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The effects of superficial wax on leaf wettability

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SUMMARY

Experiments are described which provide more information on the role played by superficial waxes in the natural water-repellency of leaf surfaces. Contact angles of water were measured on a variety of leaf surfaces, before and after removal of wax, and on smooth films of the isolated superficial waxes. The differences in wettability of leaf surfaces are not wholly accounted for by differences which occur in the chemical and hydrophobic properties of their superficial waxes.

Waxes isolated from leaves exhibiting contact angles less than 90° are usually more hydrophobic than the leaf surface itself. On most leaves exhibiting angles greater than 90° wax is the dominant factor governing water-repellency, the isolated wax normally making at least a 60% contribution to the contact angle measured on the leaf surface. Additional factors, such as roughness, responsible for the occurrence of contact angles greater than 110° on certain leaf surfaces, reside in the wax layer. The hydrophobic properties of some leaves are unaffected by chloroform washing, revealing that superficial waxes play little part in their wettability.

INTRODUCTION

The contact angles of water on leaves have been widely studied, since the magnitude of the angle determines the shape and behaviour of pesticidal spray droplets. Measurements have shown that the contact angle varies on leaves from different species, varieties of the same species, leaves of different ages of the same species and on upper and lower surfaces of the same leaf (Ebeling, 1939; Fogg, 1947; Linskens, 1950, 1952*a*; Challen, 1960; Wortmann, 1965; Hall, Matus, Lamberton & Barber, 1965; Hall, 1966; Troughton & Hall, 1967). The contact angle can also show a diurnal variation (Fogg, 1947; Linskens, 1952*b*) and be considerably altered by the addition of surface-active agents (Ebeling, 1939; Ford, Furmidge & Montagne, 1965). Contact-angle studies have also demonstrated the importance of wax in the water-repellency of some leaves. Fogg (1948) found that contact angles were considerably reduced after washing leaf surfaces with benzene. Similar effects are reported by Challen (1960) after ether washing and by Wortmann (1965) after chloroform washing. Damage to superficial wax layers on the leaf surface by wind (Hall & Donaldson, 1963), abrasion

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(Hall, 1966) and by certain herbicide treatments (Juniper, 1959; Wortmann, 1965; Gentner, 1966) produce marked effects on the contact angle.

This paper describes further contact-angle work in an attempt to deduce the separate contribution made by wax to the wettability of leaf surfaces. Such aspects have only been briefly discussed by Fogg (1948). The problem was studied on a wide variety of natural leaf surfaces which ranged from wettable to highly water-repellent. Contact-angle measurements were made on intact leaf surfaces, the surfaces after wax removal by chloroform and on smooth films of the isolated superficial waxes.

MATERIALS AND METHODS

The contact-angle apparatus and details of measurement are described in a previous paper (Holloway, 1969). Thin-layer chromatography was carried out using the methods of Holloway & Challen (1966).

Plant material. The leaves of thirty-eight species of wild and cultivated plants belonging to four monocotyledonous and twenty dicotyledonous families were obtained from plants grown in the field and in a greenhouse. Only glabrous leaves were chosen for study as trichomes can exert independent effects on leaf wettability (Challen, 1962).

Preparation of leaf surfaces for contact-angle measurements. Mature fully expanded leaves, either whole leaves or strips cut from larger leaves, were detached from living plants and measurements carried out immediately. Where possible major veins were excluded from the area of measurement. The leaf surfaces were attached to glass slides (with the required surface uppermost) by means of adhesive tape placed at opposite ends. Great care was exercised in the handling of leaves to avoid damage to the area selected for measurements. A mean equilibrium contact angle and its standard deviation were determined from a minimum of ten measurements on each of three or more independently prepared leaf surfaces.

Wax was removed from leaf surfaces by washing with three successive 5–10 ml portions of chloroform. The leaf surfaces were still attached to the slides during this procedure and the slides tilted to facilitate the drainage of solvent. Contact angles were redetermined as soon as the surface was free from solvent.

