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A Scanning Electron Microscopic Study of the Changes induced by Cement Kiln Exhaust Dust on the Phylloplane Wax Deposits of Three Plants

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The phylloplanes of control leaves and of leaves coated with the exhaust dust emanating from cement kiln were investigated with the help of scanning electron microscope in *Arachis hypogaea, Borassus flabellifer* and *Cocos nucifera*. The crystalline wax deposits easily flaked off and underwent slow disintegration in the dust-coated leaves of all the plants studied.

Key Words: Phylloplane, Wax deposits, Cement dust, *Arachis hypogaea, Borassus flabellifer, Cocos nucifera*

Introduction

Phylloplane denotes the external surface of the leaf and is generally made up of the non-cellular component, the cuticle and over it, the epicuticular waxes (Holloway 1971). These waxes affect the wettability, permeability, reflectivity, water-loss, gas-exchange, etc., which in turn condition the physiology of the leaves (Juniper & Jeffree 1983). Therefore, any change in the cuticle and epicuticular waxes could adversely affect the normal functioning of the leaf. In earlier studies on the effect of particulate dust on leaves little attention was paid to the changes in the phylloplane. Disorganization of the cuticle due to cement dust (Lerman 1972, Lerman & Darley 1975) and the removal of epicuticular waxes due to acidic pollutants (Lang et al. 1978, Evans 1979) were reported briefly. The present study deals with changes in the phylloplane wax deposits of three economically important plants which were coated with the exhaust dust emanating from the cement kiln.

Materials and Methods

Arachis hypogaea (groundnut), *Borassus flabellifer* (palmyra) and *Cocos nucifera* (coconut) were selected for the study. Cement dust-coated leaf materials were collected at random from several individual plants of these species at regular intervals throughout a period of three years near the Chettinad Cement Factory, Puliyur, about 70 km from Tiruchirapalli. Control leaf materials were collected from plants of similar age and variety growing at identical climatic and edaphic conditions from a place which is more than 30 km away from the cement plant. The size of the leaves as well as their location from the tip of the plant was kept constant throughout the collections.

Fixation was done in 3% glutaraldehyde in phosphate buffer of *p*H 7.0 at a temperature range of 0–5°C (Reed 1982), since the morphology of the epicuticular waxes was not altered in this fixative alone out of the many tried. Specimens, after wash, were coated to a thickness of approximately 200Å with gold for 2 min on a glow discharge coater which was built in the laboratory of Materials and Metallurgical Engineering of the University of Michigan, USA. Care was taken to avoid wax damage due to heating. Specimens were examined and photographed on JEOL-U3 (University of Michigan, Materials and Metallurgical Engineering Laboratory at Ann Arbor) AMR 1000 (North Carolina State University, Department of Botany) or Cambridge Stereoscan (University of Nebraska at Lincoln, Department of Botany) scanning electron microscopes.

Results

Normal Phylloplane

Wax deposits were present on both adaxial and abaxial phylloplanes in *Cocos* and *Borassus*, while in *Arachis* they were restricted to the adaxial side only. In *Arachis*, where the deposits are in the form of sparsely branched thin filaments (figure 1), the crystals were found on all epidermal cells excepting the trichomes (figure 2).

In *Borassus*, the deposits were either in the form of thin plates of varying diameter with irregular outline or as small-branched or unbranched filaments (figure 3). The subsidiary cells which were at a slightly elevated level in comparison to the sunken guard cells have characteristic wax deposits on their cell walls which are parallel to the long axis of the guard cells. These deposists are invariably in the form of either tubular or flat rods with recurved tips. The wax rods of one subsidiary cell interlaced with those of the subsidiary cell on the opposite side and formed a mesh or filter over the stomatal pore (figures 4 and 5). In certain subsidiary cells the wax crystals were partially developed (figures 6 and 7) without forming the mesh mentioned above. In some stomata broad plates of wax with ribbon-like 'branches' extended from one subsidiary cell to the opposite side covering the stomatal pore completely or partially (figure 8).

In *Cocos*, the wax deposits were in the form of very thin plates (figure 9).

Cement Dust-coated Phylloplane

In all the three plants, the wax deposits of cement dust-coated phylloplane easily flaked off from the cuticle and became misshapened; their smooth and shining texture was often lost. Invariably, these changes were followed by the slow disintegration of the wax crystals (figure 10). In *Cocos*, the thin plate-like deposits, which were parallel to the leaf surface, flaked off, became irregularly thalloid and erect (figures 11 and 12). In *Borassus*, the rod-like or plate-like wax crystals of the subsidiary cells completely broke down in a number of stomata and occluded the stomatal pore with their debris (figure 12).

