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DIRECT FOLIAR EFFECTS OF SIMULATED ACID RAIN
II. LEAF SURFACE CHARACTERISTICS

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SUMMARY

Surface characteristics and wettability of the leaves of six plant species have been examined in relation to their susceptibility to damage from simulated acid rain. The species examined differed in the type and extent of epicuticular wax deposits, surface topography of the cuticle, trichome type and distribution, and epidermal cell shape. Leaf wettability, as measured by either water-holding capacity or droplet contact angle, was significantly different among species, and is highly correlated with previous reports of damage from simulated acid rain. The leaves of *Platanus occidentalis* L. were the most wettable of the species examined and are reported to be damaged the most by repeated applications of simulated acid rain. Mature leaves of *Liriodendron tulipifera* L., with high contact angles and very low water-holding capacity, are the least damaged according to published reports. Leaf water-holding capacity and surface-droplet contact angle may be useful first indicators of resistance to acid rain, and should be included in future studies of foliar effects of acid rain.

Key words: Acid rain, cuticle, leaf surfaces, wax.

INTRODUCTION

Surface characteristics are important in determining the wettability of leaves, foliar permeability and penetration, water retention, and rates of exchange of water and dissolved substances between plant and atmosphere (Fogg, 1947; Martin & Juniper, 1970; Hallam & Juniper, 1971; Holloway, 1971; Norris, 1974; Baker & Hunt, 1981; Juniper & Jeffree, 1983). The increasing use in recent years of foliar applied herbicides, fungicides and specialized growth regulators has resulted in renewed interest in leaf surface characteristics such as trichome distribution, cuticle thickness and wax deposition and their influences on efficacy of foliar applied compounds of various types (Norris, 1974; Bukovac, Flore & Baker, 1979; Baker & Hunt, 1981).

An additional area of recent research in which leaf surface characteristics may be of particular importance concerns the effects of acid rain on plant growth and survival. Effects of simulated acid rain on leaf surface perturbations (Evans, Gmur & DaCosta, 1977; Evans, Gmur & Kelsch, 1977; Paparozzi & Tukey, 1983), foliar leaching (Evans, Curry & Lewin, 1981; Haines, Chapman & Monk, unpublished) and photosynthetic rates (Ferenbaugh, 1976; Neufeld, Jernstedt & Haines, 1985) have been reported. Sensitivity to damage by simulated acid rain has been reported to vary with species (Evans & Curry, 1979; Haines, Stefani & Hendrix, 1980; Evans, Gmur & Mancini, 1982), growth conditions (Evans *et al.*, 1982) and with

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age of leaves (Evans & Lewin, 1981), all of which are factors influencing leaf surface features (Juniper, 1959; Hull, Morton & Wharrie, 1975; Cutter, 1978).

A little-studied aspect of acid rain effects on crop and forest species is that of leaf wettability. The work of Keever & Jacobson (1983a, b) with *Glycine max* indicates that the greater leaf wettability of a glabrous soybean isoline may be a factor in its increased susceptibility to foliar injury and leaching by simulated acid rain compared with plants of a pubescent isoline. However, it appears that cuticle thickness and form and epicuticular wax distribution have not yet been considered as factors influencing the magnitude of effects of acid rain.

Because of the possibility that leaf wettability may be related to damage by acid rain in other species, and because surface features such as trichome number and distribution, cuticle thickness and pattern and wax distribution determine, at least in part, leaf wettability, these aspects of leaf structure and function were investigated. The objectives of the present study were to compare (1) leaf surface characteristics, especially epicuticular wax deposits, (2) leaf water holding capacity, and (3) leaf surface-water droplet contact angles of several species which have been subjects of previous studies of effects of simulated acid rain. The results are discussed in terms of species susceptibility to and extent of damage resulting from simulated acid rain.

MATERIALS AND METHODS

Plant material

Plants of *Liquidambar styraciflua* L. (sweetgum), *Platanus occidentalis* L. (sycamore) and *Robinia pseudo-acacia* L. (black locust) were grown from seed in 4 litre pots in a greenhouse. When the 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. (tulip poplar) and *Quercus prinus* L. (chestnut oak) were obtained as established seedlings from field populations in northeastern Georgia and southern North Carolina, respectively, transplanted into 4 litre pots, and maintained in a greenhouse, under which conditions most of the foliage and approx. 90% of the total biomass developed. Stratified seeds of *Erechtites hieracifolia* Raf. (fire weed) were germinated and grown to maturity in a greenhouse. A loamy sand mixture was used for the potting soil. Plants were kept well-watered and fertilized weekly with half-strength Hoagland's nutrient solution.

