Foliar Response of Six Clones of Hybrid Poplar

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ABSTRACT

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After exposure to simulated acid rain at pH levels from 2.7 to 3.4, lesions of several types were produced on foliage of six clones of *Populus* spp. hybrids. The types of adaxial leaf surface lesions were observed at low magnification, by scanning electron microscopy, and via leaf histology. On two clones, galls resulting from hyperplasia and hypertrophy of parenchyma cells predominated. In contrast, two clones exhibited neither hyperplasia nor hypertrophy. In these clones the upper epidermis, palisade parenchyma, and sponge parenchyma were injured in succession. In a third set of two clones, hyperplasia and hypertrophy were present in areas between injured and apparently noninjured tissues. In general, percent leaf area with lesions and percent leaves injured were similar among all six clones at all pH levels tested. At pH 2.7 up to 10% of the leaf area was injured after 5 daily exposures of 6 min each. Injury decreased to about 1.0% at pH 3.4. Lesions developed mostly near stomata and vascular tissues and occurred most frequently on leaves just prior to maximum leaf enlargement. Very young and older leaves were less affected. The results support the hypothesis that the adaxial leaf surface is the most affected after exposure to simulated acid rain.

Experiments with simulated acid rain (4, 6, 8, 14) and assessments of injury in the environment that results from exposure to acid rain (1, 2, 13) have been made, but there is need for standardization of procedures to determine levels of pollutants that influence forest plant growth, development, and productivity.

The experiments outlined herein are focused upon the effects of simulated acid rain on six hybrid clones of *Populus* spp., specifically: (i) percent of leaves injured, (ii) percent of leaf area with leaf lesions, (iii) a visual description of lesion development at low magnifications and with scanning electron microscopy, and (iv) a histological description of lesion development. Some lesions produced by simulated acid rain resemble galls, with abnormal cell enlargement and cell proliferation such as those produced by microorganisms and insects. A description of events during lesion and gall formation is presented.

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MATERIALS AND METHODS

Stem cuttings of six clones of hybrid poplar were obtained as a gift from Keith Jensen and Leon Dochinger as follows:

No. 8-hybrid of *P. nigra* L. × *P. laurifolia* Ledeb.,
No. 43-hybrid of *P. maximowiczii* Henry × *P. berolinensis* Dipp.
No. 44-hybrid of *P. maximowiczii* Henry × *P. berolinensis* Dipp.
No. 207-hybrid of *P. deltoides* Bartr. × *P. trichocarpa* Torr. and Gray.
No. 211-hybrid of *P. deltoides* Bartr. × *P. trichocarpa* Torr. and Gray.
No. 327-hybrid of *P. balsamifera* L. cv. *candicans* × *P. berolinensis*.

Basal ends of stem cuttings were dipped in an auxin compound (Hormodin 1, Merck Co., Rahway, NJ 07065, 0.1% indole-3-butyric acid in talc) and were rooted for 12 wk in a soil mix in a greenhouse equipped with activated charcoal air filters. Plants subsequently were transferred to a controlled environmental chamber (also equipped with activated charcoal filters) 1 wk prior to

experimentation. Plants were kept at 24 C, 40-50% relative humidity, with an 18-hr light period. Light intensity was 10,760 lux at plant height. Plants were fertilized weekly. Before initiation of rain applications, plants were fitted with a conical plastic hood around the base of each plant to cover the soil. During the experiments the plants were irrigated only under the plastic hoods.

Experimental plants were sprayed in a fully enclosed chamber (3.0 × 1.5 × 1.3 m) with light provided. Plants

TABLE 1. Responses of six clones of poplar after exposure to simulated rain[a]