Discussion

Although wax morphology in a plant is under genetic control (Lundquist & Von Wettstien 1962), the configuration, size and distribution of the crystalline waxes can be significantly modified by the prevailing environmental conditions (Baker 1982). An atmospheric pollutants are potential environmental agents which can modify and/or disintegrate the crystalline waxes of the phylloplane. The effect of pollutants on the epicuticular wax could be detected only from chemical analysis of the leachate of the phylloplane or through studies using transmission and scanning electron microscope (Saunders 1971). Thus, the previous studies on the histological responses of plants to atmospheric pollutants using conventional techniques were silent about this effect on the phylloplane. Acidic pollutants (acid rain, for example) had been reported to remove the epicuticular waxes fast (Lang et al. 1978, Evans 1979), but changes in crystalline epicuticular wax morphology were not known previously in cement-dusted plants.

Cement dust forms an alkaline solution (*p*H 10–12) in the presence of moisture (Saunders 1971) and can saponify the cuticle (Czaja 1962, Saunders 1971). When the alkaline solution dries, it forms a hard crust (Czaja 1962, Bohne 1963, Pathmanabhan et al. 1979, personal observations). The cuticle from such leaves invariably showed splits and tears followed by its stripping and rolling off (Lerman 1972, Lerman & Darley 1975). But how does the alkaline cement dust bring about a change in the morphology of the crystalline wax, as observed in our investigations, is not known clearly. A detailed biochemical analysis of the leachate of the phylloplane might throw much light on this.

