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Cuticle Development and Surface Morphology of Olive Leaves with Reference to Penetration of Foliar-applied Chemicals[1]

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Abstract. **Surface morphology and development of the cuticle of olive (*Olea europaea* L. cv. Manzanillo) leaves were studied in relation to some factors (wetting, retention) that influence absorption of foliar-applied substances. Cuticle deposition occurred at a uniform rate during leaf development, then ceased at full expansion. The total weight of the cuticular membrane per unit surface area remained constant during leaf expansion. The weight of the cuticular membrane of the adaxial epidermis was about 1.4 mg/cm^2 and in μg/cm^2 the principal constituents were: epicuticular wax, 243; cutin, 886; cuticular wax, 272. The thickness of the cuticle was greater on the adaxial (11.5 μm) than on the abaxial surface (4.5 μm), and both surfaces were covered with large (130 μm diameter) peltate trichomes, their number being greater on the abaxial surface (143/mm^2 vs. 18/mm^2). Stomata were present only on the abaxial surface (470/mm^2). The large number of trichomes contributed significantly to the effective surface area of the leaf, which was about 3 times greater for the abaxial than for the adaxial surface. No epicuticular wax fine-structure was observed. Both surfaces were difficult to wet. The advancing contact angle formed by distilled water was 106° for the adaxial and 125° for the abaxial surface. Retention as indexed by dipping leaves in distilled water was 0.34 μl/cm^2. When sprayed, maximum retention was 31.3 and 36.9 μl/cm^2 by the adaxial and abaxial surfaces, respectively.**

Although there is increasing interest in foliar application of growth substances in olive culture (14, 19, 30), little is known about the development of the cuticle and surface morphology of the leaf in relation to foliar penetration. The performance of foliar-applied systemic compounds depends upon retention by the plant surface, penetration through the cuticular membrane and transport to the reaction site, all of which are related to the morphology, chemistry and often to specialized features of the cuticular membrane (3, 5, 12, 18, 25).

Wettability and retention are surface phenomena and involve the physical and chemical characteristics of the leaf surface and the spray solution and interactions among them (2, 6, 10, 12, 13, 19). Retention is positively correlated with wettability, except for leaves that are easily wetted. The chemistry of the epicuticular wax, particularly the functional groups at the surface, markedly affects wetting (4, 12, 15, 16, 17, 29); surfaces rich in nonpolar groups (-CH_3) are difficult to wet while those rich in hydrophilic groups (-OH, -COOH) are easily wetted by aqueous solutions. The anatomical and morphological features of the leaf surface (trichomes, stomata, veins) and the crystalline structure of the epicuticular wax contribute to roughness and thus to the wetting properties of the leaf (4, 10, 29). Leaves with a high roughness factor are difficult to wet (4, 10, 17). The relative contributions of surface roughness and chemistry of the epicuticular wax to wettability differ markedly among species.

The cuticle is the main barrier to penetration of foliar-applied chemicals (3). Most evidence (11, 26) suggests that passage across the cuticular membrane is a diffusion process and, therefore, the rate is dependent on such characteristics as the thickness (25) and the nature and relative amounts of its constituents (1, 18, 23). Since the cuticle is not homogenous over the entire leaf surface, specialized structures such as stomata, particularly guard and accessory cells (26), trichomes (18) and specific areas within the cuticle (11, 27) may serve as preferred sites of entry.

To maximize the efficiency of foliar-applied chemicals, an understanding of the cuticle and the nature of the leaf surface is essential. However, little information exists on the morphology and anatomy of the olive leaf in relation to factors that influence the performance of foliar-applied compounds. In this paper we report on the development of the cuticle during leaf expansion, the morphological characteristics and fine-structure of the leaf surface, and discuss their relationship to wettability and retention.

Materials and Methods

Plant material. Leaves from 1- to 3-year-old 'Manzanillo' olive (Stribling Nurseries Inc., Merced, Calif.) trees grown at East Lansing in a greenhouse (min night temp controlled at 21°C during winter months) were used as the experimental material. Water and fertilizer (20N–8.6P–18.6K) were supplied through a trickle irrigation system as needed to maintain vigorous plants. Pesticides were applied when necessary and only leaves developing 2 months after the last pesticide application were used. The trees were pruned severely several times during the course of these studies to force new growth and only leaves developing on new shoots were used.

To establish the suitability of greenhouse-grown plants, leaves from trees grown under conditions described above were compared with leaves collected from trees grown in a research plot in Sevilla, Spain. No significant differences were found in leaf surface morphology, levels of epicuticular and cuticular waxes or cutin (data not presented).

Leaf expansion. The rate of leaf expansion was established under greenhouse conditions (July) concurrent with the cuticle development studies. Unfolding terminal leaves (126 in all) were selected on the basis of uniformity of size, individually tagged and their length and width measured at 2 day intervals until no further expansion was observed. Surface area was then plotted against time (Fig. 1). In a preliminary study the correlation coefficient between the product of length × width vs. leaf surface area was 0.995. This relationship is described by

