THE RETENTION OF AQUEOUS SUSPENSIONS ON LEAF SURFACES

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A method has been devised, using lycopodium, for studying the contribution of leaf surface characteristics to the wetting of leaves and the retention of particles applied as suspensions. Plants have been classified according to wettability, in relation to particle distribution and surface roughness. The recession of liquid on leaves of *Rumex obtusifolius* was studied in detail and the contributing factors analysed.

PREVIOUSLY, leaf surfaces have been classified as waxy (unwettable), or non-waxy (wettable) according to the behaviour of liquid, applied as agricultural sprays. No attempts at further classification have been made despite the considerable variation in surface characteristics of leaves. It is probable that the degree of wetting varies with the type of surface and it seemed desirable that a further classification of leaves should be attempted. Visual studies of spray particles on leaf surfaces have been made by Martin (1952, 1960) using replica techniques, but such methods suffer from the disadvantage that complete removal of particles by the replica is uncertain and the quality of replicas varies. Fluorescent tracer techniques have been used by Staniland (1960) but a range of fluorescent tracers is essential to give sufficient choice of chemicals which contrast with the natural fluorescent background.

A more suitable technique was sought, which could be used to study the detailed microscopic roughness of leaf surfaces and at the same time the deposition of standard particles. Lycopodium spores proved suitable as standard particles and were examined by direct microscopy, together with leaf surfaces, using illumination specially designed for opaque surfaces.

MATERIALS AND METHODS

Smooth Surfaces

To test the suitability of lycopodium, the deposition of spores from a suspension was first studied on glass slides in the presence and absence of a surface-active agent. Slides, previously cleaned with chromic acid, were stored in absolute ethanol and when required dried with hot air. Slides were dipped in 1 per cent aqueous suspensions of lycopodium alone or containing sodium lauryl sulphate 0·1, 0·01, 0·05 or 0·005 per cent. Slides were laid flat, allowed to dry under a bell jar, then solitary and clumped spores in ten fields were counted under the microscope. For all suspensions duplicate counts were made. Suspensions were shaken for 5 min. immediately before use. The suspension which showed maximum dispersion of spores was used for dipping tests with leaves.

Tests with Leaves of Rumex obtusifolius

Initially, dock leaves (*Rumex obtusifolius*) were dipped vertically either in aqueous suspensions of lycopodium 1 per cent alone or containing 0·01 per cent sodium lauryl sulphate. After withdrawal the leaves were laid flat lower surface downwards on a sheet of glass. The wettability of the upper surface was immediately noted visually and classified as: (i) unwettable, with no visible film of liquid; (ii) partially wettable, with a discontinuous film of liquid; (iii) completely wettable, with persistent film of liquid. As soon as the water had dried, the surfaces were examined microscopically for distribution of lycopodium, using 32 mm. and 16 mm. objectives, with a "Beck-Chapman Illuminator."

Leaves of different age, grown under glass, and leaves which had been washed with ether were also tested. The cuticle structure of untreated leaves was studied by direct microscopical examination using a 4 mm. objective and by means of cellulose acetate impressions (method of Martin, 1952). Transverse sections of leaves were treated with Sudan III and Ruthenium red solution as tests for cutin and mucilage respectively.

Tests with Leaves of Other Species

Leaves of some common weeds and plants of economic importance, were collected from the Chelsea Physic Garden and the Myddelton House Estate, Enfield, and the wettability of upper surfaces assessed, using aqueous suspensions of lycopodium alone. The surfactant was excluded from this general work as the chief object was to study the contribution of natural factors to particle retention. The cuticle structure was examined using the methods already described for *Rumex obtusifolius.* In addition the presence or absence of a waxy bloom on leaves was determined microscopically by examination before and after rubbing the surface with lens tissue. For hairy leaves, both surfaces were studied and the type and distribution of trichomes was determined from transverse sections and pieces of leaves cleared in chloral hydrate solution.

