Effect of air pollution on the leaf epidermis at the submicroscopic level

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Summary. Analysis of scanning electron micrographs of the leaf epidermis of 7 flowering plants shows that air pollution reduces the size of the stomatal pore and increases the trichome length on both surfaces of the leaf. Air pollution also increases trichome density and folding on subsidiary cells at the adaxial leaf surface.

The effect of air pollution on plants is well known, but studies pertaining to the impact of air pollution on the leaf epidermis have only recently begun.

It has been shown that air pollution affects stomatal frequency, length and density of trichomes in *Trifolium repens* L., *Trifolium pratense* L. and *Acer saccharum* Marsh[1-3].

In *Calotropis procera* R. Br., frequency of the epidermal cells, stomata and trichomes increase in response to air pollution[4].

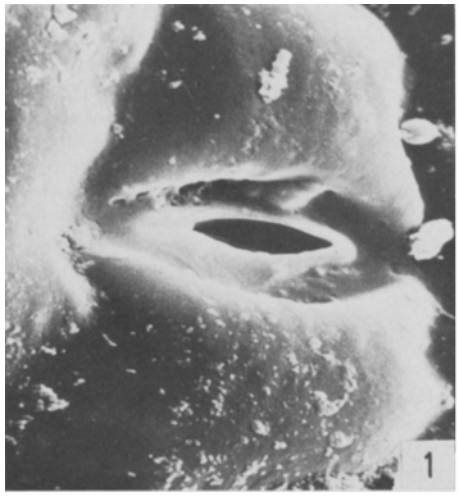
The impact of air pollution on the leaf epidermis had, until recently only been studied with light microscopy. Using the scanning electron microscope, Godzik and Sassen[5] showed that, as a result of air pollution, the leaf surface of *Aesculus hippocastanum* L. had fewer folds compared with control leaves; stomata did not have the normal appearance.

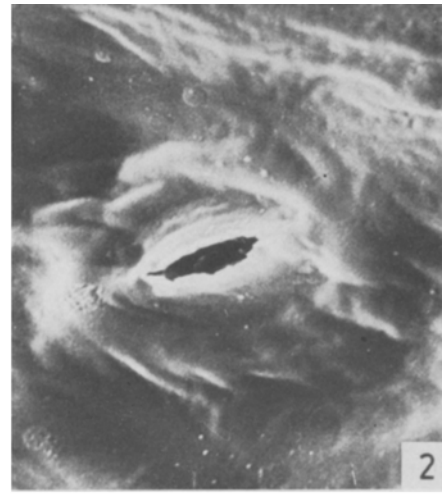
The present investigation was undertaken to examine the effects of air pollution on the epidermal features, especially stomata and trichomes, with the help of a scanning electron microscope.

Materials and methods. Leaves of *Brassica oleracea* L., *Chenopodium album* L., *Cicer arietenum* L., *Dolichos lablab* L., *Lantana camera* L., *Sonchus asper* Hill and *Withania somnifera* Dunal., of the same age and size were collected from a polluted site (P) and a non-polluted site (NP). Site P was located about 1 km east-southeast from the Indraprastha Thermal Power Station, New Delhi. It is a zone of heavy air pollution (fly ash, sulphur dioxide, carbon dioxide). Site NP was located about 6–7 km east-southeast of the power station. It is a comparatively less affected zone.

Sample preparation: epidermal peels were taken manually for studying the epidermal characteristics under the light microscope. For scanning electron microscopy, leaves were collected and washed with double-distilled water, using a soft camel hair brush. After the leaves were gently wiped with tissue paper, they were placed with their adaxial surface upward and 2 mm square samples were obtained.