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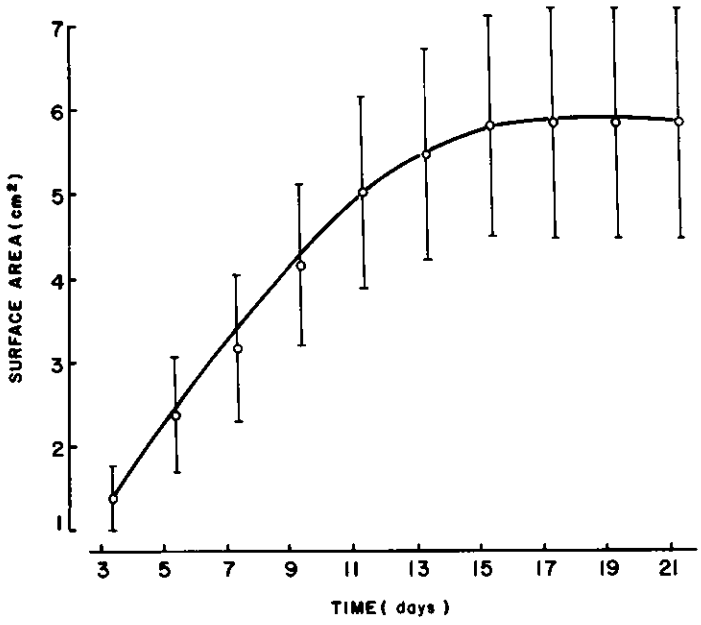

Fig. 1. Time course of leaf expansion in olive. Verticle bars denote standard deviation of the mean.

the regression equation $\hat{Y} = 0.717X - 0.095$.

Cuticle studies. Development of the cuticle was followed by assessing the change in weight of the cuticular membrane, i.e. the epicuticular wax, cuticular wax and cutin fraction during leaf expansion. A large sample of developing leaves varying in size and stage of development was collected from all portions of the trees. The leaves were then assigned to 5 size classes (Table 1), each of which was further divided into 6 subgroups of equal size (6 replications).

The epicuticular waxes were removed by dipping the leaves for 10 sec in 4 successive 100 ml portions of chloroform (7). Cutin and cuticular wax were determined on cuticles enzymatically isolated (8) from discs obtained from leaves after removal of the epicuticular wax. The discs (0.71 cm diam) were infiltrated under intermittent vacuum with 5% pectinase plus 0.2% cellulase (Nutritional Biochem. Corp., Cleveland, OH) at pH 3.6 (buffered with dibasic sodium phosphate-citric acid). Infiltration insured penetration of the enzymes into the tissue, and the infiltrated leaf discs remained submerged minimizing fungal growth during the incubation period (35°C for 15 days). The enzyme solution was renewed after 5 and 10 days. At the end of the incubation period the membranes were separated from the discs by gentle agitation in distilled water, thoroughly rinsed, allowed to dry on filter paper and transferred to a $ZnCl_2$-HCl solution (1 g $ZnCl_2$ in 1.7 ml concd HCl) to remove any cellular debris still attached to the membranes. After 3 hr, the membranes were rinsed with distilled water, air dried and weighed, yielding the weight of the cutin fraction plus epicuticular wax. At this point, 3 discs from each size class were examined with a light microscope to assure freedom from cellular debris. This procedure yielded clean cuticular membranes from the adaxial surface; however, some debris was still present on membranes from the abaxial surface. The cuticular wax was removed by refluxing with 1 methyl alcohol: 1 chloroform (by vol). Both the wax and cutin fractions (cutin) were weighed to confirm that no losses had occurred. In all cases, the total weight was within ± 3.5% of the original weight of the membranes. Extracting solvents were redistilled; chloroform was redistilled twice.

Leaf surface area was calculated from weight of outlines made on uniform grade paper by photocopying. Data for all

Table 1. Size classes and mean surface area of the leaves in each size class used to establish the relationship of cuticle development and leaf expansion.

Leaf size class[z]	Mean surface area (cm^2)
2-4	2.29
4-6	3.45
6-8	4.92
8-10	6.01
10-12	7.16

[z]Defined by the range (cm^2) of the values of the product of length × width of the leaves included in the class.

cuticular components were expressed per unit surface area.

Leaf sections for light microscopy were prepared as previously described (8, 22). Cuticle thickness above the periclinal wall of each of 5 different epidermal cells on both abaxial and adaxial surfaces of 5 mature, fully expanded leaves was measured from cross-sections.

The epicuticular and cuticular waxes (50 and 100 μg of each) were chromatographed on precoated silica gel G plates (250 μm, Analtech Inc., Newark, DE) in 90 petroleum ether: 10 diethyl ether: 3 acetic acid (by vol). Cabbage epicuticular wax (50 μg) was used for comparative purposes. Constituents were localized by spraying with 40% H_2SO_4 (v:v) and charring at 180°C.

Leaf surface morphology. Leaf surface morphology was studied by scanning electron microscopy (SEM) using fresh leaf tissue. Olive leaf tissue withstood desiccation under the coating and viewing conditions, thus minimizing alteration of fine-structure by solvents and experimental conditions normally employed in tissue preparation (24). Leaves, free of visible defects, were collected from the greenhouse immediately prior to examination. Leaf sections (10 mm^2) were mounted on aluminum stubs with a suspension of carbon in a mixture of solvents (Television Tube Koat, G. C. Electronics, Rockford, IL), coated with gold-palladium alloy (Au, 60%; Pd, 40%) and examined (MINI SEM, International Scientific Instruments) at 15 kV. Scanning electron micrographs were taken on Polaroid types 55 and 105 film.

The leaf surface beneath the trichomes was viewed after repeatedly (4–5 times) stripping with adhesive tape. The under-surface of the trichome head and the stalk were viewed by mounting a piece of adhesive tape containing trichomes stripped from the leaf surface and prepared as described for leaf tissue. Leaf cross sections for SEM were prepared by cutting small (3 mm) transverse sections of fresh leaves and mounting on edge.