Leaf surface structure

Samples of fresh leaf material of all six species were mounted on aluminum stubs with either silver conductive paint or double-sided tape. Specimens were examined directly with a Cambridge Stereoscan (Cambridge Instruments Ltd., Cambridge, England) scanning electron microscope or after sputter-coating with gold-palladium.

Water-holding capacity

Water holding capacities were determined in the laboratory for five tree species, *Liquidambar*, *Liriodendron*, *Platanus*, *Quercus*, and *Robinia*, and the herb, *Erechtites*. Individuals of each species were selected and the fourth or fifth leaf from the apex sprayed from above with distilled water for 20 s from a distance of 30 cm, using a hand sprayer. Droplet size frequency distribution was determined by the flour method of Laws & Parsons (1943). The sprayer produced droplets such that

68% (SD 8·54, $n = 3$) of the mass was between 0·59 and 1·0 mm diameter and the remainder of the mass was between 1·0 and 2·0 mm diameter. For *Robinia*, the compound leaf was detached first and placed on a horizontal surface because of difficulty in spraying when still attached to the stem. Leaves of all other species remained attached to the plant, in their natural orientations. Spraying took place in the laboratory, in the absence of air currents which would cause leaf motion. Spraying was sufficient to saturate the leaves. Water which remained on the adaxial surface was soaked up with absorbent tissue paper and weighed. Leaf areas were determined for each leaf with a Li-Cor (Li-Cor Inc., Lincoln, Nebraska) area meter. Water holding capacity was expressed as mg H_2O cm^{-2} projected leaf area.

Leaf contact angles

Contact angles were measured on adaxial surfaces of detached leaves of all six species. Angles were characterized on both rapidly expanding and recently matured leaves. To determine first if pH influenced the contact angle, 10 μl drops of either deionized (pH = 5·8) or acidified water (pH = 2·0) were placed on horizontal pieces of commercial waxed paper using an autopipette. Drop outlines were projected onto a wall using a modified 35-mm slide projector and traced on paper. Contact angles were calculated from the tracings as described by Fogg (1947). Drops of deionized water (10 μl) were then placed on adaxial surfaces of freshly detached leaf pieces, the outlines projected and traced, and contact angles calculated according to Fogg (1947).

Data were analyzed using ANOVA programs in SAS (SAS, 1982) after, where appropriate, transformation (Zar, 1974). Additional statistical calculations were performed on an HP-1000 mini-computer following procedures in Zar (1974).

Results

Leaf fine structure

Scanning electron micrographs of the leaf surfaces are shown in Figures 1 and 2. The species examined differed in type and extent of epicuticular wax deposits, cuticle surface topography, trichome type and distribution, and epidermal cell shape. Adaxial leaf surfaces of *Robinia pseudo-acacia* were characterized by the presence of uniseriate covering trichomes, flattened epidermal cells and a dense covering of crystalline surface wax deposits overlaying an apparently smooth cuticle [Fig. 1(a), (b)]. Leaves of *Liriodendron tulipifera* were glabrous. Cells of the adaxial epidermis were only slightly convex, with a dense covering of granular surface wax deposited on an unornamented cuticle [Fig. 1(c), (d)]. Leaves of *Platanus occidentalis* bore branched covering trichomes on both surfaces. The trichomes were located predominantly over the veins and occurred in extremely low frequency on the adaxial surface. Adaxial epidermal cells were convex and marked with distinct cuticular striations. Epicuticular wax was absent from the adaxial leaf surface [Fig. 1(e), (f)]. Leaves of *Liquidambar styraciflua* were glabrous. Cells of the adaxial epidermis were convex with a smooth cuticle and devoid of visible (crystalline) surface wax deposits [Fig. 2(a)]. Adaxial surfaces of *Erechtites hieracifolia* leaves were glabrous and waxless; epidermal cells were flat with a smooth cuticle [Fig. 2(b)]. *Quercus prinus* leaves bore uniseriate covering trichomes, usually along the veins. Adaxial epidermal cells were convex with a smooth cuticle, and covered by a relatively sparse layer of granular surface wax [Fig. 2(c), (d)].

