex at the beginning of the study. Other forms of damage such as wrinkling, chlorosis and abscision were noted. Abscised leaves were scored as 100% damaged. For *Robinia*, leaflets were treated in the same manner and, thus, a loss of four out of eight leaflets was scored 50%. Tree height was measured at the same time.

Four trees of each species from each pH treatment were randomly selected for measurement of their gas exchange characteristics. Measurements were done on the leaf below the older one used for estimates of damage. An open gas exchange system with 4 chambers operating simultaneously was used. Carbon dioxide concentrations were measured with a Beckmann 215B infrared gas analyzer (Beckman Inst., Houston, Texas) and water vapour concentrations with an EG&G Model 880 dew point hygrometer (EG&G Internat., Waltham, Massachusetts). Light was supplied by a Cool-lux lamp (Westinghouse Elect. Corp., Bloomfield, New Jersey). Rates of airflow were measured with rotameters calibrated against a wettest meter (ASTM, 1975). Clamp-on style plexi-glass chambers were used, with internal dimensions of  $2.5 \times 3.0 \times 1$  cm. Bypass pumps (ASF Gesellschaft für elektrotechnische Gerate mbH & Co. Kg, Munchen) recirculated air through the chambers at a rate of 3.0 l min<sup>-1</sup> to reduce the boundary layer resistance of the leaves. Leaf temperature inside the chambers was measured with copperconstantan thermocouples appressed to the adaxial leaf surface and regulated by passing water of known temperature through the top and bottom of each chamber. Air was supplied from cylinders of compressed air with carbon dioxide concentrations near 330  $\mu$ mol mol<sup>-1</sup>. Environmental conditions in the chambers were as follows: temperature 25-28 °C; photosynthetically active photon flux density (PPFD) 0.8 mmol  $m^{-2} s^{-1}$ ; water vapour concentration gradient from leaf to air (w) 8 to 16 mmol mol<sup>-1</sup>. Air was passed over the abaxial surface of all the species except for Robinia, where airflow occurred on the adaxial surface also, because the leaves were smaller than the cross-sectional area of the chamber. Only Platanus has stomata on the adaxial surface (Carpenter & Smith, 1975) so gas exchange measurements for Liriodendron and Liquidambar were probably virtually representative of the whole leaf. Measurements to be reported later in this paper show that stomatal conductance to water vapour of the adaxial surface of *Platanus* leaves is small relative to that of the abaxial surface, so measurements of abaxial gas exchange were probably nearly the same as for the leaf as a whole. Measurements on trees from all pH treatments were made twice, the day after the first rain, and after the 11th rainfall event. A temperature calibration error on the second date was not discovered until later and so conductance data for Platanus and Liriodendron on that date have had to be discarded. However, because the error was less than 2 °C, photosynthetic rates are not thought to be greatly affected, owing to the broad temperature optima for these trees (unpublished data). In fact, as reported later in this paper, rates were actually higher on this date. Photosynthesis and leaf conductance to water vapour were calculated using the equations and units of von Caemmerer & Farquhar (1981).

At the end of the rainfall period, conductance to water vapour for all trees from the pH 5.6 and 2.0 treatments was measured using a Li-Cor 1600 steady-state porometer (Li-Cor Inc., Lincoln, Nebraska). Environmental conditions were as follows: temperature 21 to 26 °C; PPFD 0.6 mmol  $m^{-2} s^{-1}$ ; w 5 to 11 mmol mol<sup>-1</sup>. The same leaves used for the photosynthesis measurements were measured. In addition, since *Platanus* was one of the species for which data had to be discarded owing to the calibration error, measurements of photosynthesis and transpiration were made on these same trees. Conditions in the chamber were the same as before.

Prior to all measurements of gas exchange, visual estimates of the percent necrotic leaf area of the abaxial surface enclosed in the chambers or porometer were made for trees from the pH 2.0 treatment. Rates were expressed on both a total leaf area basis (necrotic areas included) and on a green leaf only area basis.