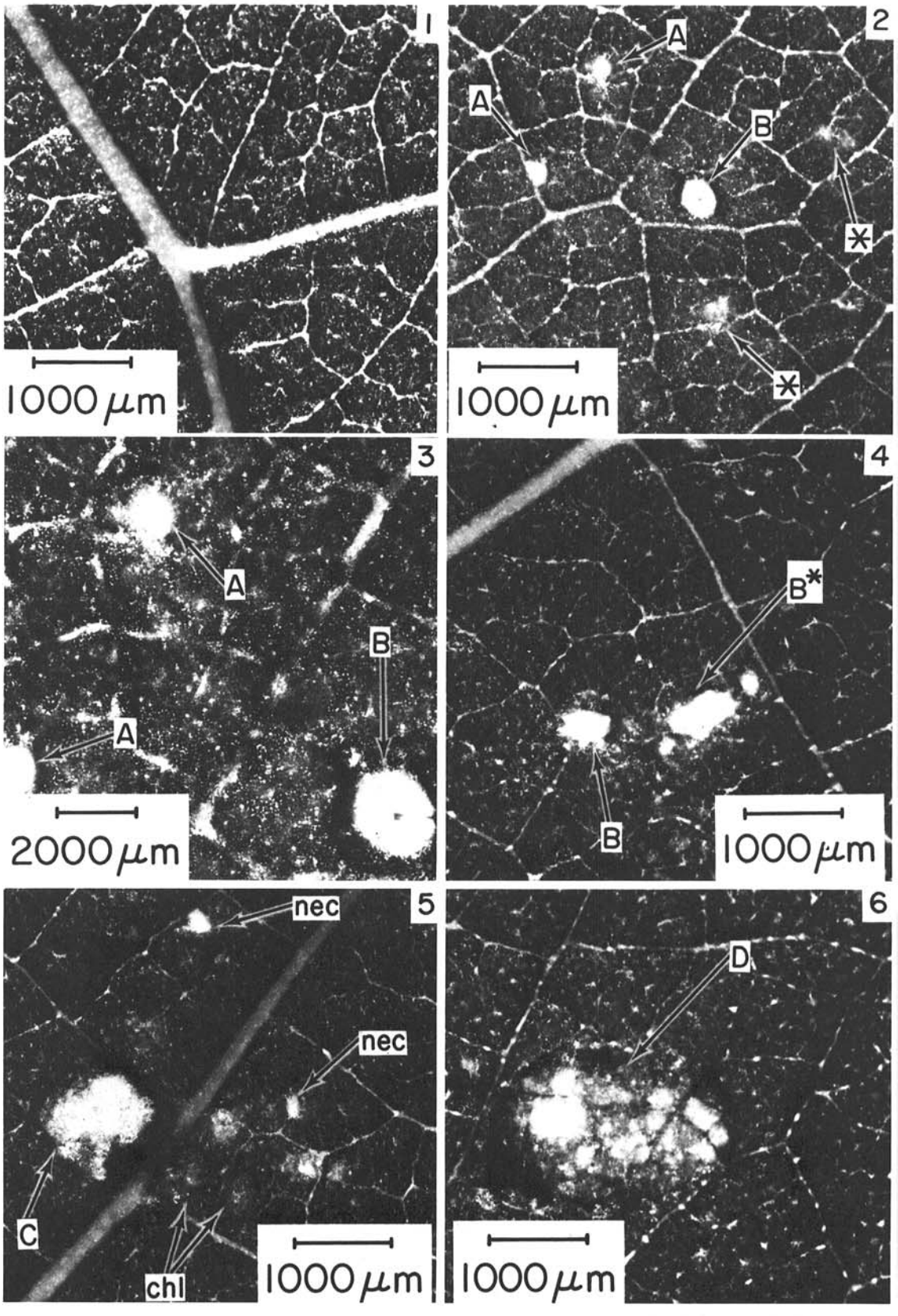
Clone no.	Reaction of simulated acid rain (pH)	Leaf area with various lesion diameters				Leaves injured[c] (%)
		<0.25 mm (%)	0.25-1.00 mm (%)	1.00-2.00 mm (%)	>2.00 mm (%)	
8	2.7	0.04[b]	0.41	0.96	1.95	91
	2.9	0.02	0.20	0.16	0.65	56
	3.1	0.02	0.08	0.16	0.00	40
	3.4	0.01	0.04	0.00	0.00	1
	5.7	0.00	0.00	0.00	0.00	0
43	2.7	0.07	0.49	1.54	4.12	69
	2.9	0.18	1.14	1.54	4.12	61
	3.1	0.07	0.49	0.51	0.00	38
	3.4	0.04	0.25	0.00	0.00	8
	5.7	0.00	0.00	0.00	0.00	0
44	2.7	0.08	0.68	0.79	2.12	71
	2.9	0.03	0.22	0.52	2.12	56
	3.1	0.03	0.22	0.24	0.00	32
	3.4	0.02	0.07	0.00	0.00	5
	5.7	0.00	0.00	0.00	0.00	0
207	2.7	0.06	0.40	0.76	2.00	80
	2.9	0.02	0.16	0.15	0.67	66
	3.1	0.01	0.04	0.00	0.00	22
	3.4	0.01	0.04	0.00	0.00	6
	5.7	0.00	0.00	0.00	0.00	0
211	2.7	0.05	0.30	1.18	2.12	89
	2.9	0.04	0.30	0.47	1.06	57
	3.1	0.04	0.15	0.00	0.00	26
	3.4	0.00	0.00	0.00	0.00	0
	5.7	0.00	0.00	0.00	0.00	00
327	2.7	0.04	0.58	1.41	3.27	60
	2.9	0.04	0.22	0.28	1.64	58
	3.1	0.02	0.15	0.28	0.00	30
	3.4	0.02	0.07	0.00	0.00	7
	5.7	0.00	0.00	0.00	0.00	0

[a]Plants were exposed to five exposures of simulated acid rain. One rain application was given daily for 6 min only. The plants were rated 1 day after the fifth exposure.

[b]Values represent estimates of mean percent leaf area with lesions from all leaves of three plants in four separate experiments.

[c]Values represent estimate of percent of leaves injured from three plants in four separate experiments.

