EXTRACUTICULAR WAX AND CONTACT ANGLE MEASUREMENTS ON WHEAT (*TRITICUM VULGARE* L.)

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[*Manuscript received August* 29, 1966]

Summary

Extracuticular wax and contact angles on wheat were studied because of their influence on the retention of chemical sprays and on disease resistance. Wax formed extensive deposits on wheat, irrespective of variety, stage of growth, or part of the plant, and these deposits overlaid or projected from the cuticle as platelets and rodlets. Platelets covered the adaxial and abaxial leaf surfaces of seedlings and some mature plants, while a net of rodlets covered the ear, culm, sheath, and flag leaf abaxial surface. Rods were occasionally present on the abaxial surface of mature vegetative leaves. Wax influenced the advancing contact angle of water droplets on wheat. Contact angles were all high, i.e. greater than 130° and generally about 150°. The contact angle on the adaxial leaf surface was higher than on the abaxial leaf surface, except on glasshouse-grown reproductive plants, where there was no difference between the two sides. Seedlings had higher contact angles than mature plants, but there was no trend in contact angle with tissue age within a leaf or within a mature plant. The contact angle on the flag leaf of glasshouse-grown reproductive Aotea plants was 24° higher than on a similar plant grown in the field.

I. Introduction

Plant surfaces play a part in regulating plant–environment interaction. In particular, the retention, reaction, and penetration of chemical sprays or pathogens may be controlled by the chemical and physical characteristics of the plant surface (van Overbeek 1956; Crafts and Foy 1958; Currier and Dybing 1959). Wheat is an important economic crop and has associated with it extensive programs involving breeding for disease resistance and spraying against disease, weeds, or insects. In spite of this, there is little information on wheat surfaces and their interaction with liquids and pathogens, and this prompted the work described in this paper.

The wettability of a solid by a liquid is measured by the contact angle (Adam 1941; Broughton 1953) and by this measurement surfaces can be compared for their ability to shed liquids. Advancing contact angles of many plant surfaces are known (Fogg 1947; Bengtsson 1961) and the angles vary with plant species, leaf age, side of the leaf, position on the plant, time of the day, and leaf turgor (Fogg 1944, 1947, 1948). Contact angles on wheat leaves have been measured at 118–152° and 140–146° (Fogg 1944, 1947), $164 \pm 0 \cdot 7°$ (Bengtsson 1961), and $160 \pm 2°$ on the adaxial and abaxial leaf surfaces (Linskens 1950).

Plant species with high contact angles normally maintain a visible wax deposit on the cuticle. Cobb (1892, 1893, 1894) described a "bloom" (bluish coloration of plant surfaces) on stems, sheaths, and flag leaves of wheat, the bloom varying in intensity between varieties. Bloom is caused by light scattering from extracuticular

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wax, but wax may be present without bloom (Hall *et al.* 1965). It is necessary, therefore, to differentiate between the extent of wax development and the visual appearance of the surface. In our results, glaucousness (called "bloom" by Cobb) indicates the visual appearance of the surface, and wax refers to wax structure indicated by electron microscopy. This confusion of terms would perhaps explain Fogg's (1948) observation that although wheat had a fairly high contact angle he could not detect a visible wax deposit, under the conditions in which he viewed the leaf. As our results show, wax is always present on wheat, but is not always extensive enough for the surface to be called glaucous. The inheritance of a "waxless" (probably meaning non-glaucous) character in wheat has been shown to be due to a simple dominant gene (Chavan *et al.* 1955; Pool and Patterson 1958; Jensen and Driscoll 1963; Driscoll and Jensen 1964).

The variation in wax between plant surfaces can be due to plant species (Juniper and Bradley 1958; Juniper 1959; Hall *et al.* 1965), variety (Daly 1964; Hall *et al.* 1965), position on the plant (Juniper 1959; Hall and Donaldson 1963), and environment (Reipma 1956; Hull 1958; Juniper 1960; Daly 1964). It is also known that wax influences plant reaction to chemicals (Crafts and Foy 1958) and physiological processes (Hall and Jones 1961), and it has been suggested that wax may increase resistance to disease. Freeman (1961) suggested "bloom" prevented disease inoculum deposition, and Berry (1959) showed that resistance to mildew by onions would be lost if the surface could be made to retain moisture. The adaxial flag leaf surface of wheat is more susceptible to rust (*Puccinia recondita*) than the sheath, and Cobb (1892, 1893, 1894) suggested that this is because there is less wax on this surface. Rust spores which germinated on a waxy surface produced hyphae which passed over open stomata, and this observation prompted Cobb to suggest that wax formed a protective net over stomata. Again, Jensen and Driscoll (1963) have suggested, on the basis of general field observations, that "waxless" wheats appear to be more susceptible to leaf and stem diseases than waxy wheats.

II. Material and Methods

(*a*) *Plant Materials*

Vegetative wheat plants were grown in controlled cabinets, at a temperature of 15 ± 1°C, a relative humidity of 80 ± 5%, and lit by M.B.T.R. mercury tungsten lamps (200 W), giving 2000 f.c. of light at the top of the plants. Reproductive plants were raised in a glasshouse, which was not accurately controlled; temperature was between 18 and 24°C, and day length was extended to 16 hr by use of two mercury tungsten lamps. Crop Research Division, DSIR, Lincoln, supplied pure lines of seed of the following wheat varieties used in the experiment: Aotea, Arawa, Hilgendorf '47 and '61, Dreadnought, Mengavi, Fortunata, 70501, F.K.N. 25, Cross 7 '61, Winglen, Sherpa, Rushmore Suppressa, Frontana, Gamenya, Mida McMurachy Exchange, Gabo, C.I. 12633, and C.I. 12633 × 5Gb.

The cabinet-grown plants were raised in perlite which was replenished daily with Hoagland's solution. Seedlings were grown in 4-in. diameter plastic pots until they had produced three leaves, i.e. they were between stages 1 and 2 on the Feekes scale (Large 1954) while mature plants were grown for 12 weeks (to an advanced stage 5 on the Feekes scale) in 10-in. diameter pots. Glasshouse-grown reproductive plants were grown in a potting mixture and watered liberally three times per week. To control aphids, insecticide was sprayed onto all parts of the plant except the flag leaf, sheath, culm, and ear, which were required for the experiment.

(b) *Contact Angles*

Wettability of the plant surfaces is determined by measuring the two advancing contact angles of each water droplet at the leaf–air–water junction with a micro-projector. A completely wettable surface has contact angles of zero and an unwettable surface has theoretical values of 180°, however, the practical unwettable limit lies at approximately 160°. Light was directed along the leaf ridges of excised plant tissue on which a water droplet had been placed by using a micropipette. The profile produced was magnified and projected onto a ground-glass screen where the contact angle was measured within 15 sec of the drop being placed. All measurements were made with relatively constant water droplet and air temperatures, humidity, and droplet size (2 mm diameter); distilled water was always used. These precautions eliminated variations of contact angle with changes of temperature and humidity at different times of day, observed by Fogg 1947. Three plants of each variety were used for the measurements.

(i) *Leaves.*—Contact angles were measured on three position, viz. the tip, middle, and base on both sides of the leaves (for results see Tables 1 and 2). Three leaves of seedlings were used and a leaf from the top, middle, and base position of vegetative plants gave a sequence of leaf ages. Only the flag leaf of reproductive plants was measured.

(ii) *Sheaths.*—It was difficult to place droplets on sheaths because the excised tissue curled on cutting and water ran off the curved surface. Due to the curved surface the contact angles measured on the sheath are not comparable with those measured on the leaf tissue kept horizontal. Contact angles were measured on three sheaths in an age sequence on mature plants. Sheaths of all glasshouse-grown wheat would not retain the water droplets and so contact angles could not be measured.