Determinations of amounts of chlorophyll and specific leaf weight were made at the end of the period of rainfall application. Eight leaf pieces from vein-free areas (total leaf area 2.56 cm<sup>2</sup>) were taken from the fifth leaf from the apex of each tree using a paper punch. Chlorophyll was extracted from four punches in 5 ml of dimethylsulphoxide at 65 °C for 1 h (Hiscox & Israelstam, 1979). After extraction samples were kept frozen at -30 °C until analyzed. All samples were analyzed within one week of extraction using a Beckman Spectronic 700 spectrophotometer (Beckman Instruments Inc., Fullerton, California). For specific leaf weight determinations, the remaining four punches were oven-dried at 65 °C for at least 72 h and then weighed to the nearest 0.1 mg.

All trees were harvested at the completion of the physiological measurements (less than one week from the last application of acid rain) and divided into root, stem and leaf fractions. For *Robinia*, the rachis was included in the leaf fraction. Biomass was oven-dried at 60 °C to constant weight and weighed to the nearest 0.01 g.

Data were analysed using ANOVA programs in SAS (SAS, 1982) after, where appropriate, being transformed (Zar, 1974).

#### RESULTS

#### Damage

No damage was observed on any of the trees investigated after simulated rain treatments above pH 2.0. At pH 2.0, necrotic lesions appeared on all of the hardwood species within 24 h after the first rain event. Flecking was noted over the entire leaf surface of *Platanus occidentalis*, with subsequent merging of small into large lesions with time. Marginal necroses were obvious, probably because drops tended to collect on the leaf margins, especially of Liquidambar styraciflua. Lesions in Robinia pseudo-acacia were primarily marginal, but those in the mid-blade area were generally quite large compared with the small flecks seen in Platanus. Liriodendron tulipifera showed sporadic flecking over the entire leaf surface. No damage was ever noted for the two pine species, and they are not discussed further. The percentage of leaf area damaged varied among species, but generally increased to an upper limit with repeated exposure to the acid rain (Fig. 1). The small decreases in percentage damage at later dates are probably the result of estimation errors. Liriodendron suffered the least damage, Platanus the most. Lower (older) leaves were damaged more severely than the upper (young) leaves of Platanus and Liriodendron, but no difference was noted in Liquidambar. In Liriodendron, the difference between age classes disappeared by the last sample date. In Robinia, the opposite pattern was evident, lower leaves suffered less damage. However, by the last sampling period this difference was no longer statistically significant.

The young leaves of all species exposed to pH 2.0 acid rain during maturation became very wrinkled (Fig. 2). This was especially true for Liquidambar and was



Fig. 1. Percent of leaf area damaged by simulated acid rain of pH 2·0 for Liriodendron tulipifera
 (○), Platanus occidentalis (□), Liquidambar styraciflua (◊), Robinia pseudo-acacia (△). Vertical bars denote standard errors of the means (n = 10). (a) Older leaves; (b) younger leaves.



Fig. 2. Representative leaf of *Liquidambar styraciflua* after exposure to simulated acid rain of pH 2.0. Note extreme distortion of leaf margins.

probably the result of disruption of lateral meristematic activity by the marginal necroses. The amount of wrinkling and the percentage of leaf area damaged did not seem closely related. After treatment at pH 2.0, loss of apical dominance was apparent in *Robinia* (in which there was death of the apical meristem) and to a lesser degree in *Liquidambar*.

Damage occurred on leaf blades, petioles and rachises. Abscission of an entire leaf of *Robinia* often occured because necrotic lesions on the rachis killed the



Fig. 3. Height growth at conclusion of experiment as a function of simulated rain pH. Vertical bars denote standard errors of the means (n = 10). Data are for (a) Liriodendron tulipifera;
(b) Platanus occidentalis; (c) Liquidambar styraciflua; and (d) Robinia pseudo-acacia.



pH of simulated acid rain

Fig. 4. Biomass at conclusion of experiment as a function of simulated rain pH. Vertical bars denote standard errors of the means (n = 10). Data are for (a) Liriodendron tulipifera; (b) Platanus occidentalis; (c) Liquidambar styraciflua; and (d) Robinia pseudo-acacia.



Fig. 5. Shoot-root ratios at conclusion of experiment as a function of simulated rain pH. Vertical bars denote standard errors of the means (n = 10). Data are for (a) Liriodendron tulipifera;
(b) Platanus occidentalis; (c) Liquidambar styraciflua; and (d) Robinia pseudo-acacia.



pH of simulated acid rain

Fig. 6. Chlorophyll a and b amounts and specific leaf weight at conclusion of experiments as functions of simulated rain pH. Vertical bars denote standard errors of the means (n = 10). Data are for (a) Liriodendron tulipifera; (b) Platanus occidentalis; (c) Liquidambar styraciflua; and (d) Robinia pseudo-acaia.

conducting tissues. This happened despite the fact that leaflets often appeared green and healthy.