Fig. 1-6. Photographs of leaf surfaces of several leaves of poplar clone No. 43 that exhibit several stages of injury after exposure to simulated acid rain. **1)** Unexposed surface of a leaf. Note sharp contrast between veinal and interveinal areas. **2)** Leaf surface of clone No. 207 exposed to several rain events. A large lesion (0.25-1.00 mm) (B) adjacent to several smaller lesions (<0.25 mm) (A). The light areas (*) represent initial injury symptoms. Initial lesions appear only slightly chlorotic. Note darkened areas (red colorations) between necrotic and healthy tissue of the larger lesion (B). **3)** Enlargement of several lesions in Fig. 2. A larger lesion (0.25-1.00 mm) (B) is shown with two lesions (<0.25 mm) (A). The darkened area between necrotic and healthy tissues of the (B) lesion (0.25-1.00 mm) represents a red coloration that occurred in clones No. 8, No. 207, No. 211, and No. 327 but not in clones No. 43 and No. 44. Red pigmentation was present in tissues that exhibited hyperplasia and hypertrophy. **4)** An elongated lesion (0.25-1.00 mm) (B*) occurred near a vein adjacent to a more circular lesion (0.25-1.00 mm) (B) in clone No. 43. A small lesion also is present near the B* lesion. Note no darkened (red coloration) areas border necrotic and healthy tissues. **5)** A large lesion (1.00-2.00 mm) (C) is present near vascular tissue in clone No. 211. Several smaller lesions (<0.25) are shown. The smaller lesions exhibit only chlorosis (chl) while other more advanced lesions exhibit necrosis (nec.). Red colorations are also present in large lesions. **6)** The large lesion (> 2.00 mm) encompasses a large area in clone No. 207 including several veins as well as interveinal tissue. In large lesions, small veins retain chlorophyll longer than interveinal areas. Large lesions were also characterized by a very thin lamina.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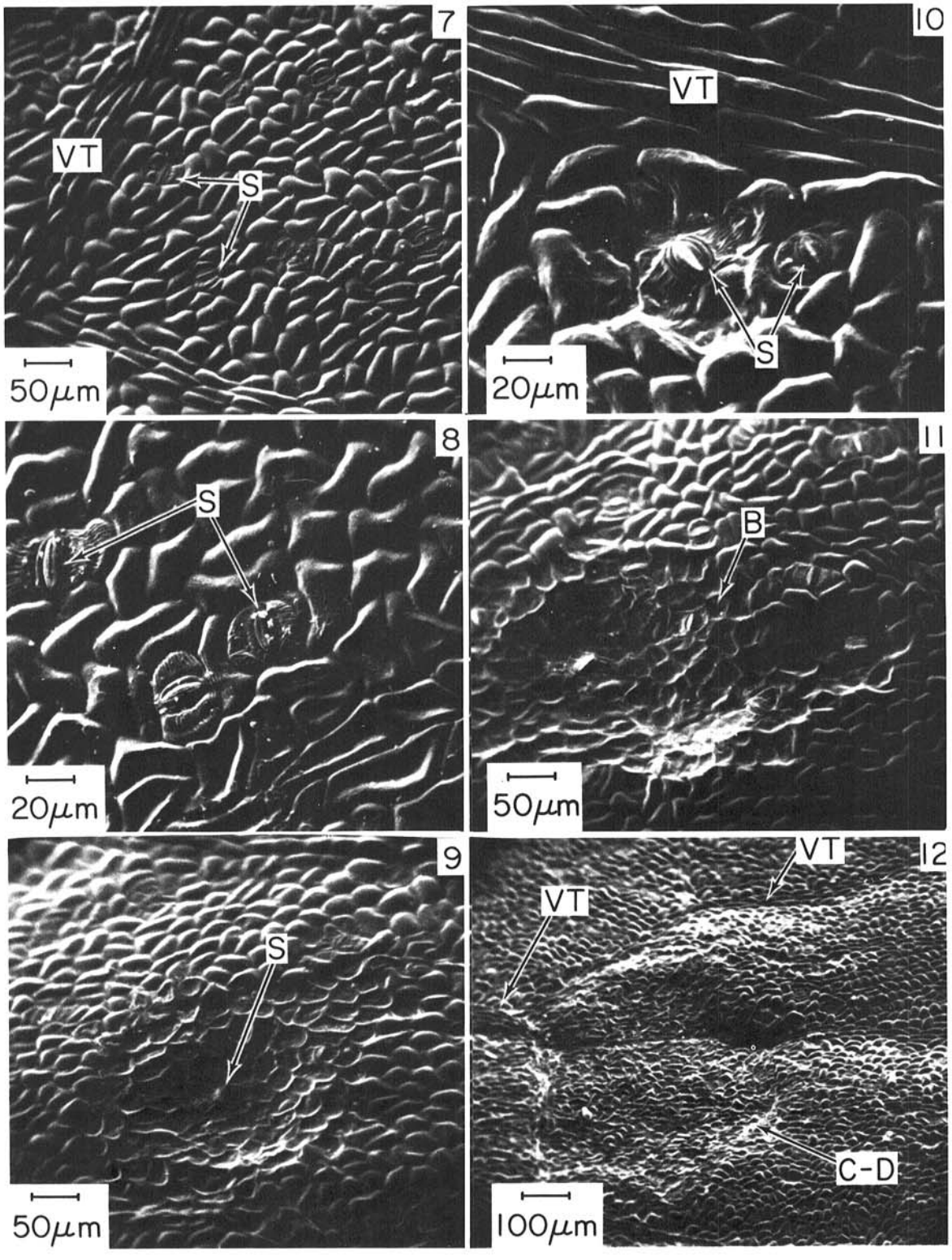
were placed on a turntable (1 m in diameter) which revolved at 2.5 rpm. Simulated rain was distributed from a set of nozzles 1 m above plant height. The nozzle system consisted of two different nozzle types (5) directed downward directly over the plants to give rain and fog drop sizes simultaneously. A vibrator assured uniform



delivery of drops over plant foliage.

In rain simulations, only one rain application of 6 min with an effective rainfall rate of 7.2 mm/hr was given daily. A rain solution (4) was created to simulate the major chemical constituents of rain of the northeastern United States. The pH of the rain was adjusted with 1 N H_2SO_4 or 1 N NaOH to obtain desired levels. Control plants were sprayed with a rain of pH 5.7.

Histology.—Samples were fixed in cold acrolein for 4 hr. After fixation, samples were dehydrated through a

