Penetration of Chemicals into the *Malus* Leaf Cuticle

An Ultrastructural Analysis

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Abstract. The adaxial leaf cuticle of *Malus pumila* was examined by electron microscopy to determine possible avenues for transcuticular movement of foliarly applied chemicals. Cutin-embedded polysaccharide microfibrils originated at the outer epidermal cell wall and occasionally extended to the cuticle surface. Lamellae, ca. 4 nm wide, usually were oriented parallel to the cuticle surface. When oriented perpendicular to the surface, they extended nearly to the subjacent wall layer from the surface. Aqueous solutions of uranyl acetate, silver nitrate and phenyl mercuric acetate applied to the cuticle surface of leaf segments floated on solutions of phosphate salts or thiocarbohydrazide (TCH) reacted within the cuticle to form insoluble electron-opaque deposits indicative of their avenues of transcuticular movement. Uranyl phosphate deposits were observed only in the polysaccharide microfibrils of chloroform:methanol-extracted leaves. Silver-TCH deposits were observed in the microfibrils of both extracted and nonextracted leaf cuticles. Phenyl mercuric acetate-TCH deposits were randomly dispersed throughout the extracted cuticle and not associated with the polysaccharide microfibrils.

Key words: Foliar adsorption – Leaf cuticle – *Malus* – Transcuticular uptake.

Introduction

In agriculture today, the plant leaf surface is treated with various chemicals. Among them are pesticides, growth regulators, and nutrients. These compounds either remain entirely on the leaf surface as in the case of many protective pesticides, or they penetrate the leaf epidermis and enter various systems of the plant. Entry into the plant is via stomata and/or the leaf cuticle. Both routes of entry are well documented for certain chemicals (Martin and Juniper, 1970; Edgington and Peterson, 1977). What is not established, however, is the actual route of movement through the cuticle. Some information is available indicating mainly that both polar and nonpolar chemicals move across the leaf cuticle in specific pathways (Franke, 1967; Martin and Juniper, 1970). Such results often are based on the amount of chemical that can be assayed in the medium beneath the cuticle, whether it is intact or isolated from the leaf. The route of transcuticular movement of various chemicals has been surmised to be through intermolecular channels or pectic pathways, and has been based on the affinities of the physico-chemical properties of both the cuticle and chemical in question.

Pathways that might be available for transcuticular movement of chemicals are the (I) cutin matrix, (II) wax channels (if they are a reality), (III) pectic and cellulose (and other polysaccharides) microfibrils, and (IV) “lamellar” pathways of unknown chemical composition. One or more of the pathways might be available to a particular chemical.

To know the actual morphological or physico-chemical route that an applied chemical would traverse would enhance our understanding of the mechanisms involved in foliar uptake of chemicals and in the function of the cuticle. Thus, the purpose of this investigation was to determine what routes are utilized by certain chemicals to diffuse across a leaf cuticle.

Materials and Methods

Preparation and Treatment of Leaves

*Malus pumila* Miller cv. Rome Beauty trees (8-10 year old) were container-grown as previously described (Hoch, 1975). The third
leaf from actively growing apple shoot apices was used in this study. Immediately after detachment, the leaves either were used fresh or were pretreated by immersion in a mixture of chloroform: methanol (CHCl₃:MeOH (1;2) for 1 h to remove surface and intracellular wax, and then rehydrated. They were dissected along the midvein and along the major lateral veins to yield four to six leaf segments 1.0–1.5 cm square. A ring of lanolin, 6-mm inside diameter, was applied to the upper (astomatous) leaf surface of each segment. Next, the leaf segments were floated on aqueous solutions of various chemicals in a covered Petri plate. In addition, ca. 0.1 ml of another test solution was placed on the leaf surface within the ring of lanolin. The two test solutions, one containing heavy metal ions, penetrated the cuticle and leaf tissue. The metal ions were precipitated when they met and reacted with the appropriate nonmetallic ions, thus indicating their route of penetration. The following aqueous solutions were used as the heavy metal donors:

a) 0.02–40 mM uranyl acetate (UO₂Ac₂),
b) 0.06–6 mM silver nitrate (AgNO₃),
c) 15 mM phenyl mercuric acetate (PMA).