(iii) *Culm.*—The curved culm surface prevented comparison of contact angles on the culm with the leaves. When excised, the culm maintained a rigid shape which allowed one in about five droplets to be retained. Contact angles were also measured on the internode region of the stem between two sheaths.

(iv) *Field-grown Wheat.*—Contact angles were measured on both sides of 40 flag leaves of Aotea wheat grown in the field at Lincoln, New Zealand. Prior to measurement the plants had been exposed to both wind and rain, as the region is prone to an adverse environment.

(c) *Leaf Widths*

Leaf width and the number of ridges on the adaxial leaf surface of vegetative plants were also recorded. Three leaves of each variety were used and three positions within each leaf were measured.

(d) *Visual Observations of Surface Wax*

Plants kept in the vegetative state in the growth cabinets were non-glaucous. Glasshouse-grown plants, however, were glaucous, and to record differences between plant organs and wheat varieties, visual estimates of glaucousness were made. An arbitrary scale of 1–5 was used based on glaucousness, and all tissues were classified on the basis of this scale. Visual observations of glaucousness are inadequate to describe the presence or degree of wax, but used with electron-microscopic examination and contact angle measurements they can elucidate important changes in wax structure occurring in the plant. Weekly observations were made on the sheath, flag leaf, culm, and ear of all varieties from prior to ear emergence until the grain was ripe.

(e) *Electron Microscopy of Surface Wax*

We examined, with the electron microscope, replicas of the adaxial and abaxial surfaces of the first three leaves of seedling Cross 7 '61 and Dreadnought plants and 11 vegetative wheat varieties (Aotea, Gabo, Cross 7 '61, Gamenya, Mengavi, 70501, Fortunata, Sherpa, Winglen, Hilgendorf '61, and Arawa), and sheath, flag leaf, culm, and floret samples of glasshouse-grown 70501, Cross 7 '61, and Aotea. Two representative samples were taken from each surface to be examined. Surfaces were prepared for electron microscopy following methods described by Hall and Donaldson (1963). Carbon was applied under vacuum to surfaces preshadowed with gold–palladium, and the composite specimen was backed with 2% collodion and allowed to dry. The replica was stripped from the plant material, mounted on a grid, and examined with a Philips EM100B electron microscope, after washing in solvents to remove collodion and wax.

III. Results

(a) *Contact Angles of Cabinet-grown Seedling Wheat Plants*

Analysis of variance was performed on the contact angle measurements of the wheat varieties, grouped according to the amount of variation. Analysis of variance established a significant difference between the sides of the leaf and between varieties on the abaxial surface, but failed to show any significant trend with leaf age, either between the first three leaves or within any leaf on either side of the leaf. Each value in Table 1 is an average of 54 readings (obtained from the results of three plants, three positions within a leaf, and from three leaves within the plant), because contact angles within the plant were not significantly different. Duncan's test was only carried out on the contact angle measurements on the abaxial surface, to establish significant differences between varieties. In Duncan's test, capital letters (A, B, C, D, etc.) indicate significance at the 1% level while small letters (a, b, c, d, etc.) indicate significance at the 5% level.

Each wheat variety had a significantly higher contact angle on the adaxial than the abaxial leaf surface (Table 1). The greatest difference was 21° on Winglen while Fortunata with 12° had the least difference. Contact angles on the adaxial surface of all varieties were similar while there was a 14° degree range on the abaxial surface. Only three varieties were outside a narrow range of contact angles (148–154°)

on the abaxial surface. Two varieties, Gabo and Winglen, were of particular interest because of their low contact angles, and Fortunata was of interest because of its high contact angle.

(*b*) *Contact Angles of Cabinet-grown Mature Wheat Plants*

Analysis of variance was performed on the contact angle measurements from a restricted number of wheat varieties. There was considerable variation in contact angles within Gabo, Hilgendorf, Frontana, and Arawa, but in contrast Sherpa was

Table 1

CONTACT ANGLES OF WATER ON ADAXIAL AND ABAXIAL LEAF SURFACES OF WHEAT SEEDLINGS GROWN IN CABINETS

Wheat Variety	Contact Angle (deg)		Duncan's Test* for the Abaxial Leaf Surface	
	Adaxial Surface	Abaxial Surface	5% Level	1% Level
Fortunata	168	156	a	A
Cross 7 '61	169	154	b	AB
Gamenya	168	153	bc	AB
Aotea	168	153	bcd	B
C.I. 12633	167	152	bcde	BCD
Rushmore Suppressa	168	151	bcdef	BCD
Arawa	169	151	bcdefg	BCD
Sherpa	169	151	bcdefg	BCD
Dreadnought	169	151	cedfg	BCD
Frontana	170	151	defgh	BCD
Hilgendorf '47	166	151	efgh	BCD
Mengavi	168	150	efgh	BCD
Hilgendorf '61	168	149	fgh	CD
Mida McMurachy Exchange	169	149	gh	CD
70501	169	148	h	D
C.I. 12633 × 5Gb	169	148	h	D
Winglen	165	144	i	E
Gabo	168	142	i	E

* Varieties without a common letter are significantly different at the significance level shown.

very uniform. Although there were trends in age of the leaf and sheath there was no trend in contact angle with tissue age. It was established that the contact angle was significantly higher on the adaxial than the abaxial leaf surface of each variety. Each value in Table 2 is the average of 54 readings of the contact angle.

Adaxial leaf surfaces supported water droplets with high contact angles and only 10° was the greatest difference between varieties. The abaxial surfaces (Table 2) had a range of 22° in contact angle: from Gabo with 130° to Hilgendorf '47 with 146° and to Dreadnought with 152°. Duncan's test established that two major groups of varieties existed—one with contact angles above 145° on the abaxial surface and one below 141°. Gabo with a contact angle of 130° on the abaxial leaf surface was significantly lower than all other varieties.

The value of the contact angle on the sheath of different wheat varieties ranged from 138 to 159°, with an average value of 147°. Sheath values are not directly comparable between varieties or with leaf data, as already noted.

The number of ridges per unit of leaf width is included in Table 2. The adaxial surface was ridged while the abaxial was not and it is possible that the difference in contact angle between the surfaces is due to the ridging. The distance apart of the

Table 2

CONTACT ANGLE MEASUREMENTS ON LEAVES AND SHEATHS OF MATURE VEGETATIVE WHEAT PLANTS AND A MEASURE OF ADAXIAL LEAF RIDGES

Plants grown in cabinets

Wheat Variety	Leaf Ridges (No./mm)	Contact Angle (deg)			Duncan's* Test (abaxial surface only)	
		Adaxial Surface	Abaxial Surface	Sheath	5% Level	1% Level
Hilgendorf '47	3·0	162	152	147	ab	A
Dreadnought	3·0	161	152	144	a	A
Mengavi	3·0	161	152	144	a	A
Fortunata	3·3	160	148	151	abc	A
Aotea	3·2	157	147	146	bc	AB
70501	3·5	160	146	148	cd	AB
Hilgendorf '61	3·0	160	146	148	cd	AB
F.K.N. 25	3·1	159	146	158		
Arawa	3·1	153	145	144	cd	AB
Cross 7 '61	3·8	159	141	140	de	BC
Winglen	2·6	160	140	142	e	BC
Rushmore Suppressa	3·5	158	140	141		
Mida McMurachy Exchange	3·4	161	139	138		
Sherpa	3·2	160	138	138	e	C
Frontana	3·6	158	137	153	e	C
C.I. 12633 × 5Gb	3·5	155	136	159		
Gabo	3·0	152	130	146	f	D

* Varieties without a common letter are significantly different at the significance level shown.

ridges will influence the contact angle, and it was thought that the difference in contact angle between varieties in our experiment may be due to the ridge spacing. However, there was no obvious relationship between the contact angle and the number of ridges per unit width of the adaxial surface.