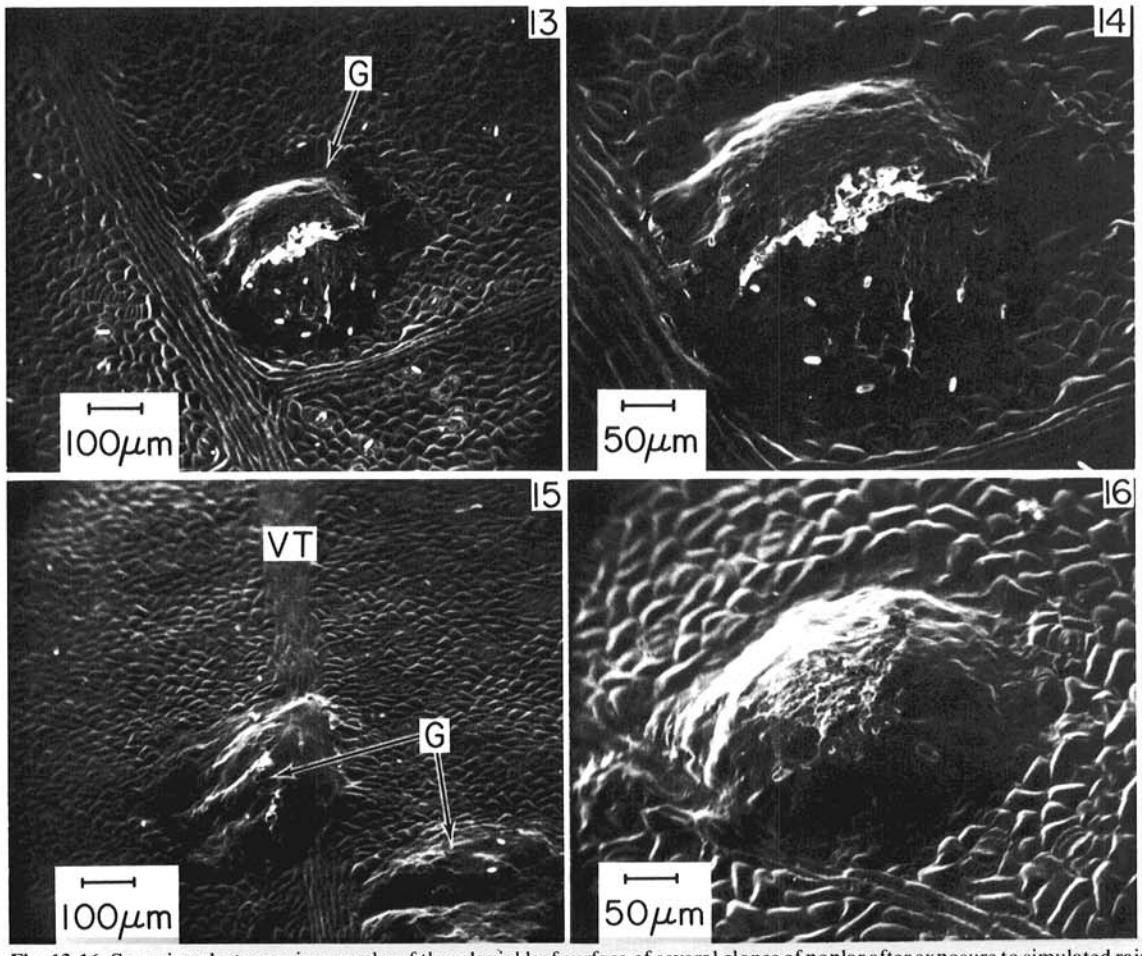


Fig. 13-16. Scanning electron micrographs of the adaxial leaf surface of several clones of poplar after exposure to simulated rain at pH 2.7. Symbols: VT = vascular tissues; G = galls produced by simulated acid rain pH 2.7. **13)** Overview of gall on the leaf surface of clone No. 327. Mound is present near vascular tissues. The gall was present on a leaf 24 hr after a single 6-min exposure to pH 2.7. **14)** Enlargement of gall in Fig. 13. Photograph illustrates mounded nature of this small gall (<0.25 mm) with completely collapsed epidermis. Epidermal cells immediately adjacent to the mound are collapsed and bounded by apparently normal epidermal cells. The light ovals on the surface of the gall are stomata. The epidermis over the gall is discontinuous. **15)** Two galls on the surface of clone No. 327. One gall encompassed vascular tissue in addition to adjacent interveinal area. This gall exhibits a depression into the leaf tissue on one side. **16)** Enlarged view of a gall on the surface of clone No. 207. Epidermal cells on the gall are no longer distinct but several stomata are present. The gall is small and the tissue is continuous compared with the discontinuous surface of the gall in Fig. 14.

Fig. 7-12. Scanning electron micrographs of the adaxial leaf surface of several clones of poplar after exposure to simulated rain at pH 2.7 unless otherwise noted. Note the absence of trichomes on all surfaces shown. VG = vascular tissue; S = stoma (ta). (Fig. 7 and 8) Leaf surfaces after four applications of simulated rain pH 5.7 only. Photographs show no leaf surface perturbations. Most epidermal cells are fully turgid. Figure **7)** view of clone No. 44; **8)** view of clone No. 8. **9)** A smaller lesion (<0.25 mm) surrounds a stoma on clone No. 44. About 20-30 epidermal cells within a radius of 100 μm of the stoma are flaccid and surround a depression. Palisade cells may be injured in the centrally depressed area near the stoma. **10)** A small lesion (<0.25 mm) after one 6-min exposure to simulated acid rain to clone No. 43. Note injury to epidermal cells near two stomata and vascular tissues. About six epidermal cells are flaccid in this view. No indication of injury to more internal cells is present. **11)** Surface of leaf with a lesion (B) (0.4 mm in length) on clone No. 43. Several stomata are present within the lesion. More internal tissues may be injured as denoted by lesion depression depth. **12)** Low magnification view of a large lesion (1.00 mm) on clone No. 44. A clear demarcation between injured and apparently uninjured area is present. A single 6-min exposure of simulated acid rain was given daily for 3 days.

series of cold alcohol solutions (7). Tissues were embedded, sectioned at 20-30 μm, and stained according to Shellhorn and Hull (12).

Scanning electron microscopy.—Leaf samples for scanning electron microscopy (SEM) were fixed in acrolein and dehydrated in ethanol. Samples were critical-point dried (4). A layer of carbon or silver was applied to each sample in a vacuum evaporator with a rotating and precessing stage. The scanning electron microscope (Model 700, Materials Analysis Co., Palo Alto, CA 94303) was operated at 5 kV or 10 kV.

Spacing of trichomes and stomata.—Spacings of trichomes and stomata were determined from positive leaf impressions by the silicone rubber technique of Sampson (11). A total of eight areas (314 mm^2) was sampled randomly on each of four leaf replicas from each clone. No estimate of stomatal functional capacity was obtained.