Extraction of superficial waxes. Upper and lower surfaces of undamaged leaves were separately washed with 5–10 ml of chloroform. The solvent was applied by means of a pipette, allowed to flow down the leaf surface held in a vertical position and drain from the leaf apex. The washings were combined, filtered and evaporated to dryness on a rotary evaporator under reduced pressure. The validity of a single rapid wash for superficial wax isolation is supported by other workers who have employed serial washing or immersion methods (Roberts, Martin & Peries, 1961; Purdy & Truter, 1963; Fernandes, Baker & Martin, 1964).

RESULTS

The wettability of the leaf surfaces is indicated in Table 1, where the measured contact angles on the intact leaf surfaces are recorded, together with the angle (expressed as a percentage reduction or increase) on the surfaces after chloroform washing. The contact angles of the isolated waxes are also listed.

Preliminary experiments established that the effects of solvent washing on the contact angle were independent of the solvent employed and the volume passed over the leaf surface. Waxes were isolated from both upper and lower surfaces of each leaf species, but only when significant differences between surfaces occurred are separate entries made in Table 1. Waxes were prepared for contact-angle measurement by fusion (Holloway, 1968), except the waxes from *Rhododendron ponticum*, *Eucalyptus botryoides*, *Epilobium montanum*, *Chamaenerion angustifolium*, *Syringa vulgaris* and *Pyrus malus* leaves, which were prepared from solutions in warm chloroform. The lower surfaces of *Pyrus malus* and *Laburnum anagyroides* leaves were not studied because they bore trichomes.

Contact angles on leaf surfaces

The contact angles on the leaf surfaces listed in Table 1 show a very wide variation. A complete range is encountered from the most wettable leaf studied, *Rumex obtusifolius* (contact angle 39–40°), to the least wettable *Eucalyptus* spp. (contact angles 160–170°). These are so hydrophobic that water droplets are accepted only with great difficulty. The standard deviations of the mean contact angles are large, reaching 5° on some leaf surfaces, but they are in agreement with the observations of Fogg (1947), Linskens (1952*a*) and Furmidge (1962).

The upper surface of most leaves is usually more hydrophobic, although some leaves show little difference between the two surfaces, e.g. *Triticum aestivum*, *Brassica oleracea;* and in a few, e.g. *Acer pseudoplatanus*, *Chamaenerion angustifolium*, the lower surface is more hydrophobic.

There was little indication of any relationship between wettability and botanical classification. Where varieties of the same species were compared (*Brassica*, *Pyrus*) or species of the same genus (*Eucalyptus*), similar contact-angle values were obtained, but the different genera of Graminae, Papilionaceae and Rosaceae showed no uniformity of results within the family.

The removal of superficial wax from leaf surfaces by chloroform washing usually alters the contact angle. Fogg (1948) has pointed out that leaves are killed by solvent treatment but that death does not appreciably change their surface properties. In Table 1 twenty-nine species could be broadly classified as having hydrophobic leaves, on which the contact angle (for one or both surfaces) was over 90°. In this group the removal of the wax by chloroform washing invariably left a surface on which the contact angle was substantially reduced, usually by 20–40%, i.e. wettability was increased. The largest reductions occurred when the intact leaf surfaces exhibited contact angles over 120°, e.g. *Clarkia elegans*, *Papaver somniferum*.

The leaves of the remaining species were more easily wettable, showing contact angles ranging from 39°–85°. In this group the effect of the chloroform washing was inconsistent. On approximately half the surfaces, removal of the wax *increased* the contact angle, i.e. made the leaf more difficult to wet. On the remaining leaf surfaces in this group, however, removal of the wax reduced the contact angle, as in the more hydrophobic species.

Table 1. *Contact angles of water on some leaf surfaces, the chloroform-washed leaf surfaces and on smooth films of their isolated waxes*

U = upper surface, L = lower surface. The standard deviations of each contact-angle determination are shown in parentheses.