(c) *Contact Angles of Glasshouse-grown Reproductive Wheat Plants*

Water droplets could not be deposited on the sheaths because of the waxy surface and the tendency for the sheath to curl when cut. Excised culms, however, maintained a rigid shape and contact angles could be measured, although with difficulty due to the hydrophobic, curved surface.

The results (Table 3) indicate that the glasshouse-grown reproductive wheat plants were extremely water-repellant. Irrespective of side of the leaf, or the position on the culm or sheath, the contact angle was over 148°. There was variation along the culm, the contact angle decreasing from just beneath the ear down to the sheath, which may be due to the length of time the culm had been emerged from the sheath. The reproductive, glasshouse-grown wheat plants had similar contact angles on both sides of the flag leaf which contrasts with the results from vegetative plants.

Table 3

CONTACT ANGLE MEASUREMENTS ON THE FLAG LEAF, STEM, AND INTERNODE REGION OF GLASSHOUSE-GROWN REPRODUCTIVE WHEAT PLANTS

Wheat Variety	Contact Angle (deg)*				
	Adaxial Surface of Flag Leaf	Abaxial Surface of Flag Leaf	Lower Stem	Upper Stem	Internode Region
Gamenya	158	161	160+	160+	160+
Hilgendorf '61	159	160	158†	160+	160+
Cross 7 '61	156	160	157†	160+	157
Arawa	160	161	160†	160	160+
Mida McMurachy Exchange	154	155	152	160+	158
Rushmore Suppressa	154	158	153	160†	‡
Aotea	155	159	160+	160+	‡
Hilgendorf '47	155	157	160+	160+	159
Gabo	154	158	155	160+	153
C.I. 12633 × 5Gb	160	161	155	160	154
Dreadnought	161	161	160+	160+	160+
60501	159	160	160	161	158
Fortunata	158	155	148	160+	‡
Frontana	159	156	151	152	149
C.I. 12633	158	156	152†	160+	‡
Winglen	158	158	160+	160	155
Sherpa	157	157	160+	160	160+
F.K.N. 25	159	155	154	160	153

* 160+ indicates that only occasional droplets would remain on the leaf area.

† These readings are the average of two plants and not three as are all other readings.

‡ No region visible.

(d) *Contact Angles of Field-grown Reproductive Plants*

The contact angles on the adaxial and abaxial flag leaf of field-grown Aotea wheat were $132 \pm 4 \cdot 7$ and $135 \pm 2 \cdot 4$ degrees, respectively. Each result is the mean of 40 readings and standard deviations are also given. There was no significant difference between these values.

(e) *Visual Observations of Glaucousness*

(i) *Cabinet-grown Vegetative Plants*

The vegetative seedling and mature plants grown in the cabinet were non-glaucous.

(ii) *Glasshouse-grown Reproductive Plants*

Two weeks prior to ear emergence the sheath of some varieties became glaucous but by the time the ear was fully emerged the plants were extensively glaucous. Degree of glaucousness differed with variety, part of the plant, and stage of growth.

(1) *Flag Leaf Sheath.*—Flag leaf sheath surfaces of all wheat varieties were conspicuously glaucous. Glaucousness increased with sheath age but by ear emergence maximum intensity had been attained.

(2) *Adaxial Surface of the Flag Leaf.*—The adaxial surface was not as glaucous as the sheath. Sherpa and 70501 had the greatest development while Dreadnought, Aotea, Gamenya, Mengavi, and Winglen were slightly glaucous. All other varieties were non-glaucous.

(3) *Abaxial Surface of the Flag Leaf.*—All wheat varieties had glaucous abaxial leaf surfaces. The glaucousness of the sheath continued onto the base of the abaxial leaf surface but decreased in intensity towards the tip, which was sometimes non-glaucous. Sherpa, 70501, Gamenya, Dreadnought, and Arawa were the most glaucous varieties. The abaxial was more glaucous than the adaxial leaf surface of all wheat varieties.

(4) *Culm.*—Observations made on the culm from ear emergence till culm extension was completed indicated that glaucousness differed with variety and position on the culm. Culms of Fortunata, Aotea, Hilgendorf '47 and '61, Cross 7 '61, Arawa, and Rushmore Suppressa were less glaucous then Winglen, Mengavi, Dreadnought, and Sherpa. Glaucousness varied along the culm, particularly on 70501, C.I. 12633 $\times$ 5Gb, and Arawa, which were glaucous on the culm below the ear but non-glaucous where the culm emerges from the flag leaf sheath. Other varieties non-glaucous at the culm base were Aotea, Hilgendorf '61 and '47, Mida McMurachy Exchange, Rushmore Suppressa, and Fortunata.

(5) *Internode Region.*—Some wheat varieties exhibited internode elongation to the extent of exposing internode tissue between the base of the flag leaf sheath and the top of the sheath of the second youngest leaf. Gamenya, Sherpa, 70501, and Dreadnought were glaucous in the internode region while Gabo, Hilgendorf '47 and '61 were semi-glaucous. Four varieties, Mida McMurachy Exchange, Rushmore Suppressa, Fortunata, and C.I. 12633 did not expose this portion of the internode under the conditions of this experiment.

(6) *Ear.*—The ears of all wheat varieties were glaucous. At emergence, the ears were non-glaucous and did not become glaucous until about 2 weeks later.

(*f*) *Electron Microscopy*

Wax, not visible to the naked eye, was revealed in electron-microscopic studies of carbon replicas of wheat plant surfaces, and the form of wax was platelet or rodlet, depending on the position on the plant. Platelets gave the appearance of finger-like lobes of wax, distributed at random over the cuticle. Rodlets occurred with platelets but it is not known if they arose at the same time as platelets or from them. Amount and form of wax depended on wheat variety, age of the leaf, position on the plant, and stage of growth (Table 4).

(i) *Cabinet-grown Vegetative Plants*

(1) *Adaxial Leaf Surfaces.*—Wax platelets of uniform density extensively covered the adaxial leaf surface of seedling (Plate 1, Fig. 1) and mature vegetative plants (Plate 1, Fig. 2). The platelets on some wheat varieties had lobes or finger-like protrusions and an example of this can be seen in the inset of Plate 2, Figure 1. All wheat varieties exhibited extensive platelet development (Table 4) though Gabo (Plate 2, Fig. 1) and Mengavi were less extensively covered than the other varieties (compare Plate 1, Fig. 2, and Plate 2, Fig. 1). Wax became more extensive on adaxial leaf surfaces as leaves aged. A succession of leaves of different ages was obtained by examining three leaves of Cross 7 '61 and Dreadnought seedlings when the youngest leaf was fully expanded and comparing seedling and mature plant surfaces of Cross 7 '61. Wax was well formed even on the youngest leaves but there was an increase in platelets from the youngest to the third leaf of seedlings and even greater wax development between seedlings and mature plants (Plate 1, Figs. 1 and 2).

(2) *Abaxial Leaf Surfaces.*—The form and extent of wax on abaxial leaf surfaces were more variable than on adaxial surfaces. Platelets with occasional rods were found on most wheat varieties but Sherpa and Hilgendorf '61 had platelets without rods on the specimens examined (Table 4). The platelets were regular in outline (Plate 2, Fig. 2) which distinguished them from the finger-like structures on the adaxial surface. Only Winglen had platelets that were different in appearance, as in Plate 2, Figure 3. Extent of wax deposition depended on leaf age, stage of growth, and wheat variety. Wax increased with leaf age in seedlings and the abaxial surfaces of mature leaves of Cross 7 '61 were more evenly and extensively covered in wax platelets than the seedlings. Platelets were most extensive on Mengavi, less extensive on Fortunata, 70601, Aotea, Arawa, and Hilgendorf '61, and least on Gabo, Cross 7 '61, Sherpa, and Winglen.