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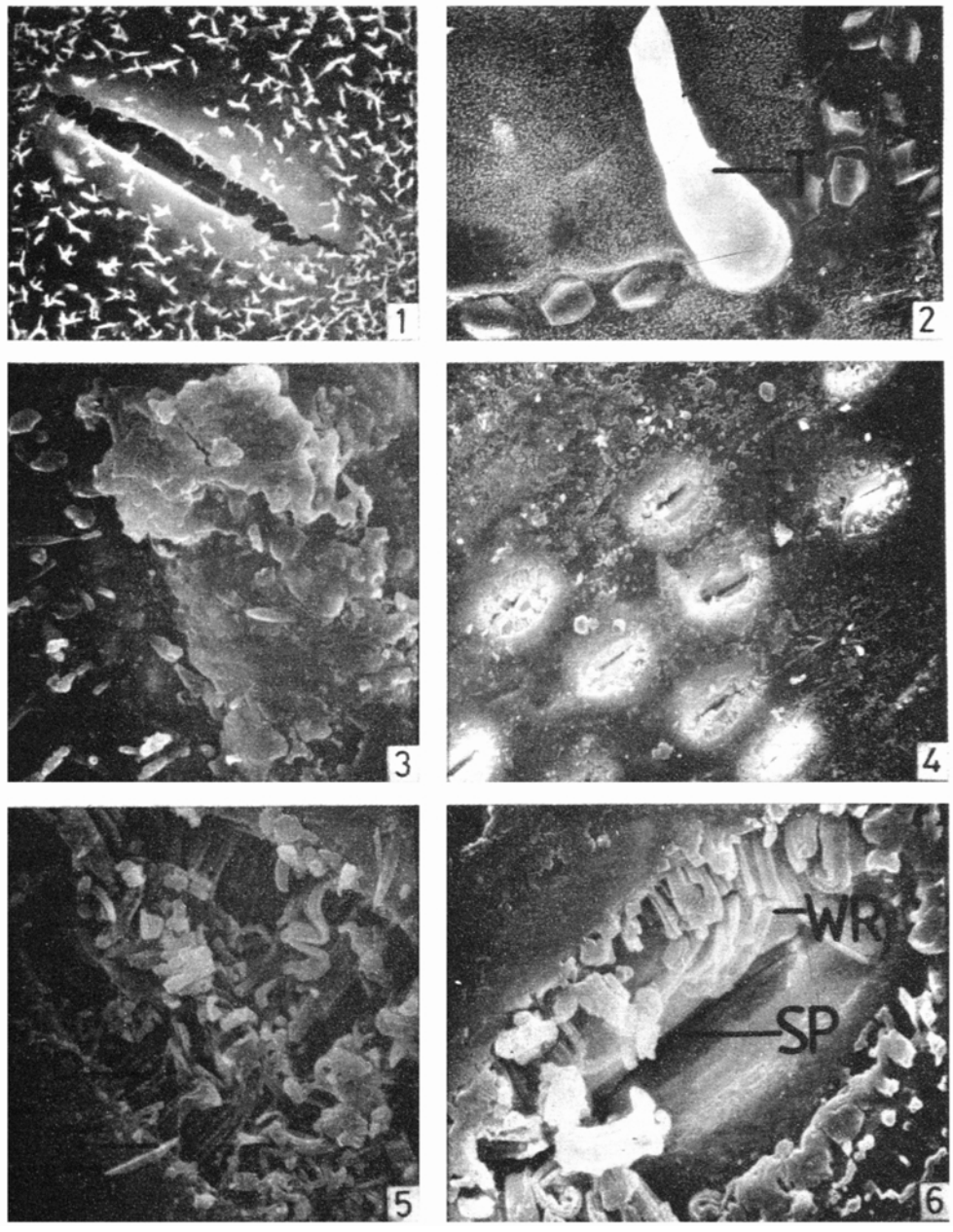
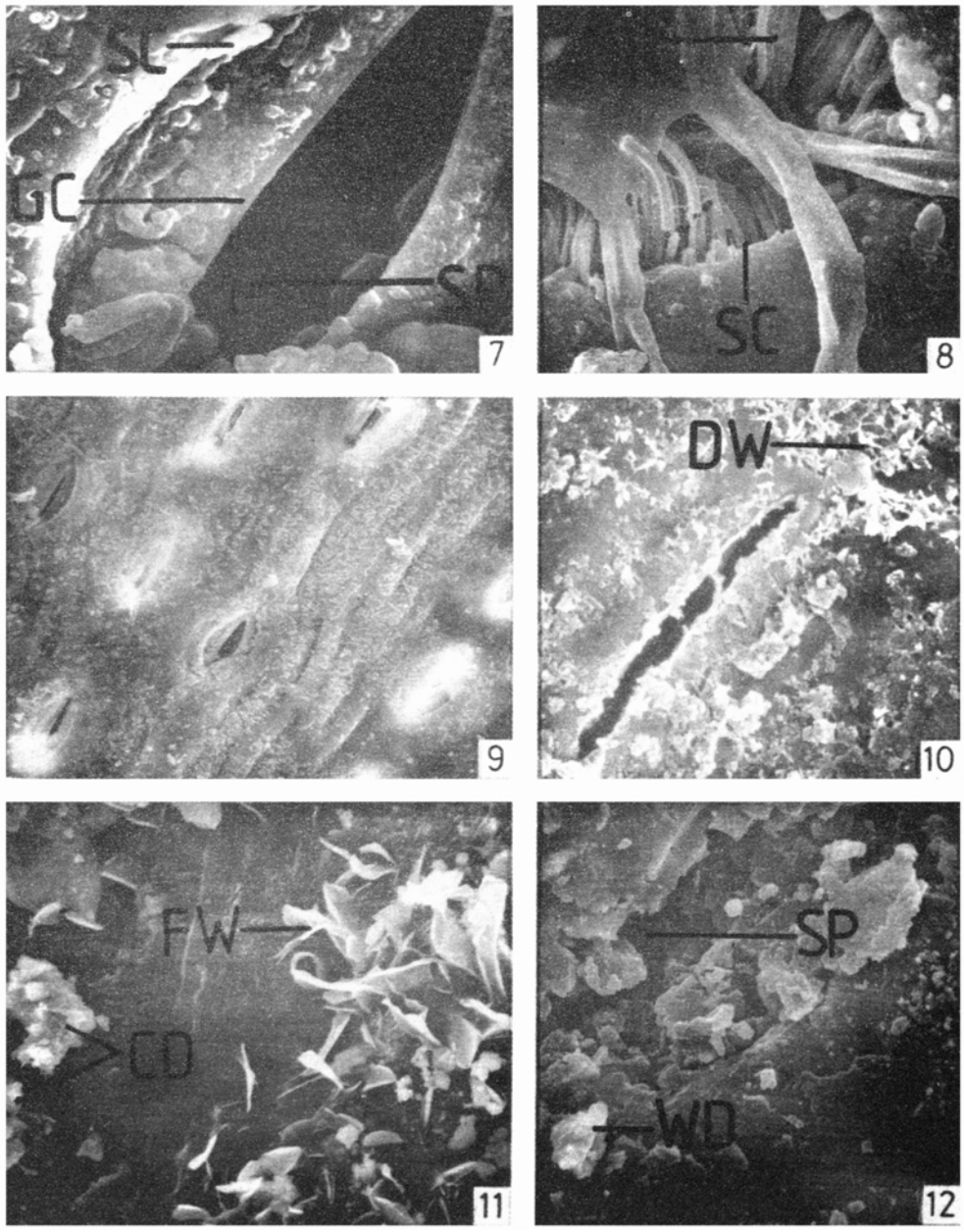