Family	Species	Surface	Contact angle	% increase (+) or decrease (−) after chloroform washing	Contact angle of isolated wax
Ranunculaceae	*Aquilegia vulgaris*	U	158° (1.4)	−32.5	104°
		L	173° (1.7)	0	
Papaveraceae	*Papaver somniferum*	U	147° (1.1)	−37.3	104°
		L	159° (1.9)	−43.1	
Cruciferae	*Brassica oleracea* (var. Greyhound)	U, L	151° (1.0)	−40.2	105°
	Brassica oleracea (white broccoli)	U	137° (1.4)	−32.2	104°
		L	146° (1.5)	−39.6	
Caryophyllaceae	*Saponaria officinalis*	U	100° (0.6)	−34.4	100°
		L	106° (1.0)	−37.6	
Chenopodiaceae	*Atriplex patula*	U	146° (0.8)	−32.6	103°
		L	155° (0.8)	−31.8	
Tiliaceae	*Tilia europaea*	U	79° (2.4)	+ 3.0	95°
		L	80° (1.0)	+12.5	
Geraniaceae	*Tropaeolum majus*	U	160° (1.3)	−45.6	106°
		L	158° (1.8)	−33.2	
Aceraceae	*Acer pseudoplatanus*	U	45° (2.0)	+123.2	100°
		L	155° (1.9)	−16.1	103°
Aquifoliaceae	*Ilex aquifolium*	U	82° (1.1)	+14.6	98°
		L	85° (2.2)	0	
Papilionaceae	*Laburnum anagyroides*	U	151° (1.8)	−20.3	105°
	Phaseolus vulgaris	U, L	50° (5.6)	U + 4.1 L + 94.4	93°
	Pisum sativum	U, L	158° (1.2)	−24.0	106°
	Trifolium repens	U	159° (1.2)	−11.9	102°
		L	71° (1.0)	−10.9	
Rosaceae	*Prunus laurocerasus*	U	91° (1.4)	−55.7	104°
		L	94° (1.3)	0	
	Pyrus malus (var. Epicure)	U	71° (1.4)	+26.5	96°
	P. malus (var. Rival)	U	70° (0.9)	+28.2	97°
Hydrangeaceae	*Syringa vulgaris*	U	71° (2.3)	−27.3	103°
		L	95° (1.9)	0	
Myrtaceae	*Eucalyptus botryoides*	U, L	168° (2.1)	U − 8.3 L − 25.1	103°
	E. gunnii	U, L	153° (1.0)	−40.0	107°
	E. globulus	U, L	170° (2.0)	−45.6	105°
	E. glaucescens	U, L	165° (3.0)	−43.9	106°
Onagraceae	*Chamaenerion angustifolium*	U	110° (1.0)	−22.6	103°
		L	168° (2.2)	−15.8	
	Clarkia elegans	U	124° (4.2)	−22.4	103°
		L	159° (1.3)	−50.4	
	Epilobium montanum	U	105° (1.3)	−22.4	99°
		L	89° (1.4)	+25.5	

Table 1 (*cont.*)

Family	Species	Surface	Contact angle	% increase (+) or decrease (−) after chloroform washing	Contact angle of isolated wax
Euphorbiaceae	*Euphorbia peplus*	U, L	157° (1·5)	U −26·3 L −32·9	103°
Polygonaceae	*Polygonum persicaria*	U L	82° (0·6) 58° (1·2)	−24·7 +54·7	100°
	Rumex obtusifolius	U L	39° (2·0) 40° (2·1)	+50·0 −75·0	101°
Ericaceae	*Rhododendron ponticum*	U L	70° (1·4) 43° (2·5)	−17·0 +41·3	105°
Oleaceae	*Ligustrum vulgare*	U L	99° (1·4) 75° (2·0)	−27·3 0	103°
Plantaginaceae	*Plantago lanceolata*	U L	74° (1·5) 40° (4·0)	0 +65·8	102°
Compositae	*Aster novi-belgii*	U L	92° (0·2) 98° (0·5)	−45·5 −15·8	99°
	Senecio squalidus	U L	90° (1·0) 92° (1·1)	−12·8 −14·7	102°
Liliaceae	*Allium porrum*	U, L	138° (3·0)	−34·4	105°
Amaryllidaceae	*Narcissus pseudonarcissus*	U, L	143° (2·0)	−37·6	106°
Iridaceae	*Iris germanica*	U, L	146° (1·0)	−24·2	103°
Graminae	*Festuca pratensis*	U L	145° (3·0) 131° (4·5)	−21·0 − 6·6	102°
	Hordeum vulgare	U, L	165° (1·5)	− 8·6	105°
	Phleum pratense	U L	141° (1·1) 113° (2·1)	−26·8 + 8·7	102°
	Triticum aestivum	U, L	170° (2·0)	−16·8	106°