RESULTS

The foliar response of clones of hybrid poplar was quantified by grouping lesion diameters as: less than 0.25 mm, 0.25 to 1.00 mm, 1.00 to 2.00 mm, and greater than 2.00 mm, respectively (4).

After exposures to simulated acid rain in which one rain application of 6 min was administered daily at five pH levels, a variety of lesion types was exhibited on all six clones (Table 1). No lesions were observed on leaves exposed to pH 5.7 rain (the theoretical control pH of unpolluted water in equilibrium with ambient concentrations, approximately 300 μliters of carbon

TABLE 2. Injury on leaf surfaces of six clones of poplar at various simulated acid rain pH levels[a]

Reaction of simulated acid rain (pH)	Leaf surface with lesions after exposure for: 1 day (%)	2 days (%)	3 days (%)	4 days (%)
5.7	0 ± 0[b]	0 ± 0	0 ± 0	0 ± 0
3.1	0.7 ± 0.0	1.8 ± 0.3	1.8 ± 0.2	2.2 ± 0.2
2.9	3.8 ± 0.2	3.9 ± 0.3	4.1 ± 0.2	4.6 ± 0.4
2.7	3.2 ± 0.4	3.5 ± 0.2	4.1 ± 0.2	4.6 ± 0.2
2.5	3.9 ± 0.2	4.4 ± 0.2	5.3 ± 0.4	5.9 ± 0.3
2.3	5.0 ± 0.3	5.1 ± 0.2	7.7 ± 0.6	8.4 ± 0.4

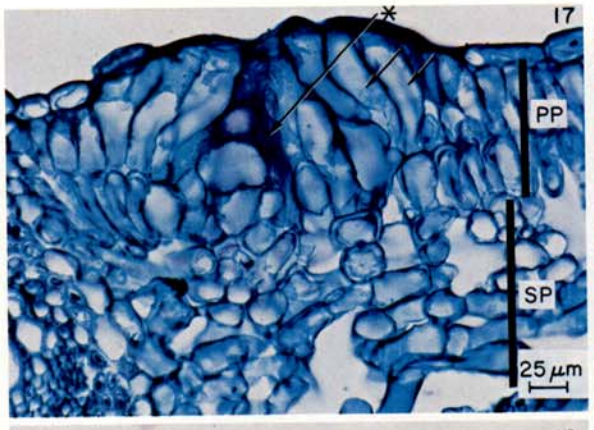
[a]Two plants of each of six clones were observed. Plants were exposed to one 6-min exposure of simulated rain per day at each pH level. Plant injury was assayed 1 day after each rain application just prior to another application.
[b]Mean and standard error of the mean, respectively.

TABLE 3. Relationship between densities of stomata and lesions caused by simulated acid rain on adaxial leaf surfaces among clones of hybrid poplar

Clone no.	Stomata per 314 mm^2[a] (no.)	Lesions per 314 mm^2[a,b] (no.)	Ratio stomata: lesions
8	336 ± 58	14.9 ± 1.9	22.6:1
43	223 ± 13	20.6 ± 2.6	10.8:1
44	160 ± 13	21.2 ± 1.5	7.6:1
207	129 ± 15	15.2 ± 3.4	8.5:1
211	170 ± 9	15.9 ± 1.6	10.7:1
327	129 ± 6	19.3 ± 2.8	6.7:1

[a]Each figure represents the average and standard error of the mean from four impressions on each leaf surface from each clone. Eight areas (314 mm^2) on each impression were scored.
[b]Plants were exposed to one 6-min simulated acid rain (pH 2.7) event daily for four consecutive days. Plants were evaluated 1 day after the last rain event. Four areas (each 314 mm^2) on five leaves from five plants were evaluated per clone. The four leaves with the most lesions were selected from each plant.