The number and size of trichomes was determined from photomicrographs of both leaf surfaces. For the adaxial surface, a Wild M-5 stereo-microscope was used. A photograph of a micrometer scale (1 μm divisions) taken with the same equipment and magnifications was used for reference. Since trichomes overlapped on the abaxial surface, they were removed as previously described and their number estimated from the broken stalks counted on micrographs (SEM) using a 400 mesh grid as reference. The number of stomata was established on the same micrographs. Five different areas of each of 5 mature, fully expanded leaves were used for each surface. Trichome diam was estimated by taking 4 measurements on each of 25 trichomes. The contribution of trichomes to the total surface area of the leaf was calculated on the basis of trichome diam and density. The change in trichome density with leaf expansion was established by SEM observations of leaves

at different stages of development (unfolding, about 50% expanded and fully expanded, see Fig. 5).

Wettability and retention. Wettability of the leaf surface was estimated by measuring the advancing contact angle formed by distilled water (6). Two narrow (2 mm) leaf strips for each surface were prepared from each of 16 different leaves and 4 contact angle determinations were made on each strip, avoiding veinal areas. One μl droplets were applied with a 50 μl syringe equipped with an automatic dispenser. The profile of the droplets was projected using a horizontal microscope and both droplet height and width of the droplet-leaf surface interface was measured. The contact angle was calculated using Mack's formula (20).

Retention by the leaf surface was estimated by 2 methods. Firstly, leaves were dipped in a 5 μCi/ml solution of ^{14}C-labelled naphthaleneacetic acid and the radioactivity retained was measured by rinsing the leaves with ethyl alcohol and measuring the activity in a Beckman LS-100 liquid scintillation spectrometer (27). Secondly, leaves were attached to a wire screen support (inclined 45^{o}) and sprayed with distilled water using a compressed air sprayer (operated at 7 kg/cm^2) from a horizontal position. Leaves were sprayed to the point of maximum retention, that is, just prior to incipient runoff, and the water retained was estimated by weighing. Retention data were expressed as μl/cm^2.

Results

Cuticle studies. Cuticle was deposited during leaf expansion and in mature, fully expanded leaves both surfaces were covered with a thick, well-defined cuticle (Fig. 2). Its thickness was greater on the adaxial (11.5 μm) than on the abaxial (4.5 μm) surface except where overlying the midrib (11.2 μm) (Fig. 2 A-E). The cuticle also covered trichomes and guard cells of stomata, extending beyond the pore and lining the substomatal chamber (Fig. 2 F). Stomata have well defined cuticular ledges (Fig. 2F).

Protrusion or pegging of the cuticle between the anticlinal walls of the epidermal cells was minimal. Extensive deposits of pectic materials were found between the cuticular membrane and the epidermal cell walls as evidenced by selective staining with ruthenium red. Birefringence was absent when leaf cross sections were viewed under plane-polarized light, and this was interpreted as a lack of molecular orientation of cuticular wax within the cutin framework.

There was no evidence that trichomes were extensions of epidermal cells. In fact, what appeared to be a thickened portion of cuticle was observed lining the bottom of the trichome stalk (Fig. 2 B). Specialized cells appeared to be associated with