Contact angles on smooth films of isolated waxes

The contact angle on a smooth film of isolated superficial wax indicates the separate contribution made by the wax to the hydrophobic property of the leaf surface. The angles thus measured fell within the relatively narrow range of 92–107°. The standard deviations of the angles usually fell between 0·4 and 0·6 but never exceeded 1·6. Only on ten of the leaves was the contact angle of the isolated wax equal to the contact angle on the intact leaf surface. Usually the contact angle is less than that on the leaf surface but always accounts for at least 60% of its value. For some leaves the isolated wax gives a contact angle greater than that of the leaf. With *Rumex obtusifolius*, for example, the isolated wax (contact angle 100·5°) is more than twice as hydrophobic as the leaf surface (contact angle 39–40°). The waxes from upper and lower surfaces of leaves are similar in their hydrophobic properties, except in *Acer pseudoplatanus*, where a significant difference occurs.

Properties of the superficial waxes

The isolated waxes show a wide variety of melting points ranging from soft, amorphous waxes melting about 50° to hard, crystalline waxes melting above 200°. These differences are reflected in their qualitative composition revealed by thin-layer chromatography. Alkane, alkyl ester, secondary alcohol, primary alcohol, fatty acid and triterpenoid classes occurred most frequently. Alkanes were detected in all the waxes studied. Triterpenoids were detected in thirty-seven of the waxes and are the major constituents of the waxes of *Eucalyptus* species, *Epilobium montanum*, *Chamaenerion angustifolium*, *Pyrus malus* varieties, *Ligustrum vulgare* and *Syringa vulgaris*. These waxes have a high melting point. The most frequently encountered triterpenoid corresponded in R_F value with ursolic acid. However, Tschesche, Lampert & Snatzke (1961) report that the isomeric oleanolic and betulinic acids also have the same R_F value. There is no apparent correlation between the contact angle measured on a leaf surface and the chemical composition of its superficial wax. Triterpenoids, for example, are found in waxes from highly water-repellent leaves, such as *Eucalyptus globulus* (contact angle 170°), as well as more wettable leaves, such as *Syringa vulgaris* (contact angle 70–90°).

DISCUSSION

Although a standardized solvent washing procedure was used for superficial wax isolation, no attempt was made to make the process quantitative, i.e. expressing results in terms of μg wax per unit area of leaf surface. A representative sample was only required for determining its hydrophobic properties and qualitative chemical composition. The waxes isolated represent the wax dissolved in a short period from the leaf and therefore are not necessarily the total surface deposits. Fernandes (1965) has reported that although highly water-repellent leaves tend to carry larger amounts of wax than more wettable leaves, water-repellency cannot always be ascribed to excessive waxiness. The problems of wax contamination resulting from solvent isolation techniques are recognized and discussed by several workers. Most workers conclude that contamination by substances from within the leaf is easily avoided by the rapid immersion of leaves in solvents at room temperature or by running solvents over the leaf surface (Martin & Batt, 1958; Richmond & Martin, 1959; Roberts, Batt & Martin, 1959; Baker, Batt, Roberts & Martin, 1962; Purdy & Truter, 1963; Fernandes *et al.* 1964; Fernandes, 1965). The similar properties of waxes isolated from a number of leaf surfaces by different methods and solvents also suggest that contamination does not occur (Holloway, 1967). Little variation occurs either with position on the plant or with age (Holloway, 1967; Purdy & Truter, 1963).