(3) *Sheath.*—Extensive deposits of wax on wheat sheaths were of a rod form on Gabo, Fortunata, Mengavi, Sherpa, and Winglen (Table 4; Plate 3, Figs. 1 and 2) and platelets with occasional rods on Cross 7 '61 and Gamenya. Rods were extensively developed as a mesh particularly on Gabo, Fortunata, and Mengavi, although Fortunata had a greater accumulation of rods than Mengavi (Plate 3, Fig. 1, *v.* Plate 3, Fig. 2).

(ii) *Glasshouse-grown Reproductive Plants*

(1) *Adaxial Surface of the Flag Leaf.*—The flag leaf produced by plants in the reproductive stage was densely covered in platelets on the varieties examined. The platelets were comparable with those produced on the adaxial surface of vegetative plants.

(2) *Abaxial Surface of the Flag Leaf.*—Wax on the abaxial surface of the flag leaf differed markedly from vegetative plants. Extensive rod production produced a mesh of wax which almost concealed the underlying cuticle surface. Platelets and rodlets occurred on Cross 7 '61 on an area which was chosen because it was non-glaucous.

(3) *Flag Leaf Sheath.*—Glasshouse-grown flag leaf sheaths of Aotea and 70501 were densely covered with wax rods (Plate 3, Fig. 3).

TABLE 4

WAX FORMS* AND CONTACT ANGLES ON WHEAT SEEDLINGS AND MATURE WHEAT PLANTS

Wheat Variety	Adaxial Surface of Leaf		Abaxial Surface of Leaf		Sheath	
	Wax Form	Contact Angle (deg)	Wax Form	Contact Angle (deg)	Wax Form	Contact Angle (deg)
			Cabinet-grown Vegetative Plants			
Seedlings						
Cross 7 '61	Extensive platelets (cf. Plate 1, Fig. 1)	169	Moderately extensive platelets	154	—	—
Dreadnought	Extensive platelets	169	Moderately extensive platelets	151	—	—
Mature plants						
Cross 7 '61	Extensive platelets	159	Moderately extensive flat platelets, with occasional rods	141	Moderately extensive platelets with bare patches, few rods	140
Gabo	Extensive platelets (cf. Plate 2, Fig. 1)	152	Occasional platelets, short rods, bare patches	130	Extensive rods	146
Gamenya	Extensive platelets	—	Moderately extensive platelets, short rods	—	Platelets, bare patches, few rods	—
Fortunata	Extensive platelets	160	Moderately extensive platelets, short rods	148	Extensive rods (cf. Plate 2, Fig. 1)	151
Mengavi	Extensive platelets	152	Extensive platelets, some short rods	150	Extensive rods (cf. Plate 3, Fig. 2)	148
Sherpa	Extensive platelets (cf. Plate 1, Fig. 2)	160	Moderately extensive platelets, bare areas	138	Moderately extensive short rods	138
Winglen	Extensive platelets	160	Moderately extensive platelets, rounded or flake-like; rods in some areas (cf. Plate 2, Fig. 3)	140	Moderately extensive short rods	142
70501	Extensive platelets	160	Moderately extensive platelets, with occasional rods (cf. Plate 2, Fig. 2)	146		
Aotea	Extensive platelets	157	Moderately extensive platelets, with occasional rods	147		
Hilgendorf '61	Extensive platelets	160	Moderately extensive platelets			
Arawa	Extensive platelets (cf. Plate 1, Fig. 2, inset)	153	Moderately extensive platelets, with occasional short rods	145		

TABLE 4 (*Continued*)

Wheat Variety	Adaxial Surface of Leaf		Abaxial Surface of Leaf		Sheath	
	Wax Form	Contact Angle (deg)	Wax Form	Contact Angle (deg)	Wax Form	Contact Angle (deg)
			Flag Leaf of Glasshouse-grown Reproductive Plants			
Aotea	Extensive platelets	159	Extensive rods	159	Extensive rods	160+
70501	Extensive platelets	159	Extensive rods	160	Extensive rods	160+
Cross 7 '61	Extensive platelets and rods	156	Platelets and rods	160	—	

* The classification of wax into different forms is based on the observations made during the experiment, and therefore only refers to wax structures produced under our experimental conditions.

(4) *Culm*.—An extensive mesh of interlocking wax rodlets characterized culms of Aotea, Gamenya, and 70501 (Plate 4, Fig. 1). Extent of rodlet development varied with the position on the culm; close to the sheath rods were sparse (Plate 4, Fig. 2) while towards the ear there was a mass of rods (Plate 4, Fig. 3). The cuticle was covered by plates of wax as well as supporting wax rods.

(5) *Ear*.—The outer cuticle of the lemma from Winglen was characterized by wax rods similar to wax development on the culm (Plate 3, Fig. 4).

IV. Discussion

There are several implications of our observations of wax on wheat and the associated contact angles. It is well known that differences in wettability of plants provide the basis for selective control by non-specific poisons (e.g. sulphuric acid) of weeds in a wheat crop but before the plant surfaces will retain or take up sprays it is necessary to overcome their hydrophobic nature. This is done by reducing the size of the droplets so that they are small enough to make partial contact with the cuticle between wax exudates or by using wetting agents or adhesives (Fogg 1944; Brunskill 1956). The main cause of the high contact angles in wheat is extracuticular wax, and knowing this will be of value when sprays are being formulated to aid their adhesion or penetration. Wax influences the rate of water loss from plants (Hall and Jones 1961) so that in wheat, encouraging wax production or preventing removal of wax would be an advantage in conserving moisture, although conversely, wax will be a disadvantage when moisture needs to be lost quickly as from the wheat grain at harvest (Pool and Patterson 1958). Martin (1964) raises the possibility of wax influencing the resistance of plants to invasion from pathogens, and the role of wax on wheat in disease resistance requires further investigation. Gabo and Winglen had the lowest contact angles we recorded, and both these varieties are known to be susceptible to leaf rust in the field. Finally, as already established, glaucousness indicates a waxy surface, but a non-glaucous appearance does not necessarily mean a non-waxy surface. Genetical studies on wax inheritance in wheat requires electron-microscopic examination of the surface or chemical analysis to determine wax, rather than relying on visual observations of bloom.

Wax was prominent on all wheat surfaces we examined, irrespective of variety, plant age, position on the plant, stage of growth, and the environment. The form of wax was independent of wheat variety but varied between platelets and rodlets depending on the stage of growth and the environment. There was marked variation in the form of wax even on the same position on the plant depending on the stage of growth. On the abaxial leaf surface of seedlings, platelets were present, on mature vegetative plants, platelets and occasional rodlets, and on the abaxial leaf surface of glasshouse-grown plants, a mesh of rodlets. By contrast the adaxial leaf surface supported platelets irrespective of the stage of growth or whether the plants were grown in the cabinets or in the glasshouse. The difference in the form of wax between glasshouse- and cabinet-grown plants may have been due to differences in light level, temperature, or stage of growth, i.e. vegetative or reproductive state. We have been able to show (unpublished data) that the form of wax differs between equivalent positions on vegetative and reproductive plants grown under the same controlled-environment condition. Day length was extended from 9 to 24 hr for the reproductive plants by using a mercury tungsten lamp which gave a light level of about 80 f.c. at the level of the plants. Rodlets covered the abaxial flag leaf surface and the sheath in reproductive plants, while platelets occupied the equivalent position on vegetative plants.