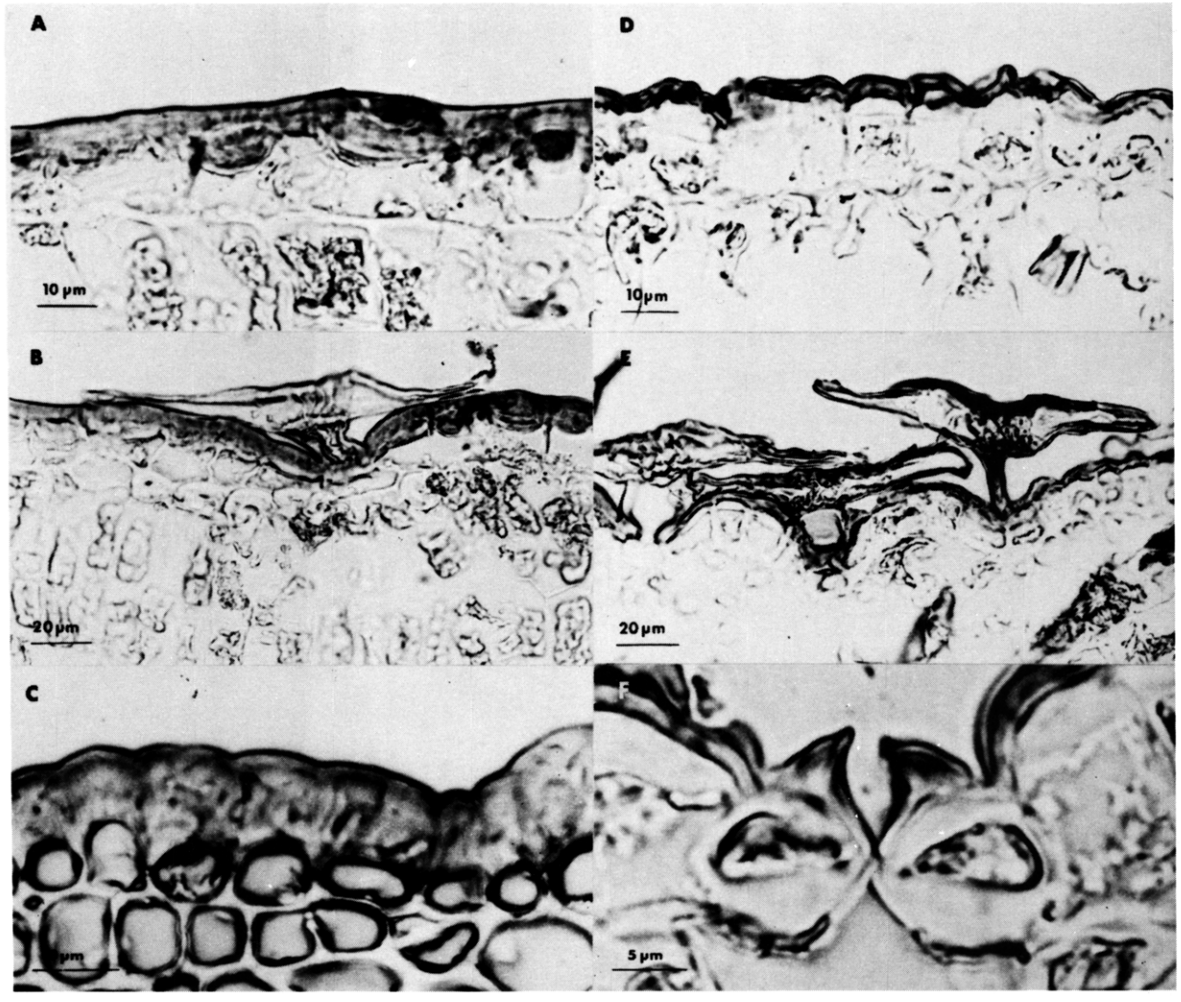

Fig. 2. Photomicrographs of cross-sections of the adaxial and abaxial epidermis of fully expanded olive leaves stained with Sudan III and IV. A and B: adaxial epidermis. C through F: abaxial epidermis. B and E: trichomes. F: stomatal apparatus.

the base of each trichome stalk; outlines of such cells can be seen in the isolated cuticle (Fig. 7 E). Whether or not trichomes are living structures in mature leaves was not conclusively established.

Even though the cuticle increased in thickness as the leaf developed, the weight of cuticular components per unit surface area remained remarkably constant throughout leaf expansion. The total weight of the cuticular membrane on the adaxial surface was 1.4 mg/cm^2, while the weight (μg/cm^2) of the principal constituents were: epicuticular wax, 243; cutin, 886; cuticular wax, 272. Good correlations were found between leaf area and the total amounts of epicuticular wax (r = 0.999), cutin (r = 0.998) and cuticular wax (r = 0.998) per leaf (Fig. 3).

The principal chemical constituents of the epicuticular and cuticular waxes were similar. However, nonpolar constituents were less pronounced in the cuticular than epicuticular wax (Fig. 4).

Leaf surface morphology. Both adaxial and abaxial surfaces of leaves were covered with large (130 μm in diam) peltate trichomes (Fig. 5, 6, 7), their number being about 8 times greater on the abaxial than on the adaxial surface of the leaf (Table 2, Fig. 6 B, F). On both leaf surfaces trichomes were more numerous over the midrib, and were also present on the edges of immature leaves. The number of trichomes was relatively constant throughout leaf expansion. Their density, therefore, decreased markedly as leaf area increased (Fig. 5).

The epicuticular wax lacked crystalline fine-structure and appeared as a smooth, amorphous layer on both leaf surfaces (Fig. 6 B, G). When a chloroform solution of the wax was plated on a glass surface and allowed to evaporate slowly, no crystalline pattern was produced.

The adaxial surface of the leaf was fairly smooth, while the abaxial surface was rough, with many ridges and depressions (Fig. 6 C, G) in which stomata and trichomes were usually found. Stomata were observed only on the abaxial surface, their density being about 470/mm^2. However, no specific distribution pattern was noted.

No significant wax fine-structure was present on the trichomes (Fig. 7 A, C, D). Because of their shape, large head size (Fig. 7 B) and high density, trichomes markedly increased the effective surface area of the leaf. If both surfaces of the trichome head are taken into account (the outer exposed to the environment and the inner facing the leaf surface and continuous with the stalk), the actual surface areas of the adaxial and abaxial surfaces of the leaf were about 1.5 and 4.5 times greater, respectively, than the apparent surface area. The effective area of the abaxial surface is therefore, potentially 3 times greater than that of the adaxial surface.

The trichome stalk on the fully expanded leaf is cylindrical (Fig. 7 F) with thick walls and is devoid of protoplasmic material.