RESULTS

Experiments with Smooth Surfaces

With the preliminary experiments using smooth surfaces, and suspensions of lycopodium in water, 51 per cent of the lycopodium was found as solitary spores and the remainder in clumps. The concentration of sodium lauryl sulphate giving the maximum dispersion of spores was 0·01 per cent, this gave 90 per cent solitary spores. Higher concentrations were too frothy and produced an uneven distribution of spores on the slides.

Tests with Leaves of Rumex obtusifolius

Immediately after the withdrawal of leaves from suspensions of lycopodium in water complete coverage of the surface was seen. Subsequently recession of the liquid film occurred and was accompanied by drifting of the particles. The deposit viewed microscopically was found to be patchy. This also occurred in the presence of 0·01 per cent sodium lauryl sulphate but although patchy deposits were produced on drying, clumping of spores was considerably reduced. Similar results were obtained with leaves of different age; on both surfaces (differing in vein prominence); with ether dipped leaves and with leaves grown in a pollution free atmosphere. Aqueous 1 per cent suspensions of kaolin were similarly tried, but the distribution of particles was difficult to assess microscopically.

Recession of water on leaves did not occur after a single application of spray drops (50–**110**–170 μ diameter) when applied from a hand operated glass atomiser, but did occur after several applications of spray, when complete coverage with a film of water had been achieved. Drops of different size (230 μ to 2 mm. diameter, from a micrometer pipette) placed at different positions on the leaf, spread uniformly and recession did not occur, but drops (2 mm. diameter) allowed to fall on the surface from a height of 100 cm. spread on impact and recession quickly followed on both upper and lower surfaces.

Microscopical examination of leaves of different age, using cellulose acetate replicas and by direct observation showed that the interneural epidermal cells in addition to cells overlying veins are roughened due to ridges and projections, mucilage and cutin were not detectable in epidermal cells treated with staining reagents.

Tests with Leaves of Other Species

These results are summarised by classifying the surfaces according to wettability as already defined; in relation to the distribution of lycopodium spores and in relation to the chief cause of surface roughness. Upper surfaces only were studied except where it is mentioned otherwise. Common names of weeds are those given by Clapham, Tutin and Warburg (1958) and varieties of cultivated plants quoted if known.

1. *Unwettable*

Cuticle waxy (surface bloom removable by rubbing with tissue paper), spores not retained, e.g. cabbage *Brassica oleracea var capitata* (Fig. 1A and 1B), clover *Trifolium repens*, opium poppy *Papaver somniferum var album*, rose (the Doctor), sowthistle *Sonchus oleraceus*, strawberry (Royal Sovereign), lower surface.

2. *Partially Wettable*

(*a*) Cuticle granular (no surface bloom), patchy retention of spores, e.g. apple (Worcester Permain), cherry-laurel *Prunus lauroceracus*, rhododendron *Rhododendron ponticum*, rose (Peace), strawberry (Royal Sovereign).

(*b*) Cuticle ridged, patchy retention of spores, e.g. bindweed *Polygonum convolvulus*, bistort *Polygonum bistorta*, cleavers *Galium aparine*, dandelion *Taraxacum officinale*, dock *Rumex obtusifolius* (Fig. 1C), groundsel *Senecio vulgaris*, horse-chestnut *Aesculus hippocastanum* (Fig. 1D), redshank *Polygonum persicaria*, tomato (Sunrise).

(*c*) Abundant trichomes ("closed" pattern, see Fig. 2), good particle retention, e.g. apple (Worcester Permain), lower surface, blackberry *Rubus fruticosus*, lower surface, elecampane *Inula helenium*, lower surface, raspberry *Rubus idaeus*, lower surface (Fig. 2C), chrysanthemum (Brenda Talbot), lower surface (Fig. 2D).