#### Height and biomass accumulation

Height growth was significantly reduced by pH 2.0 acid rain in Platanus, but

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		Number of		5-6		4-0	I	
Speci	ies	exposures to acid rain	A	81	P	81		
	linitara		$10.30 \pm 2.28$	$298\pm 95$	11-40±1-88a	$312 \pm 80$		
INI BOADDADOLIST	npilera	11*	$16.72 \pm 2.67$	100 L 100	$16.98 \pm 1.30$	I		-
Platanus occiden	ntalis	:	$8.28 \pm 0.90a$	$102\pm21$	$6 \cdot 11 \pm 1 \cdot 00a$	$52 \pm 21$		•
		•	$16.18 \pm 1.49a$	102 - 41-	$11.92 \pm 0.36b$	— — — — — — — — — — — — — — — — — — — —		
Liquidambar sty	raciftua		$10.90 \pm 0.53$ 13.48 ± 1.40 <sup>5</sup>	$1 163 \pm 418$ $387 \pm 015$	$9.1 \pm 1.528$ 14.01 ± 1.506	$10.2 \pm 44.8$ $30.4 \pm 70.6$		
			13-83 + 1-518	168+17a	13-16+1-78a	$136 \pm 258$		
Robinia pseudo-	acacia	11	$17.44 \pm 2.39a$	$1 338 \pm 54a$	14-06 ± 2-28a	259 <u>+</u> 73a		
				Acid rain pH 1	reatment			
Nu	mber of	3-0	non-second states and second sec	2.0	(1994) do a funcionaria o de anto constituição de anto en em	2.0		
ex Species to a	posures acid rain	P	ß,	4+	<i>s</i> 1t	A‡\$	g <sub>i</sub> t	Average damage (ँू)
Liriodendron tulibifera	- :	$11.68 \pm 1.40a$	409±75	$11.79 \pm 1.80a$	306±82	11-79±1-80	306±82	0
	11*	22-03 ± 3-65a		20-70±3-12a		21·72±3·16a		$-2.8 \pm 1.0$
Platanus occidentalis	1 11*	$7.54 \pm 0.80a$ $15.08 \pm 0.31a$	96±22 	$5.72 \pm 1.09a$ $6.38 \pm 1.51c$	59±13	$6.49 \pm 1.17$ 12.50 $\pm 4.38a$	67±14	12·5±1·4 42·5±6·3
Liquidambar styraciftua	- =	$10-24 \pm 1-08a$ $12\cdot79 \pm 0\cdot76a$	$175 \pm 38a$ $388 \pm 60a$	$9.00 \pm 0.68a$ 14.28 $\pm 2.03a$	115±13a 313±70a	$9.16 \pm 0.68a$ 15.44 $\pm 2.20a$	118±13a 340±78a	$1.8 \pm 0.5$ $7.5 \pm 1.7$
Robinia	-	$10.92 \pm 2.43a$	$128 \pm 33_{8}$	15-07 + 1-51g	177 + 42a	15.40 + 1.51a	178 + 40a	7.1 + 1.2
pseudo-acacia	. =	$16.06 \pm 1.84a$	$283 \pm 34a$	$17.05 \pm 3.07a$	$331 \pm 102a$	18-12±3-36a	$355 \pm 115a$	6.5±2.6
All values are $X \pm SE$ ( $n = 4$ ). * No values for $g_1$ on this c	). Means wit date owing t	thin a species for eac o a temperature cali	h exposure leve bration error, <i>n</i>	l not followed by th = 3. † Uncorrec	ted means. ‡ Co	er at the $P < 0.05$ prrected means.	level. § Values calcul	ated on basis of

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green leaf area.