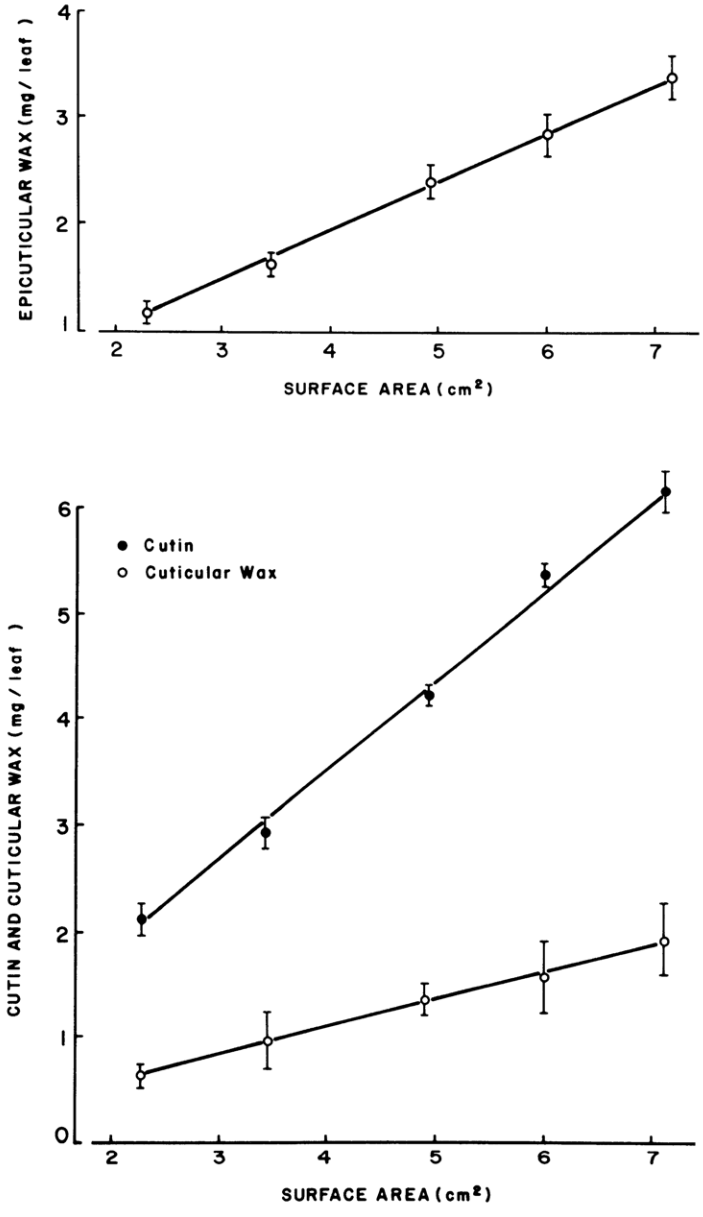

Fig. 3. Relationship between deposition of the cuticular membrane and leaf expansion. Top: relationship between total epicuticular wax level (both leaf surfaces) and leaf expansion ($\hat{Y}$ = 0.499X + 0.150). Bottom: changes in cutin and epicuticular wax levels (adaxial surface) with leaf expansion (cutin: $\hat{Y}$ = 0.846X + 0.120; cuticular wax: $\hat{Y}$ = 0.266X + 0.024). Vertical bars denote standard deviation of the mean.

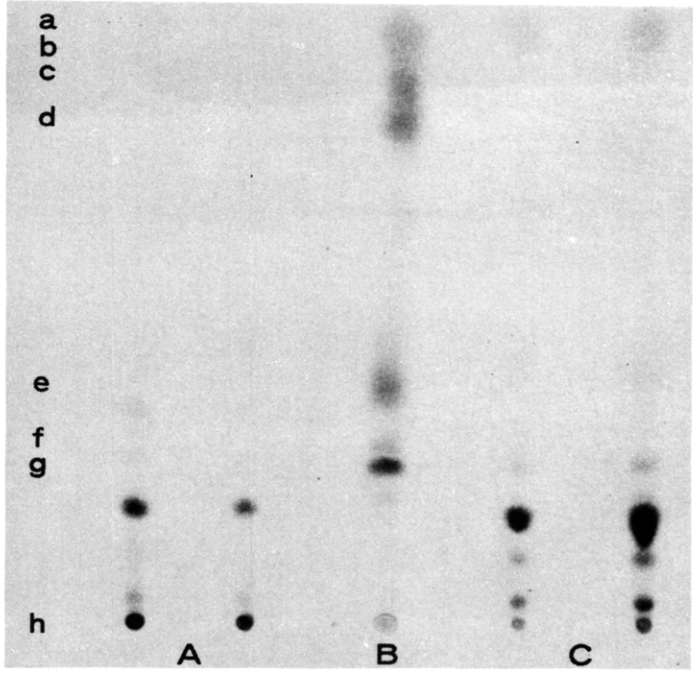

Fig. 4. Thin-layer chromatogram illustrating the separation of cuticular (A) and epicuticular (C) waxes of olive leaves and the epicuticular wax of cabbage (B) using 90 petroleum ether: 10 diethyl ether: 3 acetic acid (by vol) as the developing solvent. Fractions identified in cabbage wax: a, *n*-alkanes; b, esters; c, ketones; d, aldehydes; e, *sec* alcohols; f, ketols; g, primary alcohols; h, fatty acids. The 50 μg levels were spotted adjacent to B.