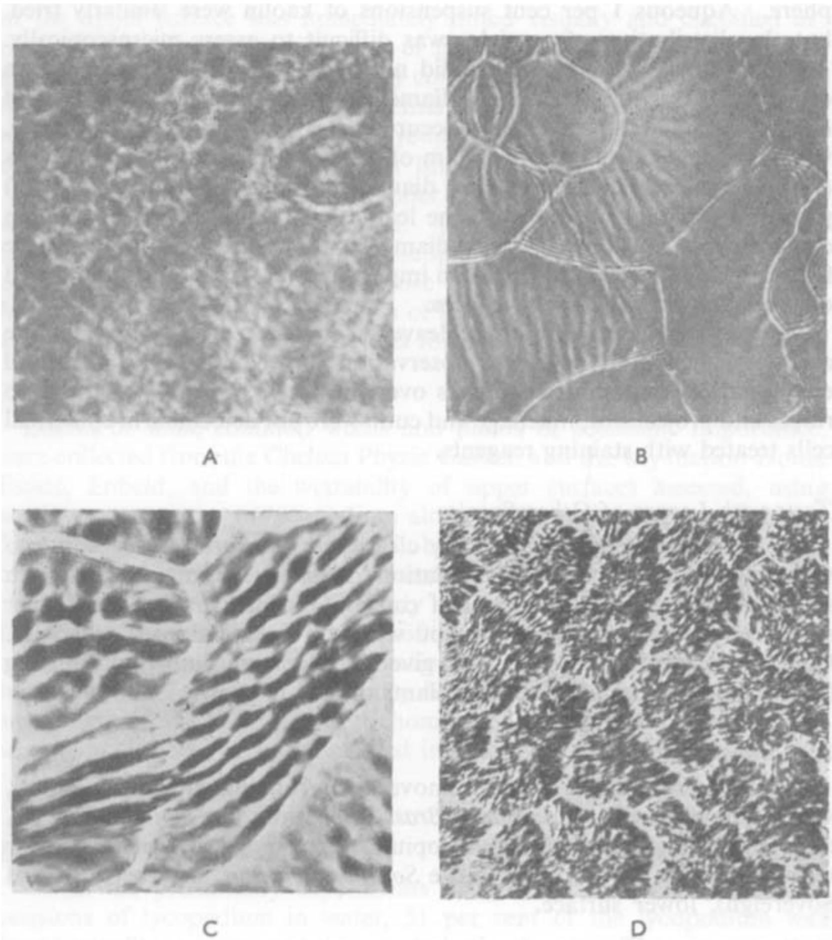


FIG. 1. Photomicrographs of replicas prepared from upper surfaces of leaves (all × 550).

A. cabbage (showing waxy "bloom"). B. cabbage (wax removed). C. dock. D. horse-chestnut.

3. *Completely Wettable*

(*a*) Cuticle smooth, good distribution of spores, e.g. chickweed *Stellaria media*, creeping butter-cup *Ranunculus repens*, dahlia (Zonnegoud), daisy *Bellis perennis*, fat hen *Chenopodium album*, french bean (Canadian Wonder), forget-me-not *Myosotis arvensis*, lucerne *Medicago sativa*, plantain *Plantago major*, raspberry *Rubus idaeus*, speedwell *Veronica officinalis*, tormentil *Potentilla erecta.*

(*b*) Abundant trichomes ("open" pattern, see Fig. 2), good spore retention, heavy deposit, e.g. foxglove *Digitalis purpurea*, lower surface, hoary cinqefoil *potentilla argentea*, lavender *Lavandula intermedia* (Fig.

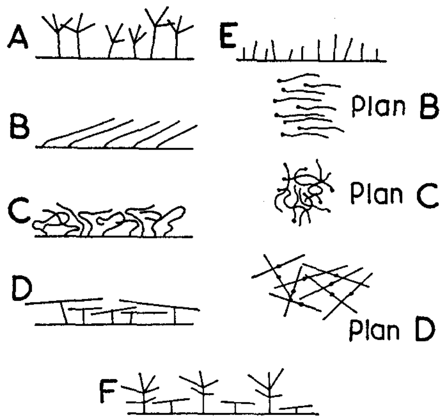


FIG. 2. Diagrams of trichome patterns, elevation and plan.
A. Lavender. B. Hoary cinqefoil. C. Raspberry. D. Chrysanthemum. E. Digitalis. F. Buddleia.