Figures 1–6. SEMs **1 & 2,** Portions of the adaxial phylloplane of the control leaf of *Arachis hypogaea* showing stellately branched wax filaments. Note their sparse distribution over the guard cells in figure 1 and their complete absence over a trichome (**T**) in figure 2 (figure 1 × 4000; figure 2 × 800.); **3,** Portion of the adaxial phylloplane of the control leaf of *Borassus flabellifer* showing large flaking plates and small filaments of wax deposits (× 3000); **4,** Portion of adaxial epidermis of control leaf of *B. flabellifer* showing stomata openings covered by the interlacing wax rods from the overarching margins of lateral subsidiary cells (× 300); **5,** Same enlarged to show a single stoma, (**WR,** Wax rods; **SC,** Subsidiary cell) (× 3000); **6,** Stoma from the same leaf epidermis showing partial covering of the stomatal pore (**SP**) by wax rods (**WR**) (× 3000)



Figures 7–12. 7, Another stoma from the same epidermis showing the absence of interlacing wax rods. (**GC**, Guard cell; **SC**, Subsidiary cell; **SP**, Stomatal pore) (×3000); **8,** Yet another stoma from same, covered by a broad plate of wax with ribbon-like branches (**RW**). (**SC**, Subsidiary cell) (×3000); **9,** Portion of the adaxial epidermis of control leaf of *Cocos nucifera* showing thin glistening wax flakes (×300); **10,** Portion of the adaxial phylloplane of the cement-dusted leaf of *Arachis hypogea*. Note that the wax deposits have easily flaked off and disintegrated (**DW**, Disintegrated wax deposits) (×4000); **11,** Portion of the adaxial phylloplane of cement-dusted *Cocos nucifera* showing irregularly thalloid and erect wax deposits formed after the flaking of the thin waxy layer; (**CD**, Cement dust; **FW**, Flaking wax deposits (×3000). **12,** Fully affected stoma of the dusted leaf of *Borassus flabellifer* showing the broken down interlacing deposits (seen in figure 5) whose debris (**WD**) fills the stomatal opening (**SP**) (×3000)

References

Baker E A 1982 Chemistry and morphology of plant epicuticular waxes; in *The Plant Cuticle* pp 139–165 eds D F Cutler, K L Alvin and C E Price (London: Academic Press)

Bohne H 1963 Schadlichkeit Von Staub ans Zementwerken fur Waldbestauda; *Aug. Forstz.* **18** 107–111

Czaja A T 1962 Uber das problem der zementstaubwirkungen auf Pflanzen; *Staub.* **22** 228–232

Evans L S 1979 Mechanisms of foliar injury; in *Proc. Int. Workshop Effects Acid Precipitn Vegetn Soils Terres. Ecosyst.* pp 11–16 eds L S Evans and R Hendrey (New York: Brookhaven National Laboratory, Upton)

Holloway P J 1971 The chemical and physical characteristics of leaf surfaces; in *Ecology of Leaf Surface Microorganisms* pp 39–53 eds T F Preece and C H Dickinson (New York: Academic Press)

Juniper B E and Jeffree C E 1983 *Plant Surfaces* (London: Edward Arnold)

Lang D S, Krupa S V and Shriner D S 1978 Injury to vegetation by sulfuric acid aerosols and acidic rain; *Ann. Meet. Air Pollut. Contr. Assocn*, Paper No. 78–7. 3. 71st USA

Lerman S L 1972 Cement-kiln dust and the bean plant (*Phaseolus vulgaris* L. Black valentine var.); in-depth investigations into plant morphology, physiology and pathology; Ph.D. dissertation, University of California, Riverside, USA (cited from Lerman & Darley 1975)

—— and Darley E F 1975 Particulates; in *Responses of Plants to Air Pollution* pp 141–158 eds J B Mudd and T T Kozlowski (New York: Academic Press)

Lundqvist U and Von Wettstein D 1962 Induction of *eceriferum* mutants in barley by ionising radiations and chemical mutagens; *Heriditas* **48** 342–362

Pathmanabhan G, Padmanabhan C and Oblisami G 1979 Effect of cement-kiln dust pollution on the weed flora; *Indian J. Air Pollut. Contr.* **2** 70–73

Reed D W 1982 Wax alteration and extraction during electron microscopy preparation of leaf cuticles; in *The Plant Cuticle* pp 181–195 eds D F Cutler, K L Alvin and C E Price (London: Academic Press)

Saunders P J W 1971 Modification of the leaf surface and its environment by pollution; in *Ecology of Leaf Surface Micro-organisms* pp 81–89 eds T F Preece and C H Dickinson (New York: Academic Press)