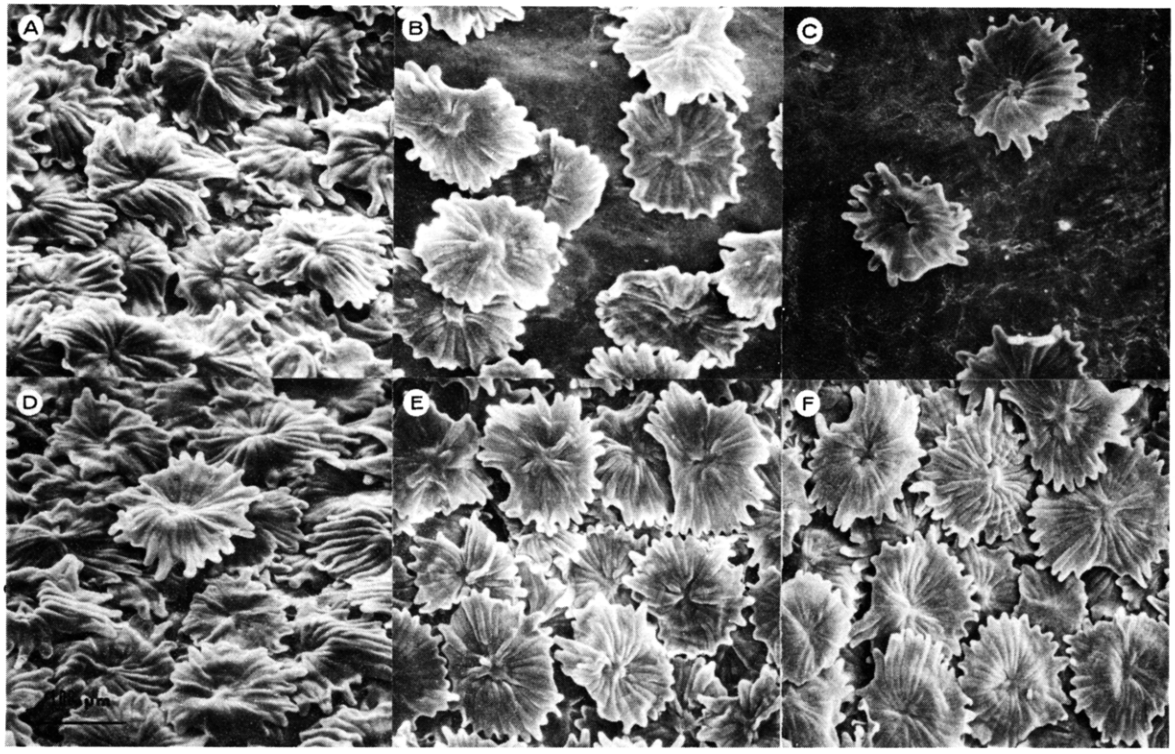

Fig. 5. Scanning electron micrographs of olive leaves illustrating changes in surface morphology with leaf expansion. A through C, adaxial surface. D through F, abaxial surface. Left to right: unfolding, about 50% expanded and fully expanded.

Wettability and retention. Both leaf surfaces were difficult to wet. The contact angles formed with distilled water were 106° for the adaxial and 125° for the abaxial surface. The difference between the 2 surfaces may be attributed to the greater roughness of the abaxial surface due to the greater number of trichomes present.

Retention of distilled water (surface tension about 72 dyne/cm), as indexed by the dipping procedure, was 0.34 μl/cm^2. This method provided an average retention value for both surfaces. Maximum retention on spraying distilled water was 31.3 and 36.9 μl/cm^2 for the adaxial and abaxial surfaces, respectively.

Discussion

Leaf development in olive takes place rapidly, reaching full expansion in about 2 weeks after unfolding. Dynamic changes in development of the cuticle and morphology of the surface occur as the leaf expands. The rate of deposition of the cuticle parallels leaf expansion, since there is a strong linear relationship (r = 0.998) between the 2 processes. No further significant deposition of cutin or cuticular wax constituents occurs after full leaf expansion, similar to other species (3, 18). The cuticular membrane on the adaxial surface appears to be more highly developed, since on a mature leaf the thickness over periclinal walls, veins excepted, approaches 3 times that found on the abaxial surface.

The cuticle extends over trichomes and guard cells, and was observed on the guard cells adjacent to the substomatal chamber (Fig. 2 G). In contrast to numerous plants (18, 21), notably pear (22), olive shows little cuticular deposition between the anticlinal cell walls of epidermal cells. The cuticular membrane over anticlinal walls of several species is often more polar than that over periclinal walls (11, 28) and has been implicated as being particularly permeable to polar substances (11, 26). In view of the extensive development (1.4 mg/cm^2) of the cuticular membrane on olive leaves and the marked barrier the cuticle poses to penetration, it would be of interest to establish the relative permeability of these areas to water soluble compounds.

The olive leaf cuticle contains about 243 μg/cm^2 of epicuticular and 272 μg/cm^2 of cuticular waxes. These levels are considerably higher than those reported for several other species (1, 3, 9, 18) and should significantly increase the diffusion resistance of the cuticular membrane. Although the level of cuticular wax was high, little or no birefringence was apparent when sections of the cuticle were viewed under plane-polarized light, suggesting a lack of molecular orientation of the wax in the cutin matrix.

The total weight on a per unit area basis of the cuticle and its constituents remained constant throughout the expansion of the leaf. This appears to be inconsistent with our microscopic observations of leaf cross-sections which clearly showed a marked increase in cuticular thickness as the leaf expanded. This apparent inconsistency is attributed to the presence of the trichomes. Trichome density is greater on immature leaves (Fig. 5); therefore, their relative contribution to the total weight of the cuticle is also greater. However, they do not contribute to the surface area as measured by leaf outlines. Irrespective, an excellent correlation exists between cuticle development and apparent surface area.

Wettability of both leaf surfaces was very low. This was probably due to the combined effects of the epicuticular wax and the roughness of the leaf surface. Since the epicuticular wax had no crystalline fine-structure, its physical characteristics were not a factor. Its chemical constituents represented a wide range in polarity, from the nonpolar *n*-alkanes to the polar acids. Since the nature of the exposed groups at the surface is not known, the contribution of the chemistry of the