2A), lady's mantle *Alchemilla vulgaris*, mouse-ear chickweed *Cerastium vulgatum*, mullein *Verbascum thapsus*, primrose *Primula vulgaris*, lower surface, witchazel *Hamamelis virginiana*, lower surface.

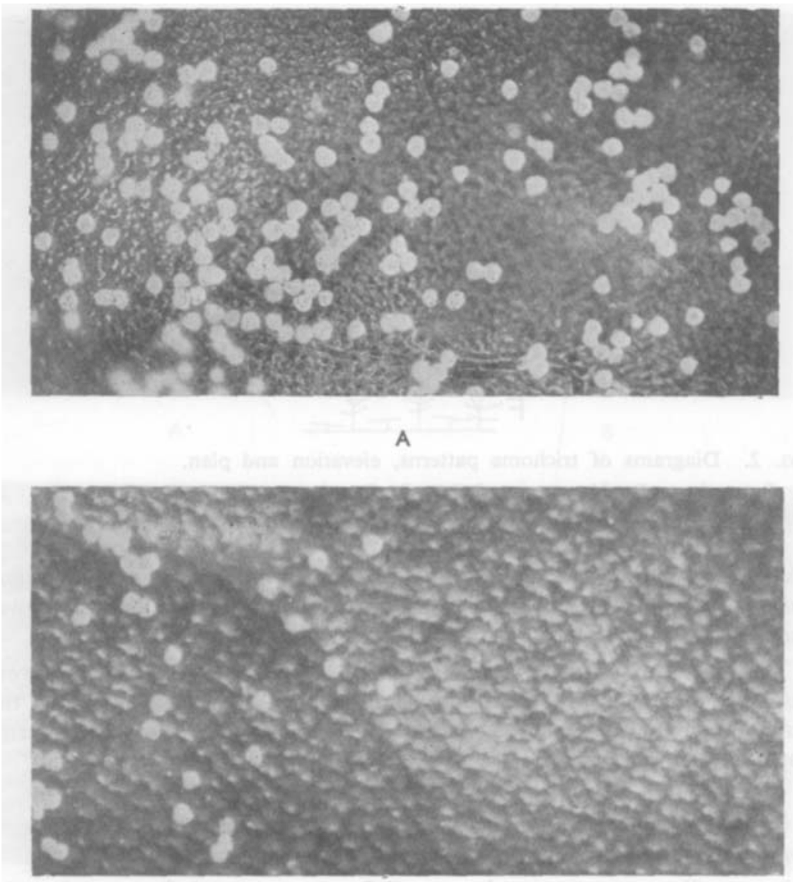
The leaves of buddleia *Buddleja davidii*, lower surface (Fig. 2F) were easier to wet than those of chrysanthemum (lower surface) and the arrangement of trichomes resembled the "open" also "closed" patterns, both "T" shaped and candelabra trichomes being present.

DISCUSSION

Preliminary Work

Lycopodium appears to be a satisfactory material for studying the influence of leaf surface roughness on the behaviour of particles when applied in the form of a suspension, as the spores are easily suspended in water and easily recognised by direct microscopical examination of the surface (Fig. 3A and 3B). The only fault is that clumping of spores occurs, but this difficulty can be overcome by the addition of sodium lauryl sulphate 0·01 per cent to the suspensions. From the tests with dock leaves, the distribution of spores does not differ from the picture obtained when no surface-active agent is incorporated provided that the concentration of 0·01 per cent is not exceeded. Although kaolin would be more appropriate for retention studies, since it is used as a pesticide diluent, lycopodium spores are more easily recognised on leaf surfaces than kaolin particles, and hence a more reliable assessment of distribution can be made.

Experiments with dock leaves show that liquid recession occurs on surfaces which are not contaminated by dirt or grease from the atmosphere and this phenomenon is not due either to the presence of extruded wax



B

FIG. 3. Photomicrographs of leaf upper surface (× 100), showing distribution of lycopodium.