Glaucousness of wheat in our experiment was associated with extensive development of randomly orientated extracuticular wax rods, which only occurred on some regions of glasshouse-grown reproductive plants. Wax was still present on apparently non-glaucous or slightly glaucous surfaces. The degree of glaucousness even varied within a plant organ, e.g. on the culm most of the surface was covered by a mesh of wax rods and was glaucous, but close to the sheath wax rods were sparse and platelets occurred, which gave the culm a non-glaucous appearance. These results support the work of Hall *et al.* (1965) which established that non-glaucous surfaces may be waxy and that glaucousness is due to the light-scattering properties of certain wax shapes on the plant surface.

We were surprised by the high contact angle (about 165°) on the adaxial leaf surface particularly on the seedlings, but our results are comparable with those of Bengtsson (1961) who measured an angle of 164° on wheat and 169° on peas, and may be explained by the combined presence of wax, and the ridges which are only on the adaxial surface. The main contributor to the high contact angles we measured would be wax, through its extensive development, surface properties, and physical form. The hydrophobic wax platelets or rods repel water drops and project sufficiently from the cuticle to prevent contact between the water drop and the polar cutin of the cuticle. A view of wax platelets projecting from the cuticle is shown in a transverse section of a leaf in Plate 5, Figure 1. The electron micrograph could only be taken because of a fold in the replica. Removal of wax reduces the contact angle (Fogg 1948; Hall and Donaldson 1963) which was evident in our results from field-grown plants [Plate 5, Fig. 3; Section III(*d*)] and would perhaps explain the value of 118° recorded by Fogg (1947) on field-grown wheat. These results are in contrast to cabinet-grown material, although the influence of the extent of wax development on the contact angle is still evident, as shown by Sherpa (Plate 1, Fig. 2) with a contact angle on the adaxial leaf surface of 160° compared with 152° on Gabo (Plate 2, Fig. 1). The physical form of the waxy surface is likely to exert the greatest influence on the contact angle. The highest known angle on a smooth surface is 120° on a methacrylic polymer (Bernett and Zisman 1962) but only 110° on paraffin (Dettre and Johnson 1963), a substance more closely related to plant wax. Apparent or observed contact angles on rough surfaces are greater than on smooth surfaces provided the angle exceeds 90° (Adam 1941) and a rough paraffin surface can have a contact angle of 158° (Dettre and Johnson 1963). Rough leaf surfaces have higher contact angles than smooth leaf surfaces and the angles increase with increasing roughness if that of the smooth leaf surface is above 100° (Ebeling 1963). Even changes in surface configuration produced by the wilting of leaves increases the angle (Fogg 1944, 1947).

Higher contact angles on the adaxial than the abaxial leaf surface of vegetative plants may be explained by plant cuticle structure as well as the extent of wax.

Ridges running from leaf tip to base were present on the adaxial but not the abaxial surface. We initially thought that perhaps the differences of the contact angle on the adaxial leaf surface between the wheat varieties was due to the number of ridges per unit per leaf width, but this was not so. Furthermore, the effect of ridges in the cuticle can be overcome by the form of wax, because on the flag leaf we could not detect differences in contact angle between the adaxial and abaxial leaf surface in spite of the differences in ridging. Both sides of the flag leaf were extensively covered in wax, but the form of wax was platelets on the adaxial and rodlets on the abaxial surface. We do not know to what extent the contact angle we measured on the flag leaf was influenced by the ridging, by the form of the wax, or even perhaps directly by its chemical composition, as little is known of the hydrophilic properties of wax from the different varieties. It has been well established that the wax forms (platelets, rodlets, etc.) have considerable influence on the wetting properties of leaves, and recent work (Hall *et al.* 1965) has shown that the chemical composition of the wax is probably one of the factors that determines their shape.

Hall and Probine (unpublished data) have shown in a study which combines electron microscopy with X-ray diffraction that the form of the wax is related to its crystal structure and chemical composition. For example, they have shown that wax on the adaxial surface of the reproductive flag leaf of Cross 7 '61 wheat, which is normally in the form of platelets, has a different crystal structure from wax on the abaxial surface which is normally in the form of rodlets. Likewise wax on the sheath of reproductive plants (normally rodlets) has a different crystal structure from wax on the sheath of vegetative plants (normally platelets). These observations suggest that the biosynthetic processes on the adaxial and abaxial leaf surface of reproductive plants differ in detail, as do the biosynthetic processes in the sheath of vegetative and reproductive plants. Baker *et al.* (1963) have shown in bananas that the wax was predominantly of a paraffin composition on the adaxial leaf surface and of an ester composition on the abaxial surface. Kolattukudy (1965) has suggested different biosynthetic pathways for different wax components in *Brassica oleracea*.

The contact angles on seedling wheat plants in our experiment were about 10° higher than on mature vegetative plants. From electron micrographs of the leaves we would have expected the contact angles on mature plants to be higher than on seedlings because of the more extensive wax development. We cannot explain our results and can only suggest that there were changes in ridging or the underlying cuticle structure with age, or that the freshly deposited wax protruded from the cuticle to a greater extent than older deposits, or that the longer period of life of the older leaves resulted in greater contamination of the surface.

We were particularly interested during this study to define areas of significantly lower contact angle which would possibly be important in explaining differences between varieties of wheat in their reaction to sprays or pathogens. Vegetative plants of all varieties grown in cabinets had lower contact angles on the abaxial leaf surfaces. Field-grown wheat with intact leaf wax should have the same characteristics but we were unable to verify this as our field studies were limited to crops which were exposed to wind and had lost some of their wax. Where wetting agents are used with sprays on undamaged field-grown wheat, spray will be retained by the adaxial surface and where the leaves are curled (and this is probably most marked in young plants) the spray will be retained more readily by the abaxial surface due to its greater wettability.

Measurements of contact angles of water on the abaxial leaf surfaces of different varieties of wheat grown in plant cabinets (in the vegetative stage) differed by as much as 22° and suggest that this could be of importance in determining the degree of retention of liquids by different varieties. Another site of variation was on the culm near the sheath, where wax production and contact angle were less than elsewhere on the culm. Entry into the plant of pathogens or chemicals, if influenced by the wettability of the stem, would find this region most vulnerable, particularly as it is associated with the ligule. At the junction of the lamina and sheath, a thin-walled parenchyma ligule encloses the emerging stem and acts as a cup, holding liquids which would otherwise have run off the plant, although at the same time it may prevent entry of liquid onto the inner surface of the sheath.

In this study we have been concerned with understanding wax structure and distribution and contact angles of different wheat varieties under controlled conditions. This method enabled us to obtain valid comparisons between varieties, although it means our results refer to the specific conditions under which the plants were grown. It would be possible to extrapolate our results on wax and contact angles to field-grown wheat if prediction of wax on field plants was possible, but it is not, because quantitative information on the relation between wax and the environment is lacking. Light level, nutrients, soil moisture level, temperature, and day length influence wax production, and abrasion (Dewey, Gregory, and Pfeiffer 1956; Bengtsson 1961) or chemicals (Dewey, Hartley, and Maclaughlan 1962) or microorganisms (Linskens, Heinen, and Stoffers 1965) remove wax. Preliminary field results on Aotea wheat [Section III(*d*)] indicate that growing the plants in the field may reduce the contact angle by 24° from glasshouse-grown plants, which was similar to the reduction in contact angle from 150 to 130° caused by wind damage in white clover (Hall and Donaldson 1963). Electron micrographs of chemically sprayed wheat (Plate 5, Fig. 2) and an abraded surface damaged in the field (Plate 5, Fig. 3) illustrate how abrasion or chemicals can reduce wax on wheat and therefore the contact angle. Even a layer of dust on the wax surface will reduce the angle. Wheat plants in the field would be more difficult to wet than is indicated by the contact angles because of the vertical orientation of the leaves and stems. We found it difficult to position water drops on the rounded sheath or culm and, in particular, on the sheath which curled after being cut off the plant, but even with leaf tissue, particularly at high contact angles, a measurement could only be made by maintaining the leaf in a horizontal position.