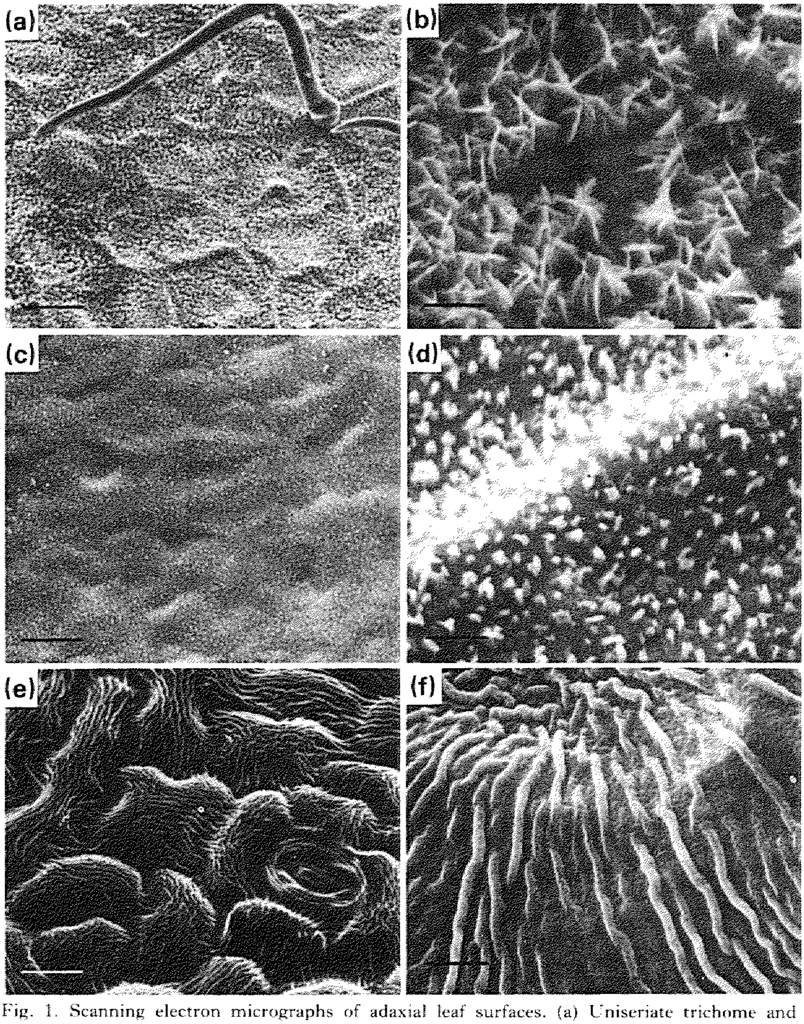

Fig. 1. Scanning electron micrographs of adaxial leaf surfaces. (a) Uniseriate trichome and wax-covered epidermal cells of *Robinia pseudo-acacia*; scale bar = 20 μm. (b) Crystalline surface wax deposits covering *R. pseudo-acacia* epidermis; scale bar = 2 μm. (c) Slightly convex epidermal cells of *Liriodendron tulipifera* covered with granular wax deposit; scale bar = 20 μm. (d) Granular surface wax on *L. tulipifera* leaf; scale bar = 2 μm. (e) Convex epidermal cells and sunken stoma of *Platanus occidentalis*; scale bar = 20 μm. (f) Cuticular striations on epidermal cells of *P. occidentalis*; scale bar = 5 μm.

Water-holding capacity

Leaf water-holding capacity, in mg H_2O cm^{-2} projected leaf area (Table 1) showed significant differences among species. Water-holding capacity was greatest in *Platanus*, *Quercus*, and *Erechtites*, and least in *Liriodendron*, with *Liquidambar* and *Robinia* intermediate in wettability as determined by this method.