Acid rain treatment (pH)	Average percent leaf damage	Uncorrected photosynthesis (µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	Corrected photosynthesis* (µmol CO2 m <sup>-2</sup> s <sup>-1</sup> )	
5.6	0	11.13 + 1.32a	11·13 + 1·32a	
2.0	42·0±3·5	$42.0 \pm 3.5$ $4.21 \pm 0.29b$		
Acid rain treatment (pH)	Uncorn leaf condu (mmol H <sub>2</sub> O	ected actance m <sup>-2</sup> s <sup>-1</sup> )	Corrected leaf conductance* (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	
$5.6   300 \pm 54a$		54a	300 ± 54a	
2.0	176 <u>+</u> 15b		$304 \pm 18a$	

# Table 2. Gas exchange characteristics of Platanus occidentalis leaves after 16 acid rain applications

Measurements made with gas exchange system. See text for details. All values are  $X \pm se$  (n = 10). Means within a treatment not followed by the same letter differ at the P < 0.05 level. \* Values calculated on basis of green leaf area.

Table 3. Leaf conductance (mmol  $H_2Om^{-2}s^{-1}$ ) after exposure to sixteen 20-min treatments with simulated acid rain

Species	Aci			
	Uncorrected means		Corrected means*	
	5.6	2.0	2.0	Average percent leaf damage
Liriodendron tulipifera	496±77a	423 ± 59a	491 ± 60a	$9.0 \pm 2.4$
Platanus occidentalis				
Adaxial	59 ± 9a	$42 \pm 4a$	70 ± 9a	$39.0 \pm 2.4$
Abaxial	240±38a	$182\pm20$ a	$311 \pm 28a$	_
Liquidambar styraciflua	463 <u>+</u> 35a	$326 \pm 23b$	355±27b	$7.2 \pm 1.1$
Robinia pseudo-acacia	190±35a	$151 \pm 30a$	$162 \pm 32a$	$7.0 \pm 1.9$

Conductances measured with steady state porometer. See text for details. All values are  $\bar{X} \pm sE$  with n = 10 except for *Robinia* at pH 5.6 (n = 6) and pH 2.0 (n = 9).

Means within species not followed by the same letter differ at the P < 0.05 level.

\* Values calculated on basis of green leaf area.

with P < 0.066 the difference was not quite significant in *Robinia* (Fig. 3). Non-significant reductions in height growth (P < 0.15) were noted also for *Liquidambar* at pH 2.0. *Liriodendron* trees set bud during the experiment, and consequently effects on height growth were minimized.

Total biomass was greatly reduced by pH 2.0 acid rain in *Platanus* and *Liquidambar* (Fig. 4). Biomass was reduced also in *Robinia*, but the reduction was not significant (Fig. 4), and no effect was found in *Liriodendron*. Lower biomass resulted mainly from reductions in dry matter in the roots and stems, but not in the leaves. Shoot-root ratios in *Platanus* steadily increased as pH decreased (Fig. 5) but, in *Liquidambar* and *Liriodendron*, no trend with pH was found. The shoot-root ratios for *Robinia* were higher at pH 2.0 than at the other treatment levels.

### Specific leaf weights and chlorophyll contents

Liquidambar had a higher specific leaf weight at pH 2.0 than at the other pH levels (Fig. 6). No other species showed treatment effects for this parameter, although *Platanus* tended toward a higher value at pH 2.0 (P < 0.0594).

Liquidambar had higher chlorophyll contents at pH 2.0 than at the higher pH levels (Fig. 6). This was due mainly to the increase in specific leaf weight, as no differences occurred on a dry weight basis. Again, the other species showed no treatment effects for this parameter. The chlorophyll a:b ratio was not affected at any pH level in any of the species.

## Gas exchange characteristics

No significant differences in photosynthesis were noted after the first application of rain (Table 1). After the 11th application, the rates were significantly lower in *Platanus* leaves from the pH 2.0 treatment. However, when recalculated on a green leaf area only basis, this difference disappeared. Leaf conductance to water vapour was variable and no treatment differences were found for any of the species on either date (Table 1). Both photosynthesis and leaf conductance were generally much higher on this date for all of the species.

When a large number of replicates was used (10), differences in both photosynthesis and conductance of *Platanus* could still be detected several days after the last application of acid rain (Tables 2 and 3). Since *Platanus* was the most heavily damaged of the species, and because of time limitations, it was the only species for which photosynthesis was measured on all trees after the pH 5.6 and 2.0 treatments. Photosynthetic rates (Table 2) were significantly lower in *Platanus* seedlings exposed to acid rain of pH 2.0 compared with seedlings exposed to rain of pH 5.6. This was also true when rates were expressed in terms of green leaf area only, but conductances were the same when expressed on this same basis (Table 3). This suggests that the reduction in photosynthesis in leaves exposed to pH 2.0 was not the result of stomatal limitation to diffusion of CO<sub>2</sub> into the leaf.