Further work, which is necessary, is to establish the relationship between disease resistance and the contact angle, firstly under controlled conditions and then on wheat plants in the field. An understanding of this relationship will also require a knowledge of a reaction of pathogens on different plant surfaces.

V. Acknowledgments

The authors appreciate and acknowledge the technical assistance of Miss M. Packwood and Mr. L. M. Adamson, both of the Physics and Engineering Laboratory, DSIR. They wish to thank the Crop Research Division, DSIR, for supplying seed, and Mr. T. Robertson, Department of Agriculture, Wellington, for statistical analysis of the results.

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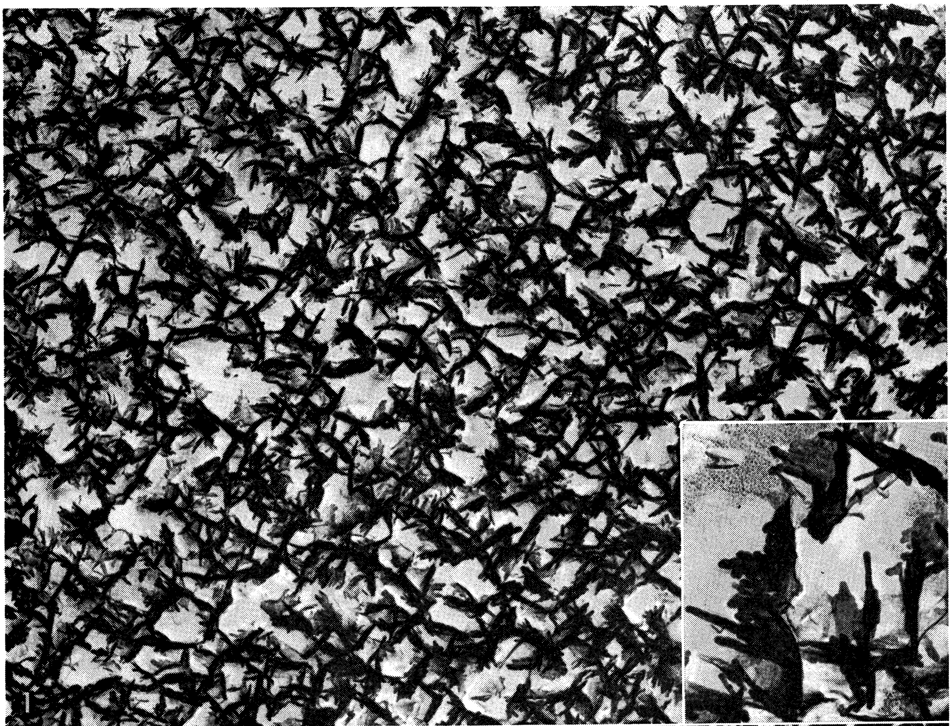

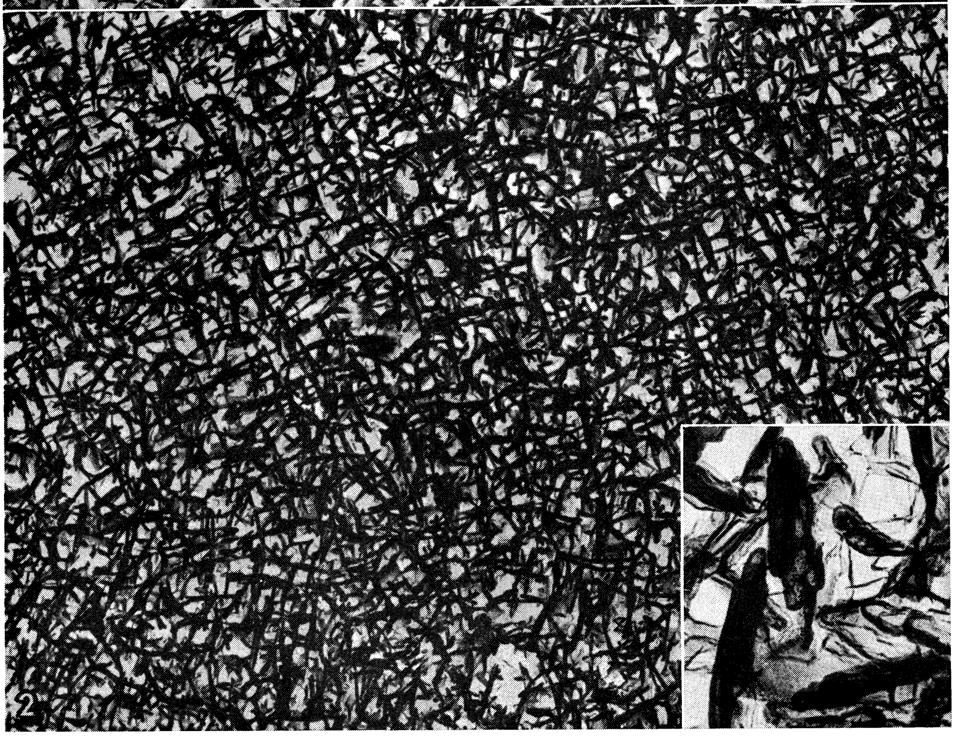

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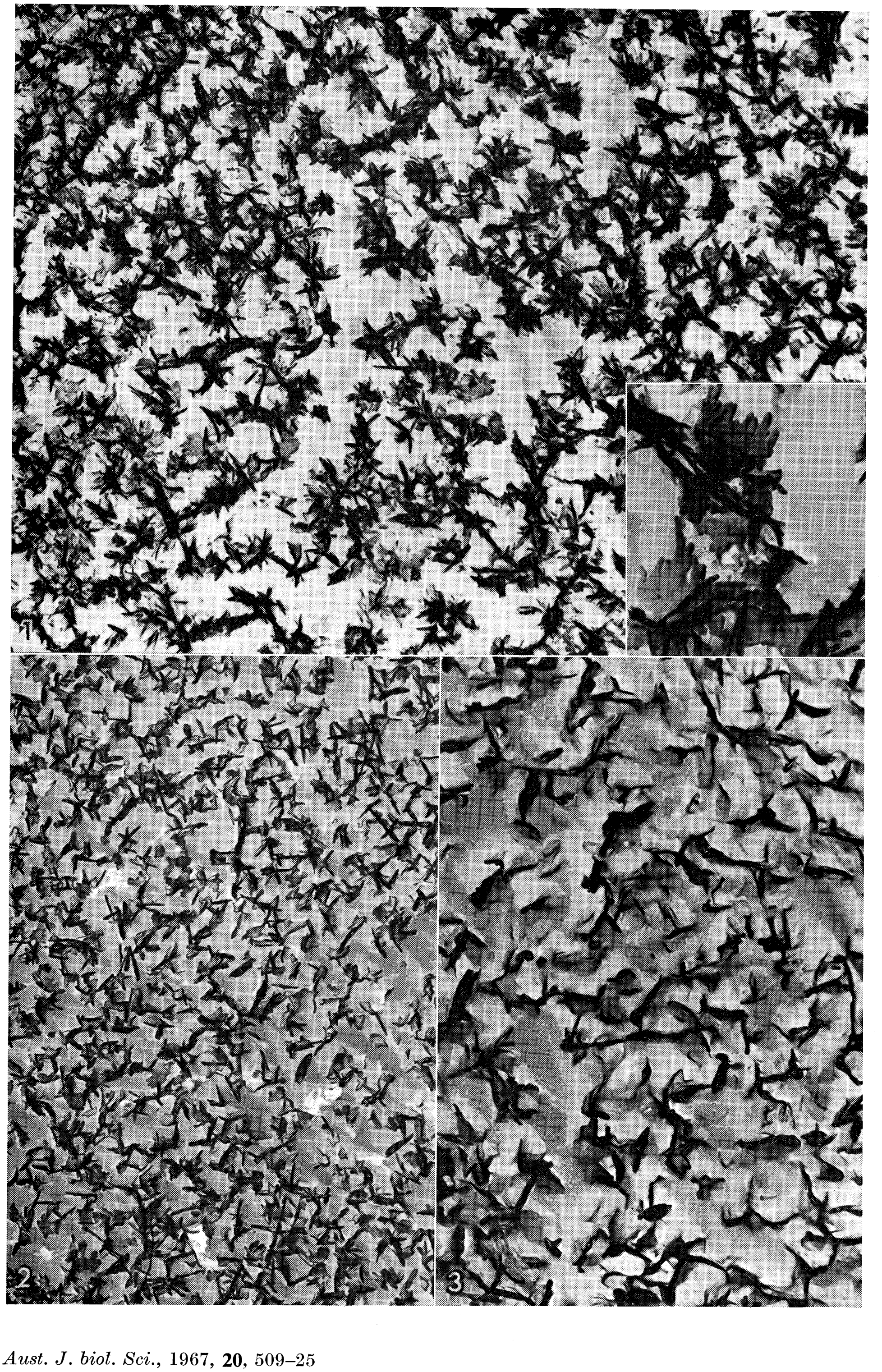