A. French bean. B. Bindweed.

or to mucilage secreted from glandular trichomes. Although histochemical tests for cutin proved negative it cannot be assumed that the epidermal walls are entirely free from it as a thin deposit would not be detected because of the limited resolving power of the miscroscope. The presence of natural water repellant surface chemicals (even a monomolecular layer) could account for recession of liquid which would be enhanced by roughness in the form of projections (Fig. 1C). Tests made to compare the dipping technique with the effect of spraying show that recession only occurs when water is present on the surface as a film and that drops carefully applied at a position close to the surface remain stationary. It was also evident that drops of water applied some distance from the surface, on impact, spread to give a thin film, followed by recession. Recession does not appear to be related to vein prominence as the phenomenon occurs on both surfaces of leaves despite differences in macroscopical appearance.

Different Species

The value of a better classification of leaf surfaces in terms of wettability is borne out by the results obtained with different types of leaves. For instance the leaves of one variety of rose have no waxy bloom, are only partially wettable and particle retention is poor (cf. variety with waxy bloom, unwettable). It has been assumed by Juniper (1960), because of the surface characters of dock and horse-chestnut leaves, seen by electron microscopy, that there would be no difficulty in wetting these surfaces. The latter conclusions do not appear to be adequate as it is essential to differentiate between "spreading" and "wetting" especially when recession of the liquid occurs. The latter was demonstrated with tests on horse-chestnut and dock leaves, where wetting and particle distribution was poor, despite initial spreading, which was good. Juniper (1958) also states that the surface of dock leaf is smooth, but direct and replica investigation of the surface shows that it is ridged and also roughened by projections. This roughness could be overlooked when using the limited field of view of electron microscopy. Juniper (1959) further believes that the ultra microscopic roughness of the waxy extrusions of cabbage and other species largely determine wettability. The influence of waxy extrusions cannot be neglected but the present work shows that cuticle which is roughened by ridges is only partially wettable. It can be seen from (Fig. 1B) that the cuticle of cabbage leaf is ridged and consequently it is difficult to judge the contribution of either microscopic or ultra-microscopic roughness or both, to water repellency.

The differences in behaviour of water on hairy leaves of the "open" and "closed" pattern suggests that chemicals applied as solutions would be retained and distributed efficiently on the first type of surface but not on the second type (without a wetting agent being used). Leaves with trichomes exhibiting the "open" pattern illustrate a form of surface roughness, which actually enhances wetting, possibly due to the influence of capillarity. As the upper surface of chrysanthemum leaf is less hairy than the lower, better wetting can be achieved on the upper surface; there being less tendency for air pocket formation. Water repellency may therefore vary between varieties of chrysanthemum depending on the density of trichomes per unit area. With all but one class of leaf surface, spores were distributed according to the distribution of water. Where recession occurred spores were carried with the receding film, hence particles did not appear to be retained in preference to liquid or vice versa.

The exception to the above general conclusion arises in the case of suspensions applied to hairy leaves with a "closed" pattern of trichomes, where spores were retained despite difficulties in wetting the surface and particles were probably trapped by the trichomes. In addition to variations in roughness attributed to different trichome patterns and densities, surface roughness of individual trichomes often occurs, and together with natural water repellent chemicals could influence wetting of leaves.

REFERENCES

Clapham, A. R., Tutin, T. G., Warburg, E. F. (1958). *Flora of the British Isles*, Cambridge: University Press.
Martin, J. T. (1952). *Ann. Rep. Long Ashton Res. Sta.*, 71–74.
Martin, J. T. (1960). *Proc. 4th Int. Congr. Crop Prot.*, 1957, **2**, 1087–90.
Juniper, B. E. and Bradley, D. E. (1958). *J. Ultrastructure Res.*, **2**, 16–27.
Juniper, B. E. (1959). *Endeavour*, **18**, 20–25.
Staniland, L. N. (1960). *J. Agric. Eng. Res.*, **5**, 42–81.