Leaf samples were fixed in 3% glutaraldehyde (in phosphate buffer, pH 7.2) post-fixed in 1% osmium tetraox-





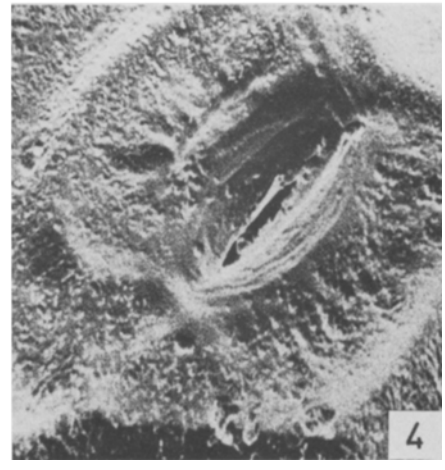


Fig. 1. Adaxial leaf surface of *Withania somnifera* from site NP. ×2100.

Fig. 2. Adaxial leaf surface of *Withania somnifera* from site P. ×2100.

Fig. 3. Abaxial leaf surface of *Chenopodium album* from site NP. ×2200.

Fig. 4. Abaxial leaf surface of *Chenopodium album* from site P. ×2200.

ide (in phosphate buffer, pH 7.2), dehydrated in 35, 50, 85, 95, 99% ethanol, 3 changes of 5 min each before placing in amyl acetate for 15 min (Krause, 1976)[6]. They were fixed on a specimen holder with 'Quick-fix', and coated with a layer of silver, approximately 200 Å thick, by the vacuum evaporation method under 10^{-5} Torr. The preparations were scanned using a Cambridge Steroscan model S4-10 electron microscope. Scanning electron micrographs of stomata and trichomes were taken for both the adaxial and the abaxial leaf surfaces.

Results. Plants from site P had smaller stomatal pores compared with the plants from site NP. Measurements of the length and breadth of stomatal pores on the adaxial leaf surface showed that they were reduced approximately by 1/3 and 1/4, respectively, both in *Withania somnifera* and *Brassica oleracea.* In *Chenopodium album* and *Dolichos lablab* the reduction in the length and breadth of stomatal pores on the abaxial leaf surface was less in comparison to the other 2 species. It seems that the adaxial leaf surface was more affected than the abaxial surface in plants from site P. The scanning electron microscope study supported the observations made with the light microscope (table 1). In addition,

Table 1. Length and breadth of stomatal pores of plants grown at non-polluted (NP) and polluted (P) sites

		Site NP Length (µm)	Breadth (µm)	Site P Length (µm)	Breadth (µm)
Withania somnifera	(Ad)	11.1±1.9	2.8±0.3	8.2±1.1	2.2±0.4
Brassica oleracea	(Ad)	13.0±0.9	3.5±0.4	9.0±0.5	2.6±0.2
Chenopodium album	(Ab)	11.0±0.7	2.5±0.2	8.0±0.4	2.0±0.1
Dolichus lablab	(Ab)	17.0±1.2	3.0±0.3	14.0±0.8	2.7±0.2

Values expressed as mean±SD; Ad, adaxial leaf surface; Ab, abaxial leaf surface. Measurements made under the light microscope.

Table 2. Density and length of trichomes of plants grown at non-polluted (NP) and polluted (P) sites

		Site NP Density (No./cm²)	Length (µm)	Site P Density (No./cm²)	Length (µm)
Lantana camera	(Ad)	120±21.2	160±16.4	220±31.2	200±22.6
Sonchus asper	(Ad)	140± 7.2	152±12.3	210±21.2	245±29.7
Cicer arietenum	(Ab)	130±16.2	175±12.8	170±17.4	220±14.2

Values expressed as mean±SD; Ad, adaxial leaf surface; Ab, abaxial leaf surface. Measurements made under the light microscope.