Fig. 17-24. Cross sections of leaves of clones No. 327, No. 207, No. 8, and No. 211 of hybrid poplar that exhibit (1) gall formation and (2) lesion development with associated cell hyperplasia and hypertrophy in response to simulated acid rain exposure of pH 2.7. **17)** Initial gall response of clone No. 207 to simulated acid rain injury. Collapse of epidermal cells with distortion of palisade parenchyma (pp) cells appears darkly stained (*). Elongation of injured palisade parenchyma cells produces a slight elevation of adaxial leaf surface (arrows). No apparent injury to spongy parenchyma (sp) cells occurs. **18)** Initial gall formation of clone No. 8. Cells of both the palisade and spongy parenchyma exhibit early stages of hypertrophy and hyperplasia in response to simulated acid rain exposure. Note the degree of elongation in altered spongy parenchyma cells (arrow). An elevation of both the adaxial and abaxial leaf surface occurs (*). Collapse of the epidermal cells occurs on the adaxial leaf surface. Normal epidermis = N.E.; Vascular tissue = V.T. **19)** Micrograph of a small gall (<0.25 mm) in clone No. 8. The leaf mesophyll consists of altered parenchyma cells in late stages of hypertrophy and hyperplasia. Note the degree of elongation in several mesophyll cells (arrows). A region of hyperplastic activity occurs in the palisade parenchyma (*). No tissue layers are distinguishable. Elevation of the leaf surface with collapse of the epidermal cells is apparent on both adaxial and abaxial leaf surfaces. **20)** Micrograph of a gall (0.25-1.00 mm) in clone No. 327. Collapse of epidermal and palisade parenchyma cells occurs on the adaxial leaf surface (*). Zones of hyperplastic cell activity surround the elevated leaf surfaces (A). Large elongated cells lie in the central zone of cell hypertrophy (B). Normal tissue = N. **21)** Cell hypertrophy in a small lesion (0.25-1.00 mm) of clone No. 211. Malformations of both epidermal and palisade parenchyma cells occur in the lesion (A). Sponge parenchyma cells below the lesion exhibit cell hypertrophy (B). Cell enlargement of the spongy parenchyma cells in the area of the lesion (C). **22)** Higher magnification of cell hypertrophy surrounding a small lesion (0.25-1.00 mm) of clone No. 211. Palisade and spongy parenchyma cells exhibit the hypertrophic activity responsible for the elevation of the leaf surface (arrows). Cell types are indistinguisable in the zone of hypertrophy. Epidermal and palisade parenchyma cells are destroyed in the lesion. **23)** Cell hypertrophy and hyperplasia surrounding a large lesion (1.00-2.00 mm) of clone No. 207. Collapse of the epidermal, palisade and spongy parenchyma cells within the lesion results in a depression of the leaf surface (A). Mesophyll tissue surrounding the lesion is subject to cell hypertrophy and hyperplasia which results in an elevation of the leaf surface on both the adaxial and abaxial sides (B). **24)** Enlargement of Fig. 23 that exhibits the zone of hyperplasia and hypertrophy surrounding a lesion (1.00-2.00 mm). Note the proliferation of parenchyma cells (hyperplasia) which appears as a darkly stained band adjacent to the lesion (arrow). Hypertrophic cells lie to the left of this zone of hyperplasia. Only remnants of parenchyma cell walls are evident in the lesion proper.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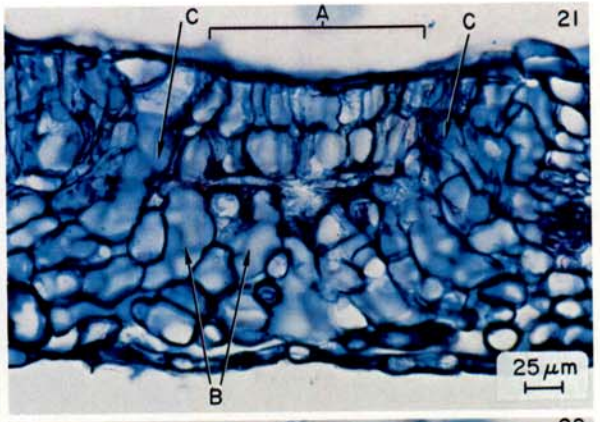
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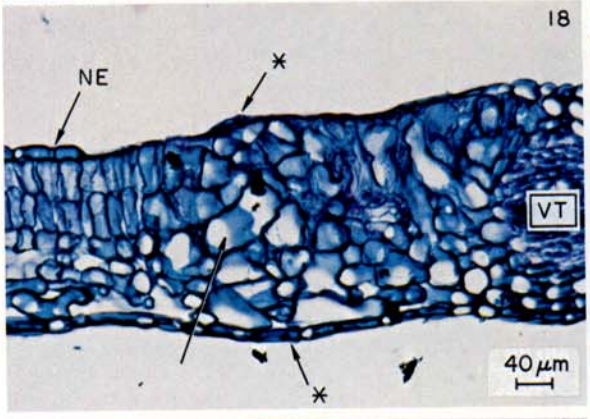
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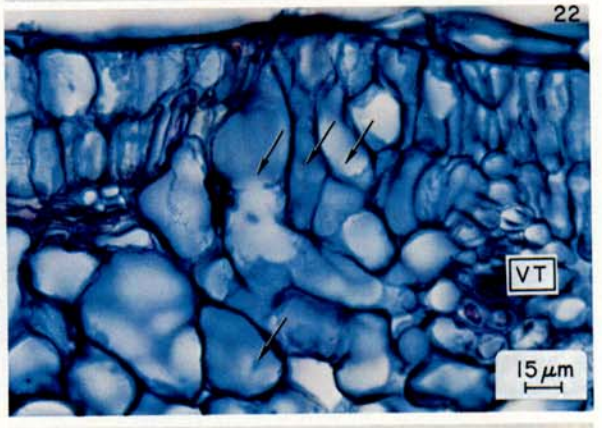
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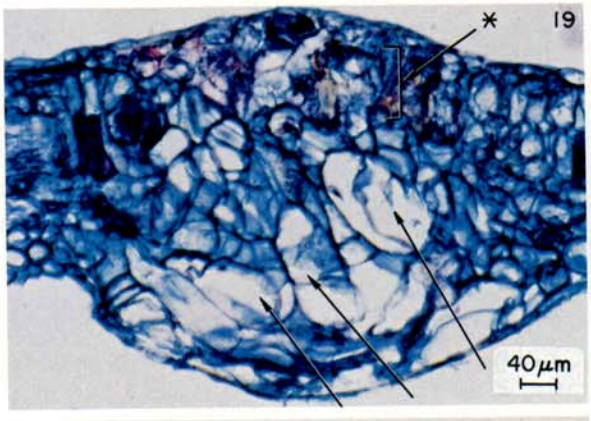
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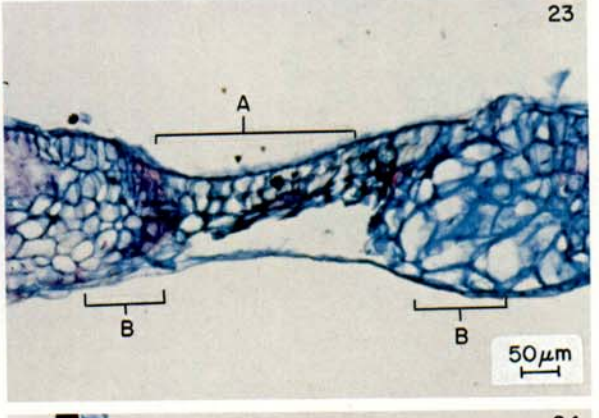
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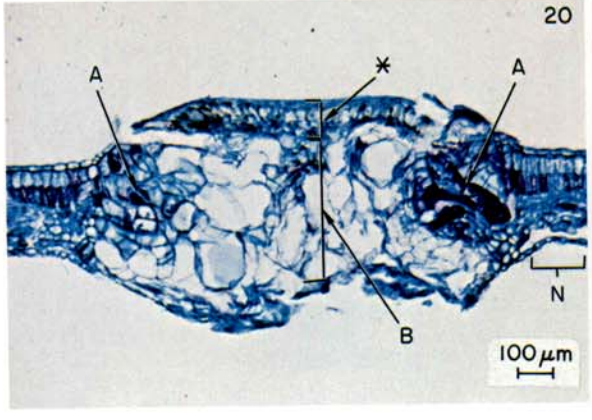
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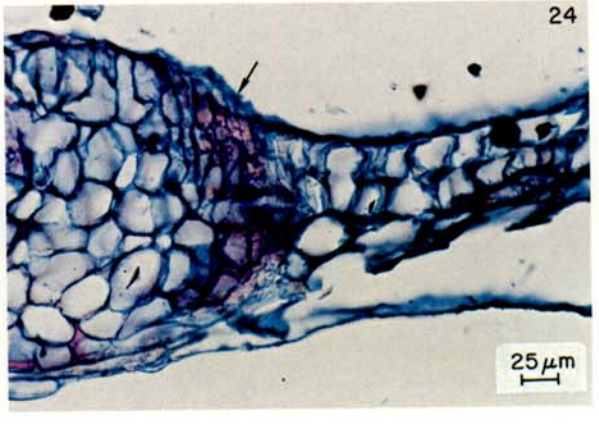
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dioxide per liter). However, several clones exhibited some small (<1.0 mm) lesions after exposure to simulated rain of pH 3.4. The percent leaf area with lesions and percent leaves injured both increased as the simulated rain pH decreased. At pH 2.7, the percent of leaf area injured was 4 to 6% of the leaf surface and 60 to 91% of the leaves exhibited lesions.