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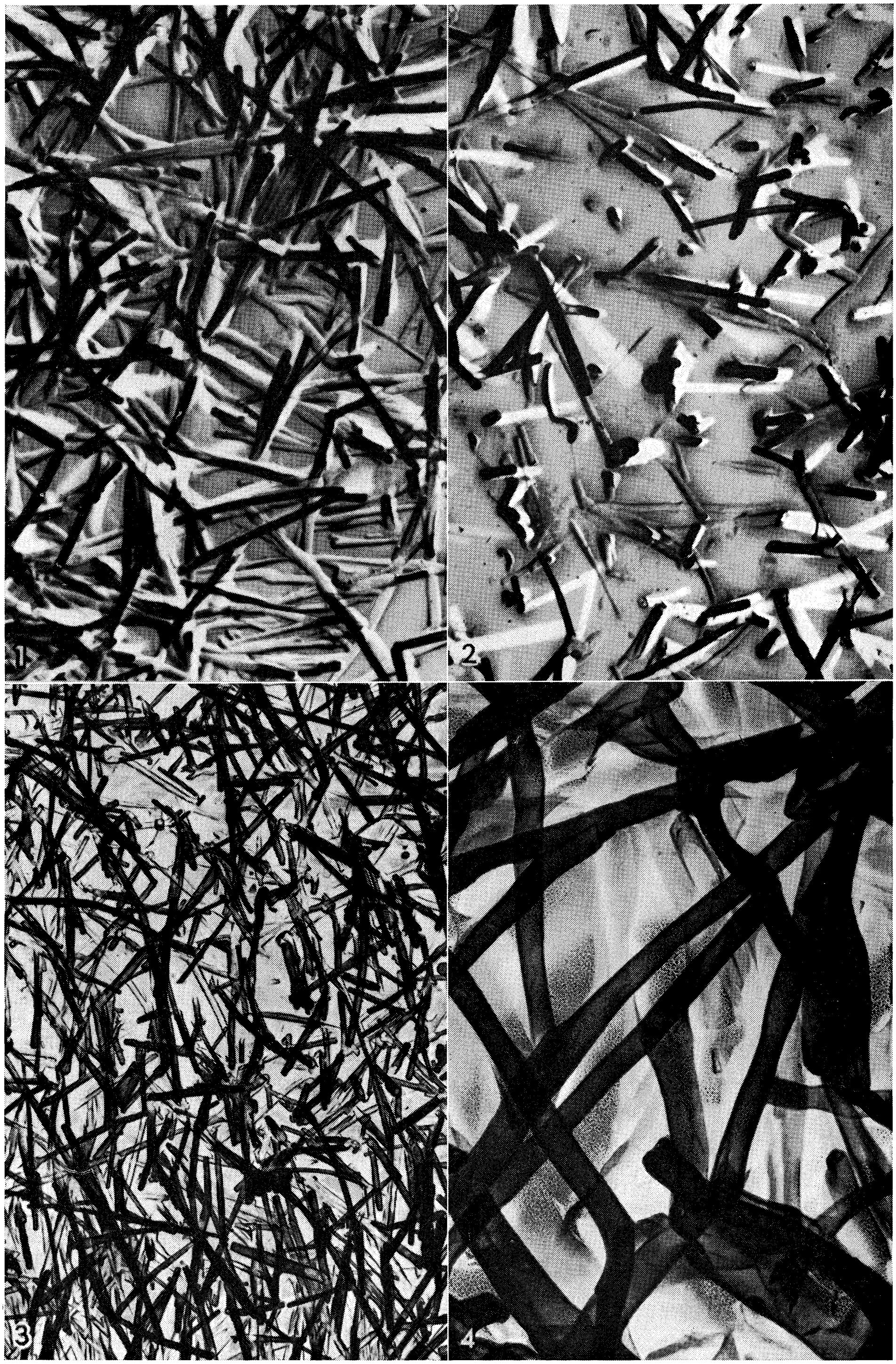

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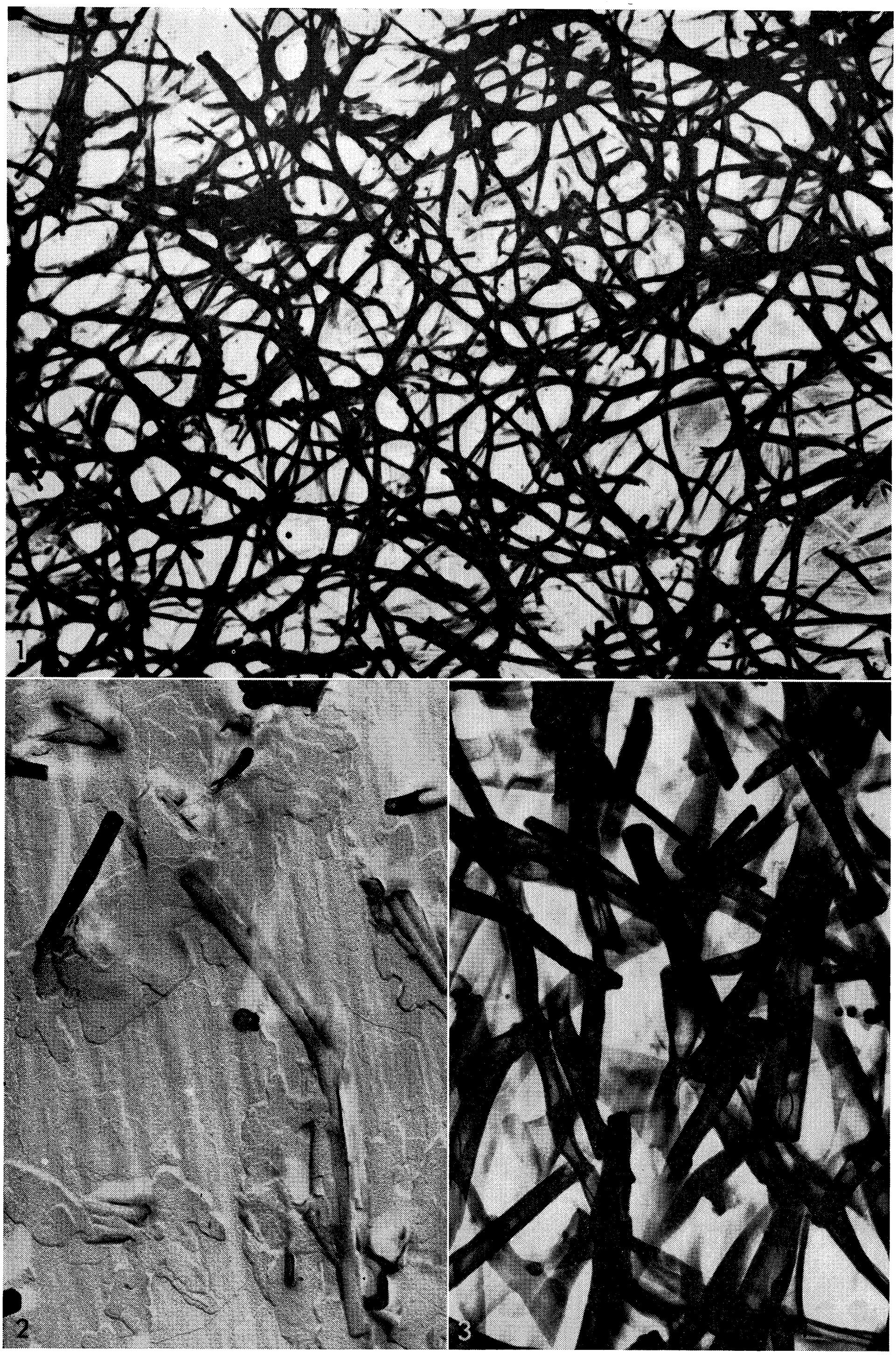