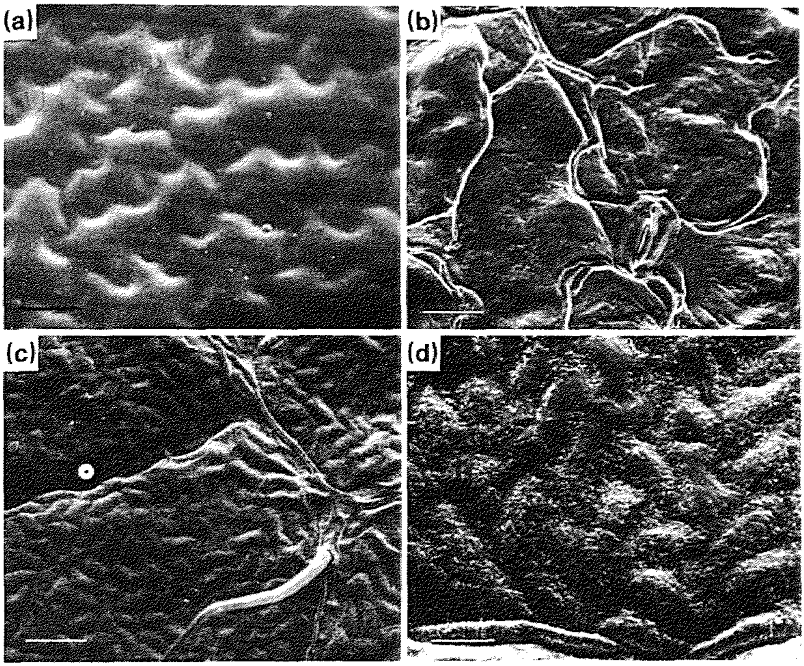

Fig. 2. Scanning electron micrographs of adaxial leaf surfaces. (a) Convex epidermal cells with smooth cuticle and no surface wax, *Liquidambar styraciflua*; scale bar = 20 μm. (b) Flattened, waxless epidermal cells of *Erechtites hieracifolia*; scale bar = 20 μm. (c) Uniseriate trichome and sparse wax covering of *Quercus prinus* leaf; scale bar = 50 μm. (d) Sparse layer of granular surface wax of *Q. prinus* leaf; scale bar = 20 μm.

Table 1. *Mean contact angles and mean water-holding capacities* (*mg H_2O cm^{-2}*) *of adaxial leaf surface*§

	Contact angle		Water holding capacity	
Species	$\bar{\theta} \pm$ SE	*n*	$\bar{X} \pm$ SE	*n*
Robinia pseudo-acacia	131·6 ± 2·2a	5	15·4 ± 3·2b	5
*Liriodendron tulipifera**	124·8 ± 1·6a	5	3·9 ± 0·8a	5
*Quercus prinus**	100·9 ± 6·2b	5	29·5 ± 3·7†	3
Liquidambar styraciflua	97·8 ± 4·7b	5	19·7 ± 0·8b, c	5
Platanus occidentalis	80·7 ± 8·5c	5	25·0 ± 1·9c	5
Erechtites hieracifolia	70·1 ± 2·7c	5	24·9 ± 3·6c	5
Wax paper pH 2‡ (acidified H_2O)	119·7 ± 2·6a	5	—	—
Wax paper pH 5·8 (deionized H_2O)	116·0 ± 2·2a	5	—	—

* Transplanted as seedlings to the greenhouse, under which conditions all the foliage used in this study and approx. 90% of the total biomass developed.

† Not included in Duncan's multiple range test because of insufficient plants.

‡ Means for the wax paper comparison were analyzed using a Student's *t*-test (Sokal & Rohlf, 1981).

§ Means not followed by the same letter differ at the $P < 0·05$ level, as determined by a Duncan's multiple range test (SAS, 1982).

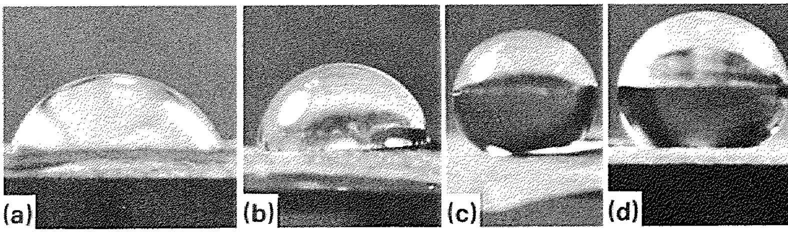

Fig. 3. Side views of 10 μl droplets of deionized water placed on adaxial leaf surfaces; contact angles increasing from (a) to (d). (a) *Platanus occidentalis*, $\theta = 82{\cdot}2°$. (b) *Liquidambar styraciflua*, $\theta = 88{\cdot}9°$. (c) *Liriodendron tulipifera*, $\theta = 118{\cdot}6°$. (d) *Robinia pseudo-acacia*, $\theta = 126{\cdot}3°$.