Uranium was insolubilized with PO₄ from 0.1 M PO₄(K⁺) buffer, pH 6.8, while silver and PMA were insolubilized with an aqueous solution of 1% thiocarbohydrazide (TCH) (Polysciences, Inc., Warrington, Penna.). The principle of the PMA-TCH complex is analogous to that previously described by Smith and Fishman (1969). All treatments were conducted in the dark for 1–72 h depending on the particular experiment. Control treatments consisted of various combinations of the test solutions without the complexing ions.

Electron Microscopy

Treated leaf segments were carefully removed from the Petri plates so that the two solutions did not intermix and rinsed under a stream of distilled water. Next, the leaf tissue within the ring of lanolin was excised and cut into 1-mm² segments in 4% glutaraldehyde in either 0.1 M PO₄(K⁺) buffer, or 0.1 M cacodylate (Na⁺) buffer, pH 6.8. Subsequent treatment was similar to that previously described (Hoch, 1975). Some samples were postfixed with OsO₄. The sections (60–90 nm thick) were either not stained or they were stained with barium permanganate (Hoch, 1977) and/or with saturated aqueous uranyl acetate followed by lead citrate (Reynolds, 1963) and examined with a JEOL 100 B electron microscope. Nonstained sections (80 or 200 nm thick) were examined and analyzed with a Hitachi H-500 H microscope operated at 75 kV for elemental analysis and X-ray mapping, courtesy of N. Shikashio (Scientific Instruments, Mountain View, Calif.).

Results

The adaxial Malus leaf cuticle as referred to in this paper consists of that layer of wax, cutin, and cutin-embedded cellulosic and/or pectic microfibrils that is usually separated morphologically from the more uniform and discrete epidermal cell wall (Figs. 1–5). It is also that layer which is separated from the epidermal cell wall by the methods previously described for obtaining isolated leaf cuticles (Hoch, 1975). The cuticle used in this investigation varied from 0.3 μm to 1.0 μm in thickness. The innermost region of the cuticle, adjacent to the epidermal cell wall, contains numerous microfibrils that extend from the cell wall into the cutin matrix (Figs. 1–3). As an anastomosing network, the microfibrils commonly extend from the epidermal cell wall to within 35 nm of the outer cuticle surface (Figs. 1–3). Finer extensions of the microfibrils often extend to the surface (Fig. 2). The microfibrils, as well as the cell wall material, were not contrasted in nonstained sections, whether postfixed with OsO₄ (Fig. 4) or fixed only in glutaraldehyde (Fig. 5).

Within the cuticle, and particularly in the outer regions, are electron-translucent streaks often referred to as lamellae (Sitte and Rennier, 1963; Hoch, 1975; Sargent, 1976) that are ca. 4 nm wide. These lamellae, generally oriented parallel to the cuticle surface (Fig. 4), are also oriented perpendicular to the surface (Figs. 1 and 3), particularly over or near anticlinal walls. When oriented perpendicular to the surface, the lamellae frequently extend three-quarters or more of the distance into the cuticle (Fig. 3).

Epicuticular wax has been removed either intentionally during the CHCl₃:MeOH extraction treatments or unintentionally during the dehydration and embedding procedures. Nevertheless, outlines of the wax surface due to natural surface contaminates were observed in some specimens (Figs. 1–3).

Transcuticular Movement of Chemicals

Movement of chemicals into and through the Malus leaf cuticle occurred more readily in CHCl₃:MeOH-extracted cuticles than in the natural non-extracted cuticles.

CHCl₃:MeOH-extracted leaf segments, floated on phosphate buffer, to which UO₂Ac₂ was applied, indicated that the heavy metal salt moved transcuticularly via the cutin-embedded polysaccharide microfibrils (Figs. 6–9). The close correlation of the UO₂HPO₄ deposits with the extension of the cutin-embedded polysaccharide microfibrils that extend from the epidermal cell wall is most evident when the thin sections were postsection-stained with uranium and lead salts (Fig. 7). Cutin-embedded polysaccharide microfibrils are not visible in Fig. 6 due to the omission of postsection-staining procedures (compare Figs. 4 and 6). The limited extent of the transcuticular movement of UO₂Ac₂ before precipitation with PO₄ in Fig. 6 and 7 is attributed, in part, to the faster movement of PO₄ through the subjacent leaf tissue. The pattern of UO₂HPO₄ deposition in Fig. 9 indicates that the PO₄ reached the outer region of the cuticle over the anticlinal wall faster than in regions over periclinal walls. The faster migration of PO₄ was due to the wick-like nature of the anticlinal wall, and/or from more polysaccharide microfibrils within the inner regions of the cuticle.