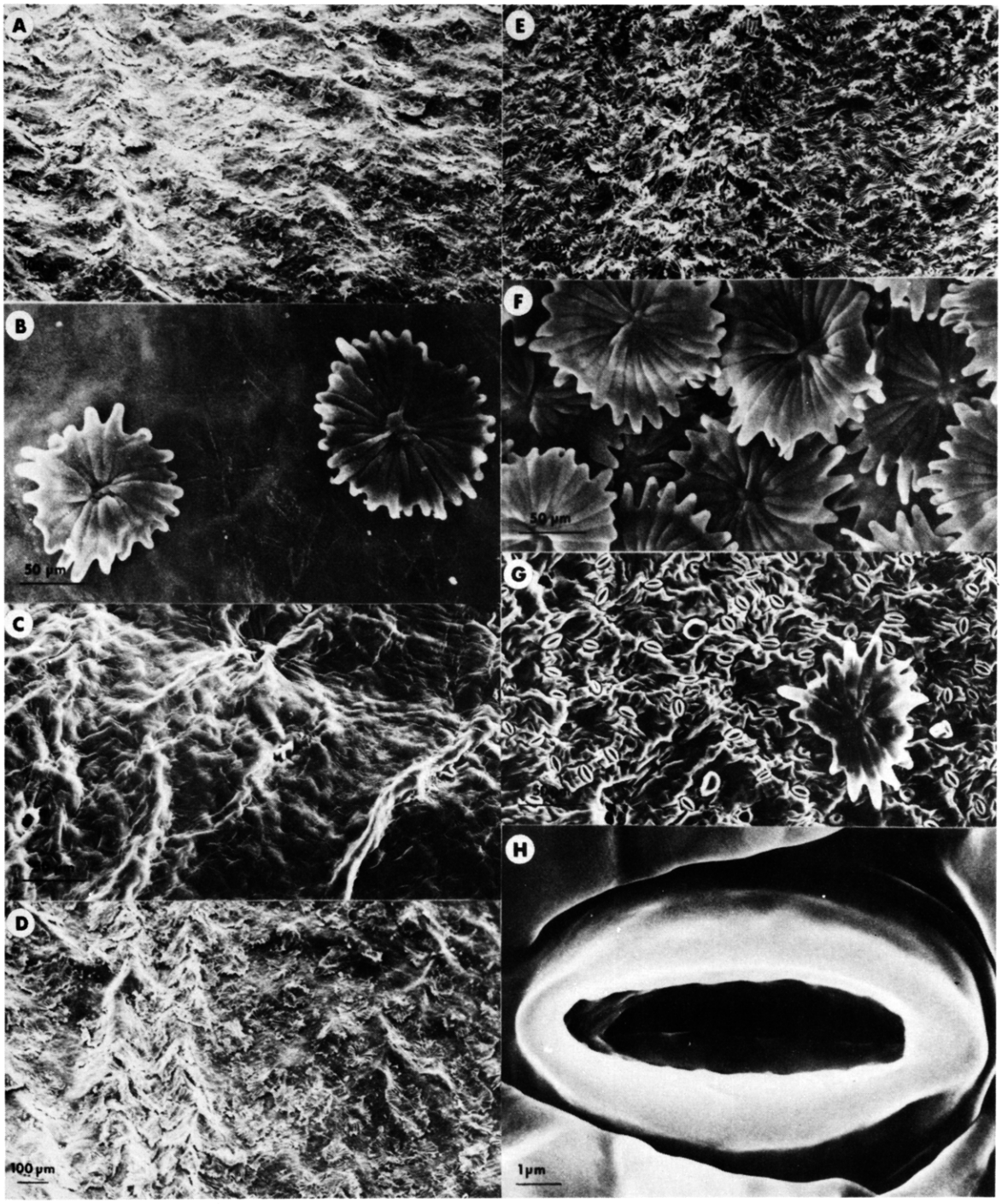

Fig. 6. Scanning electron micrographs of the adaxial (left) and abaxial (right) surfaces illustrating the mature, fully expanded olive leaves. A and E: general view. B and F: relative trichome density. C and G: leaf surface after removal of trichomes. D: over vein. H: stoma.

epicuticular wax to the wetting properties of the leaf could not be documented. Nevertheless, the large contact angles obtained (106° and 125° for the adaxial and abaxial surfaces, respectively) indicate a significant contribution of the chemistry of the epicuticular wax to water repellency (4, 15, 16, 29). Trichomes contribute the major component of surface roughness. Their numbers were greater on the abaxial surface and thus account for its lower wettability. Although the relative contributions of epicuticular wax chemistry and surface roughness could not be conclusively established, both contribute significantly to the repellency of water. The contact angle obtained for the abaxial surface is greater than that expected from chemistry alone (16) suggesting that the trichomes contribute to produce a composite surface (2, 4, 10, 17), that is, a liquid, air, solid interface. This low wettability was reflected in low retention values when the leaves were dipped in distilled water. The relatively high values obtained for retention when the leaves were sprayed represent only the maximum amount of water that can be potentially retained by the leaf and not necessarily the amount that would be retained under