Leaf contact angles

Leaf-water droplet contact angle measurements and the effect of pH on contact angle are shown in Table 1. The mean contact angles of droplets of deionized water (pH 5·8) and acidified water (pH 2·0) placed on commercial wax paper were not significantly different ($P < 0{\cdot}05$). Mean contact angles of deionized water on young and mature leaves of all species examined were not significantly different and were pooled for analysis. Mean contact angles for adaxial surfaces of *Platanus* and *Erechtites* leaves were less than 90° and the low spreading droplets covered a comparatively large area of the leaf surface [Fig. 3(a)]. Mean contact angles for *Robinia* and *Liriodendron* exceeded 120° and only a small area of the adaxial surface was covered by these rounded droplets [Fig. 3(c), (d)]. *Quercus* and *Liquidambar* leaf contact angles were of intermediate magnitudes; droplet shape and coverage were also intermediate [Fig. 3(b)].

DISCUSSION

The degree of damage which trees and herbs suffer when exposed to acid rain varies widely among species (Wood & Bormann, 1974, 1975; Evans & Curry, 1979; Lee & Weber, 1979; Haines *et al.*, 1980; Neufeld *et al.*, 1985), but the underlying mechanisms are poorly understood at present. Leaf wettability may be an important determinant of foliar damage by acid rain, and physical and chemical characteristics of the leaf surface determine the wettability of most leaves (Martin & Juniper, 1970; Evans, 1982; Shriner, 1983). An estimate of the potential wettability of a leaf surface can be obtained by measuring the angle of contact between a water drop and the surface on which it rests (Fogg, 1947). The higher the contact angle, the more hydrophobic the leaf, while the lower the angle, the more wettable it is. Water holding capacity of leaves, while influenced by such factors as leaf motion and orientation, is also generally indicative of wettability, with more wettable leaves retaining more moisture than more hydrophobic leaves.

In the present study the contact angles of leaf surface-water droplets and laboratory estimates of water-holding capacity were somewhat positively correlated. Of the tree species examined, mature leaves of *Platanus* retained the most water and had the lowest contact angles. At the other extreme, the leaves of *Liriodendron* had the lowest water-holding capacity and high contact angles. Although *Robinia* leaves had the highest contact angles, they were intermediate in water-holding capacity, while leaves of *Liquidambar* were intermediate in both measures of wettability. The relatively high water-holding capacity of *Robinia* may have been because these leaves were not sprayed *in situ*. Differences in contact angles among tree species could be due to the distribution and nature of waxes overlying the cuticle. Both the chemical composition and orientation and pattern of surface waxes on the cuticle are important determinants of leaf wettability (Holloway, 1969a, b; Leece, 1978).

In addition to the effects of superficial waxes, contact angles may be altered by surface roughness (Wenzel, 1936; Gray, 1965). The presence of hairs may decrease contact angles (Boize, Gudin & Purdue, 1976), but this can be dependent on the basic contact angle. If the angle is originally less than 90°, increased surface roughness further lowers the angle, but if it is greater than 90°, then it raises the angle (Wenzel, 1936; Fogg, 1947). Surface roughness may partially explain the low contact angles observed for *Platanus*. Numerous cuticular striations covered the adaxial surface of *Platanus* leaves, and branched covering trichomes and sunken stomata occurred as well, all of which might act collectively to lower the contact angle. *Robinia* leaves showed the highest contact angles, as might be expected from the presence of trichomes and a dense covering of crystalline wax deposits on adaxial surfaces, although the flattened epidermal cells and abundant trichomes may have also contributed to the intermediate water-holding capacity. No attempt was made in the present study to estimate the relative contributions to the determination of leaf contact angles made by epicuticular wax chemistry and distribution and leaf surface features such as trichome type, number and position or stomatal position. Nonetheless, it is probable that there are primary and secondary effects of each on leaf contact angles, and that the relative importance of the various features varies with the species.

Four of the tree species of this study are subjects of a previous report on the effects of simulated acid rain on leaf damage, growth and gas exchange (Neufeld *et al.*, 1985). Although sample size is small, percent damage among species is highly correlated with both the water-holding capacity of the leaves and with contact angles. *Platanus* leaves, the most wettable by our present determinations, were damaged the most by repeated applications of simulated acid rain. Mature leaves of *Liriodendron*, with high contact angles and very low water-holding capacity, were the least damaged, in terms of percent necrotic leaf area, wrinkling and abscission (Neufeld *et al.*, 1985). *Liquidambar*, which showed intermediate wettability in the present study, also showed intermediate degrees of damage. *Robinia* shows a somewhat anomalous response. Although it had the highest contact angles, in order of damage it was second among these species (Neufeld *et al.*, 1985). This may have been due to the method by which damage was assessed. For *Robinia*, entire rachises and leaflets often abscise after exposure to the acid rains, even though leaflets themselves appeared green and healthy. Loss of a rachis was scored as 100% leaf loss. Actual damage to leaflet laminae was quite low, which would correlate with the high contact angles measured for this species.