Leaf surface photographs (Fig. 1 to 6) document lesion development on various clones. Small lesions (<0.25 mm) (Fig. 2, 3, and 5) may develop after one 6-min exposure to simulated rain at either pH 2.7 or 2.9. These lesions initially exhibited chlorosis followed by necrosis. Chlorotic lesions exhibited a slight depression in the leaf surface (Fig. 2). Larger lesions (0.25 - 1.0 mm) exhibited necrotic tissues usually in a circular pattern. In some clones (No. 8, No. 207, No. 211, and No. 327) a red coloration developed in peripheral areas of large (>1.0 mm) lesions, although colorations were not present on either clone No. 43 or No. 44 (Fig. 4). Figure 5 shows a large lesion with several smaller lesions (<0.25 mm) denoted as either chlorotic (chl) or necrotic (nec). The large lesion is about 1 mm in diameter and exhibits a darkened area between healthy and necrotic tissues. A large lesion (2.0 mm in length) (Fig. 6) encompasses several small veins that retain chlorophyll longer than interveinal areas.

Scanning electron microscopy of leaf surface also was performed on healthy and noninjured leaf samples of various clones of poplar (Fig. 7 to \$6). Only clone No. 44 exhibited a low density of trichomes on both abaxial and adaxial leaf surfaces. In treated leaves, lesions initially were localized near stomata (Fig. 9 and 10) and generally were localized near vascular tissues. More internal leaf tissues probably were injured as estimated by depression depth (Fig. 11).

The responses of clones No. 8 and No. 327 to simulated acid rain was markedly different from the responses of the other clones. In these two clones, exposure to simulated acid rain resulted in gall-like outgrowths even after one or two, 6-min exposures daily (Fig. 13 through 16). The epidermis of the gall and the tissues that surround the gall collapse (Fig. 17). The collapse of epidermal cells around the gall forms a depression on the leaf surface. This depression was present in interveinal tissues but not if galls were near or within vascular tissues (Fig. 15 and 16). On occasion, galls were observed on clones No. 207 and No. 211 but none was present on clones No. 43 and No. 44.

In a later stage of gall formation, cells of both the palisade and spongy parenchyma areas exhibited hypertrophy (abnormal cell enlargement) and hyperplasia (abnormal cell proliferation) in response to simulated acid rain after collapse of the adaxial epidermis. These cell abnormalities generally caused elevations on both adaxial and abaxial leaf surfaces (Fig. 18 to 20).

The responses of clones No. 207 and No. 211 were intermediate between: (i) those of both No. 43 and No. 44, and (ii) those of clones No. 8 and No. 327. Clones No. 8 and No. 327 exhibited galls with abnormal cell enlargement and cell proliferation, but clones No. 43 and No. 44 did not produce either abnormal cell enlargement or abnormal cell proliferation (Fig. 21 to 24).

Galls were infrequent in small lesions on leaves of clones No. 207 and No. 211. Usually the adaxial epidermis collapsed initially with some injury to the palisade parenchyma. As lesions enlarged after additional exposures to simulated acid rain, palisade cells in the lesion usually collapsed completely. However, cells of the spongy parenchyma of lesions (0.25 - 1.00 mm) exhibited hyperplasia that usually preceded the presence of hyperplasia and hypertrophy in spongy and palisade parenchyma in zones adjacent to the lesion (Fig. 21 and 22). This latter zone of hyperplasia and hypertrophy produced a ridge of tissue surrounding the lesion (Fig. 23 and 24).

All six clones of poplar exhibited a similar response at each pH level of simulated acid rain, as indicated by the low standard error of each mean (Table 2). In general, the percentage of leaf surface area with lesions increased with an increase in number of exposures at each pH level. In addition, after each rain event, the percent of total leaf area with lesions increased with an increase in acidity of simulated rain.