Leaf surfaces may be roughly divided into two groups: those having contact angles below 90° and those where the angles are above 90°. The range of angles measured on leaf surfaces is very much wider than the range measured on smooth films of their isolated superficial waxes, therefore differences in the hydrophobic properties of superficial waxes, which in turn also reflect differences in chemical composition, cannot satisfactorily account for the considerable variations of leaf wettability.

Contact angles below 90° suggest that wax is not a prominent feature of the leaf surface. The wettability of a leaf surface devoid of wax will be governed by the available chemical groups exposed on the cuticle surface. The non-waxy components of the cuticle—cutin, pectin and cellulose—are more polar than waxes and consequently more hydrophilic. Bartell & Ray (1952) report cellulose as being completely wettable by water (contact angle 0°). Pectin is also likely to exhibit similar properties. The wettability of cutin has not been examined directly but has been suggested to be intermediate between wax and cellulose (Fogg, 1948). Van Overbeek (1956) and Hall & Donaldson (1963) both agree that cutin will be less hydrophobic than waxes because of the presence of polar groups capable of attracting water through hydrogen bonds. However, chloroform washing leaves of this category normally reduces the contact angle, showing that wax must play some part in wettability. The isolated waxes from these leaves are also more hydrophobic than the intact leaf surface. Such a situation could arise from a leaf surface incompletely covered with wax, allowing water droplets to come into contact with the more hydrophilic cuticle. This effect has been suggested by Hall & Donaldson (1963) and observed by Juniper (1959) on pea leaves following trichloroacetic acid treatment. Similar properties could also derive from a surface devoid of superficial wax but instead exposing some cuticular wax on the cuticle surface.

Increases of contact angle after chloroform washing are observed on a number of leaf surfaces with contact angles less than 90°. These increases are large in some cases, for example *Acer pseudoplatanus*, but the resultant angles are never greater than 100°. At first sight such behaviour seems to indicate that the surface is covered with a hydrophilic layer of material removed by chloroform to expose a more hydrophobic layer underneath. However, the chloroform extract is a normal wax which is considerably more hydrophobic than the intact leaf surface. A rational explanation of the phenomenon appears impossible. Similar effects have been recorded by Fogg (1948) on *Prunus laurocerasus* and *Nymphaea* leaves after benzene washing.

Contact angles above 90° recorded on leaf surfaces suggest that wax plays a significant part in the hydrophobic properties of the surface. Angles in the range 90–110° indicate that the surface is probably covered with a smooth layer of wax. Angles over 110° signify the presence of additional factors which are capable of modifying the hydrophobic properties of the leaf surface. It is generally agreed that the principal factor is roughness, which also provides a satisfactory mathematical explanation of the phenomenon. The largest contact-angle reductions after chloroform washing are encountered on these leaf surfaces, demonstrating the importance of the wax. Contact angles measured on smooth films of the isolated waxes can only account for about 60% of the angle measured on the leaf surface. These results also show that the additional factors, such as roughness, responsible for the large contact angles, must reside within the wax layer itself. Leaf surfaces in which a smooth film of the isolated wax accounts for the measured contact angle on the leaf surface are only occasionally observed, e.g. *Saponaria officinalis*.

The experiments also reveal that the cuticle surface free from superficial wax is very variable in wettability. Some leaf surfaces—for example, *Acer pseudoplatanus*, *Trifolium repens*—show little alteration of contact angle after chloroform washing. Water-repellency in such instances must also be a property of the cuticle itself, independent of superficial wax deposits. If the solvent-washed leaf surface is comprised solely of cutin, as suggested by Fogg (1948) and Hall & Donaldson (1963), then a reasonably constant contact angle would be anticipated. In the present investigation the contact angles on the chloroform-washed cuticle surfaces varied between 10° and 154°. Angles above 90° indicate that wax must still be involved at the cuticle surface and angles above 110° suggest that roughness factors must still be present. Support for the former hypothesis is given by the electron-microscope studies of Mazliak (1963) and Fernandes (1965). Chloroform-washing of apple fruits and leaves and *Exochordia* leaves revealed a layer of triterpenoid compounds remaining on the cuticle surface. The presence of such layers lying beneath the superficial wax would certainly account for some of the variations in the wettability of solvent-washed leaf surfaces.

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