When stomatal conductance to water vapour was measured with the steady-state porometer on all trees from the pH 5.6 and 2.0 treatments, significantly lower conductances were found only for leaves of *Liquidambar* from the pH 2.0 treatment, both on a total and green leaf area only basis (Table 3). For *Platanus*, adaxial stomatal conductances in both treatments were much lower than abaxial conductances but there were no treatment effects on either leaf surface.

#### DISCUSSION

The degree of damage suffered by trees exposed to acid rain varies widely among species (Wood & Bormann, 1974, 1975; Evans & Curry, 1979; Lee & Weber, 1979; Haines *et al.*, 1980), but the underlying mechanisms are poorly understood at present. Foliar damage has been reported on deciduous tree species at pH levels as high as 3.25 (Forsline, Dee & Melious, 1983) although an average of the H<sup>+</sup> ion concentrations based on a survey of the literature yields a pH of 2.30 as the threshold for damage. Conifer species may be more resistant, but at present, data exist only for three species. For older *Pinus strobus* seedlings with mature foliage, damage was not found until pH 1.0 (Haines *et al.*, 1980) whereas with rapidly expanding needles of young seedlings, damage occurred at pH 2.3 (Wood & Bormann, 1975), and at pH 2.0 for *Pinus ponderosa* and *Pseudotsuga menziesii* (McColl & Johnson, 1983).

In the present study, no foliar damage was found on any species at pH 3.0 or above. At pH 2.0, necrotic lesions formed on the leaves of the deciduous species after one 20-min rainfall event. Although the two pine species were not included in the final data analyses, they did not show any signs of foliar damage even at pH 2.0. These results corroborate and extend the observations made by Haines et al. (1980) on various southeastern tree species, in which they found no damage at pH 2.5 on any hardwood species. Thus the threshold for damage in southern hardwood tree species apparently resides between pH 2.0 and 2.5. Evans, Gmur & Mancini (1982) suggested that plants grown in the greenhouse may be more susceptible to foliar damage by acid rain than field grown plants. If so, then the findings of this study suggest that present levels of acidity in ambient rainfall are not severe enough to cause direct foliar damage. Currently the mean volume weighted pH of rain in Georgia is 4.19 (Haines, 1979) and a substantial decrease of at least 1.9 pH units (a nearly hundred-fold increase in H<sup>+</sup> ion concentration) would be needed to cause foliar necroses. The lowest recorded pH in one storm so far has been 3.2. Thus at present, acid rain by itself is probably not causing direct foliar damage to trees in the southeastern United States.

In the present study, recently matured leaves were most susceptible to damage by rain of pH 2.0 with the exception of *Robinia*. Similar trends have been observed on a variety of plant species (Evans, Gmur & DaCosta, 1977; Evans, Gmur & DaCosta, 1978; Evans & Curry, 1979; Ferenbaugh, 1976; Paparozzi & Tukey, 1983). In other plants, damage has been found to be most severe in rapidly expanding leaves (Wood & Bormann, 1974, 1975, 1977; Keever & Jacobson, 1983a, b) or in older, mature leaves (Keever & Jacobson, 1983c; Paparozzi & Tukey, 1983). Species-specific differences in leaf wettability during maturation resulting from changes in wax and cuticle development, and changes in epidermal surface features such as number and density of trichomes may be involved (Paparozzi & Tukey, 1983). In addition, the physiological sensitivity of epidermal and mesophyll cells to acidic solutions could possibly change with age.

Tamm & Cowling (1976) suggested that foliar damage by acid rain may alter the gas exchange characteristics of plants through effects on guard or subsidiary cells. Evans *et al.* (1981) showed that adaxial stomatal resistance was lower in leaves of *Phaseolus vulgaris* exposed to acid rain of pH 2.7 compared with leaves exposed to rain of pH 5.7. In our study, after 16 exposures to acid rain (320 min of exposure) *Liquidambar* had lower abaxial conductances (higher resistances) in leaves exposed to rain of pH 2.0 than in leaves exposed to pH 5.6. These differences persisted when conductances were recalculated on a green leaf area only basis, suggesting that the reductions actually were due to stomatal closure.