Since most lesions were associated with stomata, experiments were focused on the density of stomata on abaxial and adaxial leaf surfaces. Density of stomata on abaxial leaf surfaces was about three times that of adaxial surfaces. The number of lesions per unit area on adaxial leaf surfaces after four consecutive daily simulated rain events was compared with the density of stomata per unit area on adaxial leaf surface (Table 3). The density of stomata and density of lesions per unit adaxial leaf area were not correlated. For example, clone No. 8 had the highest density of stomata but had the lowest density of lesions, whereas clone No. 43 ranked second in both categories.

Experiments were focused on the response (density of

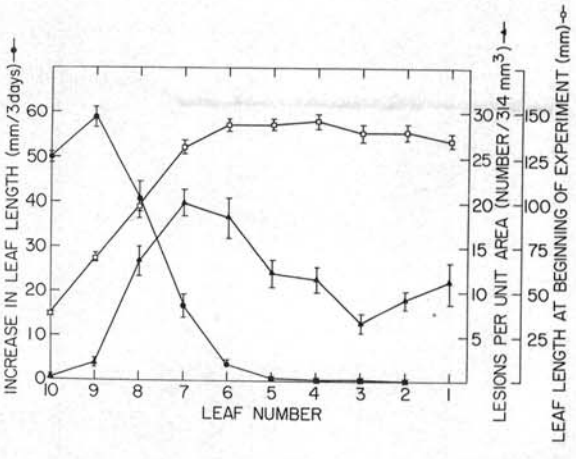

Fig. 25. Leaf length, increase in leaf length, and number of lesions per unit area on leaves of poplar clone No. 8. Increase in leaf length occurred during the rain period of 3 days. The number of lesions per unit area was measured one day after the last of three 6-min daily rain events. Measurements of the number of lesions were made on four areas per leaf. Characters represent mean and standard error of the mean from 10 plants. Leaves were numbered from oldest (leaf No. 10) to youngest (leaf No. 1).

lesions) with stage of leaf development in clone No. 8. The increase in leaf length occurred during a rain period of 3 days in which plants were exposed to one 6-min simulated rain event daily for 3 consecutive days. The number of lesions per unit area was determined 1 day after the last rain application. Lesions were most frequent on leaves 5, 6, 7, and 8 which were undergoing leaf expansion or had just become fully expanded (Fig. 25).

DISCUSSION

The experimental approach was aimed at a prediction of the acute and long-term responses that foliage of a variety of plant species may have after exposure to acidic precipitation in the environment. The experimental results with leaves of *Phaseolus vulgaris*, *Helianthus annuus*, and hybrid clones of *Populus* suggest that the density of lesions was not correlated with numbers of leaf development. The density of lesions remains low before the stage of most rapid leaf enlargement. In poplar, the density of lesions per unit area is greatest during maximum leaf expansion. In older leaves the density of lesions declines. A similar decline in lesion development with foliage maturation on fronds of bracken fern had been noted (Evans et al., *unpublished*). The presence of acidic rain during May, June, and July may have the most detrimental effects on deciduous tree and crop plant foliage.

Simulated acid rain produced more lesions per unit area at a particular pH on clones of hybrid poplar than occurred on foliage of *Phaseolus vulgaris* and *Helianthus annuus*(4). The sensitivities of these plants are markedly higher than that exhibited by needles of coniferous trees. Coniferous needles usually do not exhibit visual lesions above pH 2.5 (Evans, et al., *unpublished*). Thus the foliage of predominantly deciduous forests of the northeastern United States may be more sensitive than the coniferous forests of Scandinavia at similar acidity levels (3, 9, 10).

The features of deciduous tree and crop plant leaves that determine maximum sensitivity to simulated acid rain remain unknown. Among the clones of hybrid poplar used, the density of lesions was not correlated with numbers of stomata per unit leaf area, even though most lesions develop adjacent to or at stomata. In addition, density of lesions was nor correlated with numbers of stomata or trichomes per unit leaf area even though 95% of all lesions occur at these sites in *Phaseolus vulgaris* (5). Some other factors must be involved in predisposing these leaf structures and, possibly other areas of the leaf, since maximum sensitivity to simulated acid rain is correlated with maximum leaf expansion rate in all three plants tested. This developmental stage of maximum sensitivity may be correlated with a temporary decrease in cuticle thickness in some leaf areas as a result of differential leaf growth, or may involve synthesis of different types of cutin or cell wall components for short periods.

In two clones, No. 8 and No. 327, injured areas produced mostly galls after one or two exposures to simulated acid rain. In contrast, leaf cells of clones No. 43 and No. 44 underwent no hyperplasia and hypertrophy after exposure to simulated acid rain. In two clones, No. 207 and No. 211, hyperplasia and hypertrophy were limited to peripheral areas surrounding lesions. Both galls and the more limited response in peripheral areas of lesions were responses to exposure to simulated acid rain.

In accordance with lesion development on other plants, lesions of the hybrid clones of poplar exhibited an increase in depth and lateral enlargement with number of exposures to simulated acid rain. With continued exposures to rain solutions of low pH, the number of lesions increased, but lesion enlargement over the 6- to 7-day period of exposure reached a final diameter of several millimeters only. As a result, at any one time there was a steady state in the progression of small to large lesion diameters.

Our results, coupled with those of other investigators, demonstrate that simulated sulfate acid rain preferentially affects the leaf indumentum near trichomes, stomata, and vascular tissues. After initial injury, subsequent lesion development shows a progressive deterioration of the adaxial leaf surface to more abaxial structures. Because lesion frequency was not correlated with either stomatal or trichome frequency, the results suggest that epidermal cell morphology during development predisposes leaves to lesion development. These results with simulated rain suggest that differential sensitivity of plant taxa in nature to acid rain may be influenced chiefly by characteristics of the leaf indumentum.

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