It does not appear that leaf wettability is correlated with the susceptibility threshold of tree leaves to acid rain as much as with percent damage that occurs. According to Neufeld *et al.* (1985), the four hardwood species exhibited damage at approximately the same pH level (less than pH 3·0). In Keever & Jacobson's studies (1983a, b) contact angles were also more closely related to the degree of damage than to the threshold for damage. It must therefore be concluded that the susceptibility threshold and resistance to damage are not necessarily linked.

In the present study, contact angles of young and mature leaves of *Platanus*, *Liquidambar*, *Liriodendron* and *Robinia* were not significantly different and consequently were pooled for subsequent analysis. However, in other studies, species specific differences in leaf wettability occurred during maturation, resulting from changes in wax and cuticle development (Juniper, 1959; Martin & Juniper, 1970; Baker & Hunt, 1981). For example, young leaves of one isoline of *Glycine max* had lower contact angles than older leaves, and this was correlated with greater damage to younger leaves by acid rain (Keever & Jacobson, 1983b). As emphasized by Keever & Jacobson (1983b), rapidly expanding leaves may have low rates of wax production and thereby develop discontinuities in the coverage of waxes over the leaf surface (Baker & Hunt, 1981). Since the cuticle is more hydrophilic than the surface waxes, this results in lower contact angles (Holloway, 1969a). Furthermore, rainfall acidity is often higher in spring and summer (Hornbeck, Likens & Eaton, 1977), a period coinciding with rapid leaf expansion, and the potential for foliar damage would therefore be greatest at this time of the year.

The leaching of nutrients and other substances from leaves in response to acid rain has received attention recently (Evans *et al.*, 1981; Keever & Jacobson, 1983). Because the wax component of the cuticle is thought to be responsible for reduction of nutrient losses by leaching (Martin & Juniper, 1970), leaf surfaces and wettability were examined on two species for which rates of mineral element leaching in response to simulated acid rain have been documented. Haines, Chapman & Monk (unpublished) showed that the highest leaching rates occurred in the herbs *Erechtites hieracifolia* and *Erigeron canadensis*. Our results show that *Erechtites* leaves had the lowest mean contact angle ($\bar{\theta} = 70{\cdot}1°$) of the six species examined. Adaxial leaf surfaces of *Erechtites* are glabrous and waxless, and composed of flattened epidermal cells with a smooth cuticle. The resulting highly wettable leaves of *Erechtites* seem to be more subject to leaching than another species examined, *Quercus prinus*. Haines *et al.* (unpublished) reported 10 to 100-fold differences in leaching rates between *Erechtites* and *Quercus*, which is consistent with expectations based on leaf surface characteristics. Leaves of *Quercus* had a mean contact angle of 101·5°, and adaxial surfaces are covered with a sparse but distinct layer of wax granules, indicating a lower degree of wettability than *Erechtites* leaves. Keever & Jacobson (1983a) were able to correlate foliar damage of soybeans from simulated acid rain with foliar leaching and leaf contact angles. They observed that their glabrous isoline of *Glycine max*, which had more wettable leaves than the pubescent isoline, showed more damage to acid rain and was more prone to foliar leaching of ^{86}Rb. They suggest that the greater wettability of the glabrous isoline, as indicated by lower mean contact angles, may have been a factor in the observed differences in foliar sensitivity.

Besides our own research and that of Keever & Jacobson (1983a, b), we know of no other studies relating either leaf water-holding capacity or contact angle to damage by acid rain. The effects of increased retention time of acid precipitation on wettable leaves (i.e. water-holding capacity) and extent of surface area covered by rain droplets (i.e. leaf contact angle) on susceptibility to damage by acid rain may provide insight into the mechanism of foliar damage by acid rain. One or both of these characteristics should be quantified in future studies because of the possibility that these simple measurements may be useful first indicators of the resistance of plants to acid rain.

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