There are several possible explanations for lower stomatal conductances in response to rain of low pH, and these are not mutually exclusive. Firstly, preferential loss of turgor by guard and/or subsidiary cells may occur as a result of acid rain-induced plasmolysis, causing stomatal closure. Guard cells are thought to have uncutinized areas on the anticlinal walls of the stomatal pore complex, which are probable sites of peristomatal transpiration (Appleby & Davies, 1983) and preferential sites for penetration of liquids into leaves (Dybing & Currier, 1961; Leece, 1978). Secondly, entry of large amounts of H<sup>+</sup> ions may disrupt the ionic balance of the guard cells, and cause stomatal dysfunction (Raschke, 1975; Zeiger, 1983). Thirdly, guard cells may be sites of nutrient leaching from the leaf. The loss of K<sup>+</sup> ions may be especially critical, since stomatal movements have been linked to K<sup>+</sup> fluxes into and out of guard cells (Raschke, 1975) and maximum stomatal

apertures are reduced if there is a deficiency of  $K^+$  ions (Fischer & Hsiao, 1968). Losses of  $K^+$  ions due to exposure to acid rain have been reported in several tree species (Wood & Bormann, 1975, 1977), and Evans *et al.* (1981) have suggested that  $K^+$  ions may even be preferentially leached from foliage. Tissue damage at low pH may accentuate the losses (Wood & Bormann, 1975), whereas if no damage occurs, losses may not be detectable (Haines, Chapman & Monk, unpublished data). Results of the latter study may partially explain the lack of a stomatal response at the higher pH levels in this study. Finally, reductions in photosynthetic capacity may lower stomatal conductance (Wong, Cowan & Farquhar, 1979). However, in this study, abaxial stomata of *Platanus* were not affected by exposure to rain of pH 2·0, even though photosynthesis was reduced, so this mechanism is probably not important.

Photosynthetic rates were reduced in *Platanus* leaves exposed to rain of pH 2.0. Rates calculated using only green leaf area were also lower than rates in leaves exposed to rain of pH 5.6. Because stomatal conductances were so much greater on the abaxial than the adaxial surfaces, the bulk of the  $CO_2$  diffusing into the leaves probably occurred through the abaxial surfaces. Since stomatal conductance of the abaxial surface was not affected by the rain of pH 2.0, the reduction in photosynthesis in this treatment was probably due to a lower mesophyll conductance to  $CO_2$ .

Mechanistic explanations for reductions in mesophyll conductance after exposure to acid rain are presently lacking. Penetration of acid solutions into mesophyll cells may upset the homeostatic pH balance of the cells, stimulating respiratory CO<sub>2</sub> loss as the cells attempt to maintain the status quo (Smith & Raven, 1979). Ferenbaugh (1976) found that photosynthesis rose in leaves at pH 2·0, but that growth was inhibited, and reasoned that photosynthesis was uncoupled from photophosphorylation. Additionally, pH imbalances may alter enzyme conformation and carboxylation reactions and increase mitochondrial respiration (Smith & Raven, 1979), all of which would contribute to lower net photosynthesis.

Since the final measurements of photosynthesis in *Platanus* were made 5 d after the last acid rain application it is unlikely that the leaf cells had remained acidified by the rain over this period of time, and a more satisfactory explanation for the long term reductions is needed. Prior acidification may have rendered irreparable damage to the chloroplast membranes. Paparozzi & Tukey (1983) have shown disruption of thylakoid membranes in chloroplasts of cells from injured areas of leaves of *Betula alleghaniensis*. No mention was made of possible disruption in areas of leaves devoid of visible lesions, but Adams, Dengler & Hutchinson (1983) have recently shown the existence of damage at the microscopic level when no injury was detectable at the macroscopic level. Further studies of cell ultrastructure are therefore needed.

Accelerated rates of senescence have been reported in older leaves of *Phaseolus* vulgaris after exposure to acid rain (Johnston et al., 1982). Tree leaves reach their maximum photosynthetic capacity near the time of completion of leaf expansion (Richardson, 1957; Dickmann, 1971; Dougherty et al., 1979) and premature senescence due to exposure to acid rain might act to reduce this capacity.