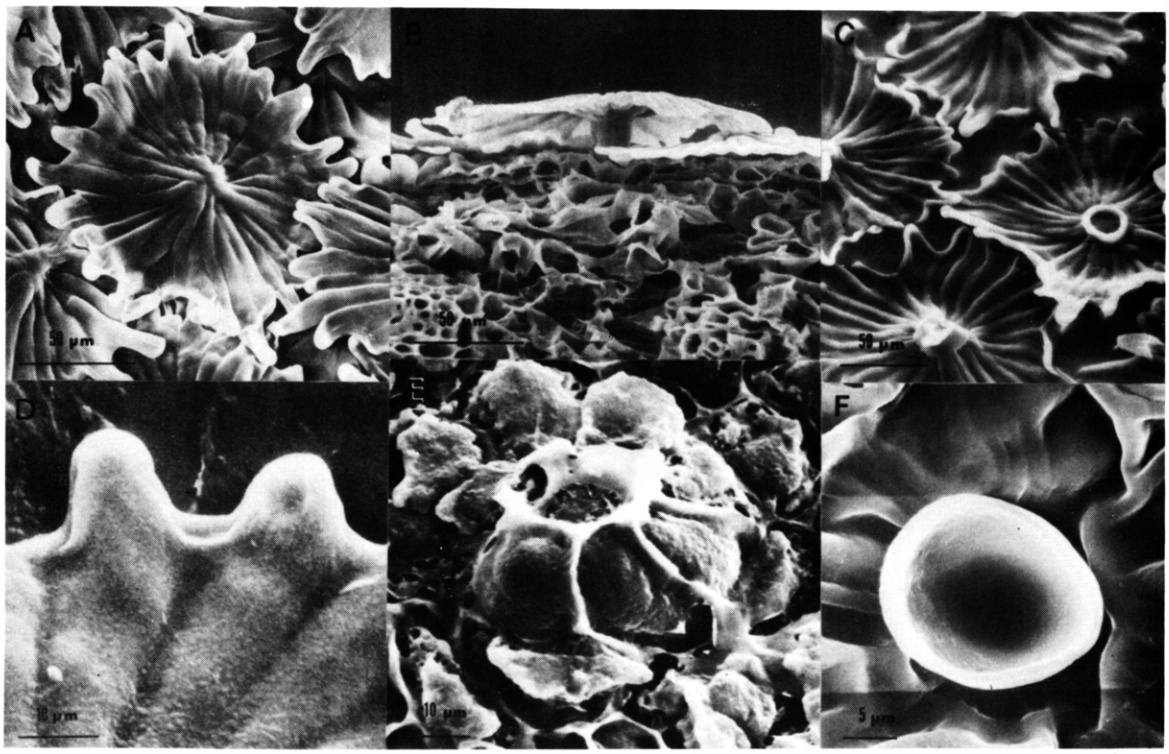

Fig. 7. Scanning electron micrographs illustrating the nature of trichomes of olive leaves. A and B: top and side view, respectively, of trichomes on abaxial surface. C: view of the underside of the trichome head. D: edge of trichome head showing absence of wax fine-structure. E: inner (cell-wall side) surface of cuticle from adaxial epidermis isolated with pectinase showing outlines of accessory cells associated with trichome base. F: trichome stalk on abaxial surface after removal of the head.

Table 2. Morphological and wetting characteristics of fully expanded olive leaves.

Measurement	Leaf surface	
	Adaxial	Abaxial
Stomata (no./mm^2)	0	470 ± 7.8[z]
Trichomes (no./mm^2)	18 ± 0.4	143 ± 2.4
Relative surface area	1.0	3.2
Cuticle thickness (μm)	11.5 ± 0.2	4.5 ± 0.1
Contact angle (^{o})	106.2 ± 0.4	125.4 ± 0.6
Water retention (μl/cm^2)	31.3 ± 1.8	36.9 ± 1.8

[z]Values represent mean ± SE of the mean.

field spraying conditions. Movement of the leaves due to wind may cause most of the water to fall off. Thus, the complex nature of the olive leaf surface presents certain limitations that need to be overcome in developing foliar application practices. Direct penetration studies are needed to assess the significance of the factors discussed here and to establish the permeability characteristics of the olive cuticular membrane.

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Silver Ion Inhibits Ethylene Synthesis and Action in Ripening Fruits[1]

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Abstract. **Ag(I), applied in aqueous solution as $AgNO_3$, inhibited ethylene synthesis and ripening of mature green banana fruit slices and pericarp discs of mature green tomato fruit. It also inhibited ethylene production by cortical tissue from post-climacteric apples. Concentrations of Ag(I) which reduced ethylene synthesis had an inconsistent effect on CO_2 production: no effect on banana slices (1.0 mM Ag^+), stimulation in apple tissue (0.1 mM Ag^+), and inhibition in tomato tissue (0.3 mM Ag^+). This was accompanied by a slight amount of tissue necrosis at these concentrations. Of the 7 metallic salts tested, only Ag(I) inhibited ripening and ethylene synthesis at observed non-phytotoxic levels. Inhibition of ripening and ethylene synthesis by Ag(I) was evident in tissue treated with sufficient exogenous ethylene to elicit both responses in control tissue. The inability of applied ethylene to overcome the inhibitory effect of Ag(I) suggests that the silver ion may interfere with the primary action of ethylene in the tissue.**

Ethylene, whether exogenously applied or endogenously produced, promotes the ripening of mature fruits and vegetables (5, 10, 13). Reducing the endogenous concn of ethylene by hypobaric ventilation (3), with inhibitors of ethylene synthesis (10), or by reducing the activity of ethylene with elevated concn of CO_2 (4) delays ripening. Beyer (2) recently showed that the silver ion, Ag(I), applied foliarly as a solution of $AgNO_3$, was far more effective than 9 other metal ions at inhibiting the effect of exogenous ethylene on etiolated pea stems, cotton plants, and orchid flowers. A short dip in a 50 ppm $AgNO_3$ solution extended the longevity of carnation flowers by reducing their sensitivity to ethylene (8). In contrast, impregnating the stem with 1000 ppm silver solutions extended flower life by inhibiting the growth of microorganisms (12). Lau and Yang (9) have reported that ethylene production by post-climacteric apple tissue and by etiolated mung bean hypocotyl tissue was inhibited by Co^{++}, and to a lesser extent by Ni^{++}.

In this paper we report the inhibitory effect of Ag(I) on ethylene synthesis by apple tissue and on ethylene synthesis and ripening of banana and tomato fruit tissue.

Materials and Methods

Preparation of tissue. Cylinders (3 × 1 cm diam) cut from the cortex of post-climacteric apples (*Malus domestica* Borkh. cv. Ida[illegible]) were soaked for 30 min in 3 changes of 0.5 M glycerol, blotted dry, weighed, and placed in test tubes with 10 ml of 0.4 M mannitol plus or minus $AgNO_3$, $Ca(NO_3)_2$, or KNO_3. Salt concn from 0.02 to 60 mM were employed. Glass beads were used to keep the cylinders immersed. The test tubes were placed in a 10 liter desiccator, which was evaluated 3 times to 25 mm Hg and maintained each time for 1 min. The cylinders were removed, blotted, re-weighed, placed in plastic gas-tight syringes which were set to 10 ml and capped with rubber serum stoppers. Gas samples were taken after 1 hr and analyzed for ethylene and CO_2 by gas chromatography.

Commercially obtained mature green banana fruits (*Musa* sp. cv. Valery) were surface-sterilized in Clorox (5.25% sodium hypochlorite) diluted 1:9 with water, and rinsed in sterile water. Although all fruit were mature green and selected for uniformity, each lot of fruit varies slightly in its degree of ripeness. All cutting, washing and other manipulations were

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