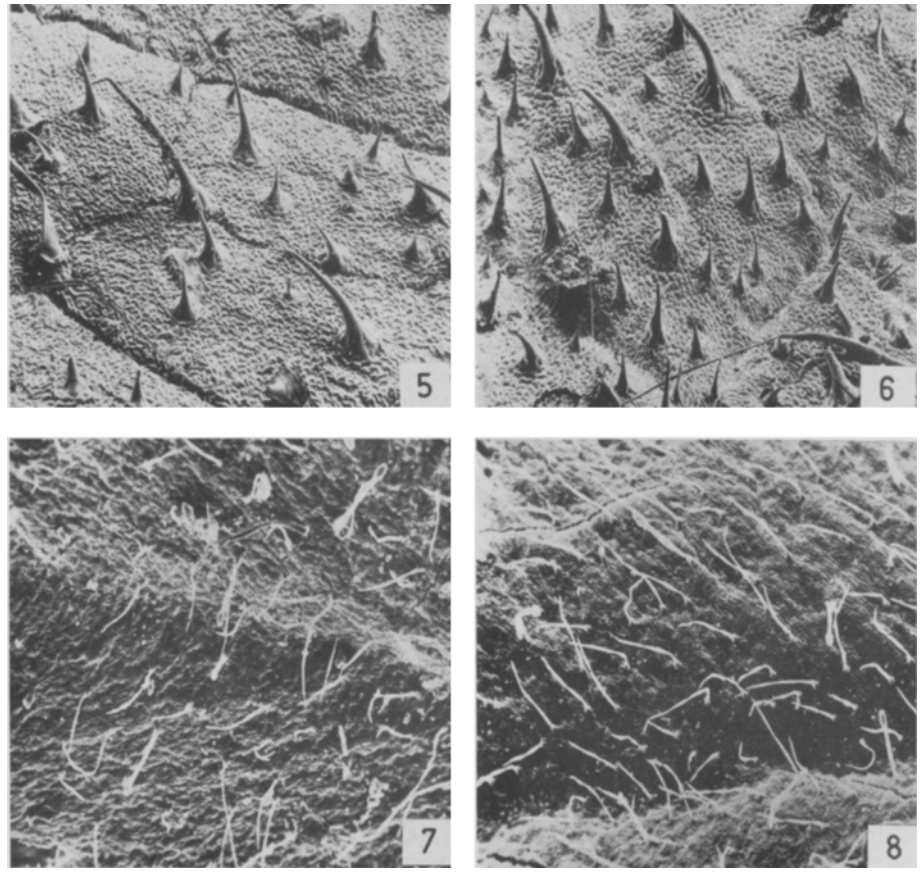


Fig. 5. Adaxial leaf surface of *Lantana camera* from site NP. ×56.

Fig. 6. Adaxial leaf surface of *Lantana camera* from site P. ×52.

Fig. 7. Abaxial leaf surface of *Cicer arietenum* from site NP. ×53.

Fig. 8. Abaxial leaf surface of *Cicer arietenum* from site P. ×53.

an uneven boundary of the stomatal pores and a rugged surface of subsidiary cells were quite prominent in plants from site P (figures 1–4).

The adaxial leaf surface of *Lantana camera* and *Sonchus asper,* as well as the abaxial surface of *Cicer arietenum* from site NP and site P, were scanned to study the effect of air pollution on trichome characteristics (figures 5–8). Density and length of trichomes were relatively greater in plants from site P. There was more variation in trichome density and length on the adaxial surface than on the abaxial surface of site P plants. The electron microscope study supports the observations made with the light microscope (table 2).

Discussion. Air pollution apparently reduces the size of the stomatal pores on both the adaxial and the abaxial leaf surfaces. Sharma and Butler[1–3] have (on the basis of light microscope studies) reported a slight reduction in the sizes of stomatal pores in *Trifolium repens, Trifolium pratense* and *Acer saccharum* growing in polluted areas. According to these authors, a reduction in size of stomatal pores of plants from a polluted site could be considered as a favourable adaptation, as it might help in reducing the absorption of gaseous pollutants.

The subsidiary cells on the adaxial leaf surface of plants from the polluted site have numerous folds. Our observations do not match those of Godzik and Sassen[5], who reported a reduction in the number of folds on the outer epidermal cells in plants from a polluted area. However, they indicated that a variation in folds together with the changed ultrastructure of the outer cell wall of the epidermis may contribute to the loss of elasticity of leaves.