Loss of chlorophyll after exposure to acid rain, particularly chlorophyll a, has been suggested as a cause of lower photosynthesis in the moss *Tortula ruralis* (Sheridan & Rosenstreter, 1973). Similar reductions in chlorophyll for higher plants were reported by Ferenbaugh (1976), Hindawi *et al.* (1980) and Jaakkola *et al.* (1980). At pH 3·0, however, Ferenbaugh (1976) reported increased chlorophyll amounts in *Phaseolus vulgaris*. A similar increase was found by Irving & Miller (1980) for *Glycine max*. In the present study, only *Liquidambar* showed an effect of treatment on the amount of chlorophyll, and this is explained as resulting from an increase in specific leaf weight. Thus, the reduction in photosynthesis observed for *Platanus* at pH 2.0 probably was not due to loss of chlorophyll.

Photosynthesis may have been lowered because of alterations in source-sink relationships between leaves and other plant parts, or because of lower phloem loading rates. In the latter case, phloem loading is known to require energy inputs in the form of ATP (Herold, 1980) and acidification of the cytoplasm may have lowered the amount of available ATP, which in turn would have reduced the capacity to translocate carbohydrates out of the leaf. Physical damage to the leaf may also disrupt the export of photosynthates.

Altered hormonal levels may have affected photosynthesis (Treharne & Stoddart, 1968; Tamas *et al.*, 1974; Buggeln & Bal, 1977; Erkan & Bangerth, 1980). Ferenbaugh (1976) has suggested that acidification of the cytoplasm may reduce auxin levels in leaves. The observed reductions in root growth might have resulted in lower cytokinin levels which in turn could have lowered photosynthesis in the *Platanus* leaves (Wareing, Khalifa & Treharne, 1968). It is not possible in the present study to separate potential effects of H<sup>+</sup> ions from those of  $SO_4^{2^-}$ . Excessively high  $SO_4^{2^-}$  loadings could have contributed to lower photosynthesis (Lechowicz, 1982; Paparozzi & Tukey, 1983).

Enhanced rates of nutrient leaching from leaves exposed to acid rain may depress photosynthetic rates. Numerous studies have shown a strong dependence of net photosynthesis on foliar nutrient levels (Osman, Goodman & Cooper, 1977; Ishihara *et al.*, 1979; Kabaki, Saka & Akita, 1979; Longstreth & Nobel, 1980; O'Toole *et al.*, 1980; Mooney *et al.*, 1981). Both increases (Wood & Bormann, 1977) and decreases (Proctor, 1983) in foliar N amounts have been found in trees exposed to acid rain. Evans *et al.* (1981) reported enhanced leaching of nitrate, calcium, and sulphate by low pH rain, and Wood & Bormann (1975) reported foliar losses of calcium, magnesium, and potassium. No losses of N were, however, found in the *Platanus* leaves in this study (unpublished data).

Finally, altered leaf development may affect photosynthetic capacity. Ferenbaugh (1976) reported that cell sizes were smaller and intercellular leaf spaces reduced in leaves of *Phaseolus vulgaris* exposed to acid rain. Mesophyll conductance to  $CO_2$  has been linked to internal leaf surface area (Nobel, 1977) and decreases in intercellular leaf area would be expected to lower mesophyll conductance to  $CO_2$ , resulting in lower photosynthesis. However, the lack of a pH effect on specific leaf weight suggests that this may not be important.

Reductions in biomass by rain of low pH were the result mainly of decreased allocation to stem and root, and not so much the result of leaf loss. However, viable leaf area was greatly reduced, and this was probably the primary reason for lower stem and root biomass. The one exception was *Robinia*, which lost many leaves because rachises tended to abscise with green, apparently healthy leaflets. These results suggest that one of the major effects of acid rain on plants may be to alter the patterns of translocation of photosynthates. Similar alterations in biomass allocation due to acid rain have been reported by Troiano *et al.* (1982) and Lee *et al.* (1981). The trend toward higher shoot-root ratios, coupled with reduced root biomass, might predispose acid rain-stressed trees to additional stresses, such as drought. Interactions between drought stress and acid rain are currently being investigated (Johnson, 1983).

This study is, to our knowledge, the first actually to link reductions in biomass

# Foliar effects of simulated acid rain I.

due to acid rain to reductions in photosynthetic capacity. Further studies of net photosynthesis are needed to see if this is a general phenomenon. In addition, the time course of changes in photosynthesis and of the mechanism behind the reductions should be investigated. In this investigation, non-stomatal effects were implicated for one species, suggesting that the basic alterations in photosynthetic metabolism persist after exposure to acid rain.

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