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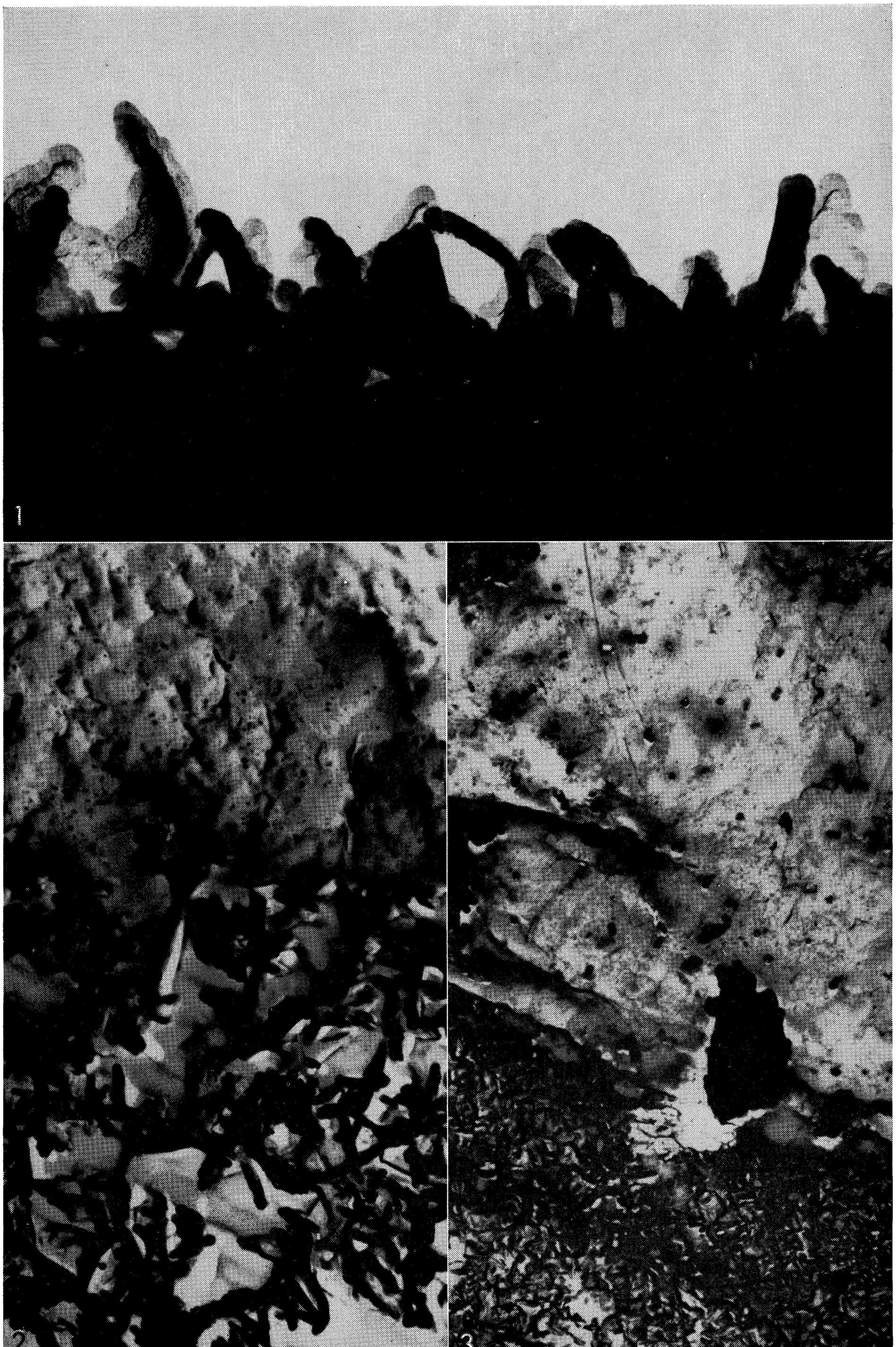

Explanation of Plates 1–5

Plate 1

Fig. 1.—Adaxial leaf surface of a Cross 7 '61 seedling showing open platelet structure. ×6000. *Inset*: platelets, as in Figure 1. ×21,600.

Fig. 2.—Adaxial leaf surface of a mature vegetative Sherpa plant with the platelets densely packed. ×6000. *Inset*: platelets on a similar surface of Arawa. ×28,800.

Plate 2

Fig. 1.—Adaxial leaf surface of a mature vegetative Gabo plant, showing open platelet structure. ×6000. *Inset*: platelets lying flat on, or projecting from, the cuticle surface. ×21,600.

Fig. 2.—Abaxial leaf surface of a mature vegetative 70501 plant. ×8000.

Fig. 3.—Wax on the abaxial leaf surface of a mature vegetative Winglen plant. ×6000.

Plate 3

Fig. 1.—Sheath of a mature vegetative Fortunata plant. ×6000.

Fig. 2.—Sheath of a mature vegetative Mengavi plant. ×6000.

Fig. 3.—Flag leaf sheath of a reproductive Mengavi plant. ×4000.

Fig. 4.—Rodlets on the lemma of a Winglen wheat ear. ×21,600.

Plate 4

Fig. 1.—Typical appearance of wax on a wheat culm, cv. Gamenya. ×6000.

Fig. 2.—Wax plates and occasional rods on newly emerged culm of Aotea wheat. ×14,400.

Fig. 3.—Densely packed wax rods on the culm just below the ear of Aotea. ×14,400.

Plate 5

Fig. 1.—Transverse view of an Aotea wheat leaf. Wax structures project into the air, away from the leaf surface. ×28,800.

Fig. 2.—The adaxial surface of a Sherpa leaf showing damage caused to wax by an insecticide. ×14,400.

Fig. 3.—Adaxial leaf surface of a field-grown Aotea plant, indicating extensive damage of extracuticular wax and the cuticle caused by abrasion. ×4000.