The length and density of trichomes on the adaxial leaf surface were greater in plants from the polluted site compared with those from the non-polluted site. At the abaxial surface, an increase in length only of trichomes was observed in plants from the polluted area. Sharma and Butler[2] suggested that these changes could have adaptive significance as the high density of trichomes may protect the leaf from direct exposure to the sun's rays, thus lowering the leaf temperature and hence reducing the rate of metabolism. In constrast, Eller[7] suggested that there would be an increase in surface temperature as dust on a leaf is responsible for high absorbance and low reflectance of IR-waves.

In short, air pollution reduces the length and breadth of stomatal pores, augments folding of the subsidiary cells and increases the length and the density of trichomes. These changes appear to have some adaptive significance, because ozone-resistant varieties of *Petunia* also have small stomatal pores and high trichome density compared with its sensitive varieties[8].

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Photoperiodism and morphogenesis of the protonema of *Ceratodon purpureus* (Hedw.) Brid

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Summary. Elongation of *Ceratodon* protonema is a long-day phenomenon. Branching is a short-day phenomenon. The two morphological systems are antagonists.

Light, which has very diverse effects on the protonema of bryales[2] controls morphogenesis by its influence on: 1. photosynthetic activity: a certain amount of trophic light is necessary for protonema to develop into a caulonema branched system. The spectrum of light activity on branching and growth of filaments can be shown to be virtually identical with the absorption spectrum of photosynthetic pigments in vivo[3]. 2. Phytochrome action, which intervenes the positioning of lateral buds branches from which originate. Its role depends on the existence of a substrate, previously synthesized during the course of photosynthesis[3]. 3. The effect of the cellular division factor, which is only present if cultures are submitted to a certain amount of light. This factor circulates in both the acropetal and basipetal directions from the same filament, and is responsible for the perpendicular mitosis generating the main axis[4].

During the course of these experiments, the notion of independence between the growth length of protonema and the branching of axes emerged on several accasions. Thus, the optimum amount of light, was required for lengthening the filament (mitosis rate)[5]. A unilateral and perpendicular light on the axes of protonema filaments is always less favorable for branching than multidirectional light of equal energy, although this has no effect on the mitotic rate[4]. In contrast to the cellular division factors, the branching factor (or factors) seems to be motionless in protonema.

Little data is available concerning the photoperiodic responses of branched filamentous systems. Precise studies carried out on the rhodophyte, *Acrochaetium,* enabled us to reach the conclusion that this organism reacted like a higher plant of the long-day type[6]. For bryales at the protonematic stage only the sexualization phenomena have been studied until now[7–9]. In an endeavour to fill this gap, we decided to undertake this experiment.

Materials and methods. Protonema of *Ceratodon purpureus* (Hedw.) Brid. were grown under sterile conditions at 23 °C ± 1 °C on Kofler A medium[2] under multi-lateral light, of 2250, 4500 ergs cm^{-2} sec^{-1} and 11,000 ergs cm^{-2} sec^{-1} provided by fluorescent tubes (Mazda 'Blanc brillant de luxe' type).

The photo-periods chosen were 3, 6, 9, 12, 15, 18 and 24 h per 24 h. The parameters measured on 12 day-old cultures and characterizing development are the following: average cell length in µm, growth speed of the main axis per 24 h, branching density ($R/N \times 100$: R = number of lateral branches, N = number of cells composing the main axis).

Results. Photoperiodic effects on the elongation of the main axis: Cellular elongation does not appear to vary significantly according to the photo-period, no matter what energy is considered (figures 1, B and 2, B).

Growth speed (function of perpendicular mitoses), reached a maximum at a photo-period of 15 h light by 24 h at 4500 ergs cm^{-2} sec^{-1}, and in continuous darkness (etiolation). If