Review of Herbicide Penetration through Plant Surfaces

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The known bases of herbicidal selectivity are enumerated and those relating to foliar penetration are emphasized. The process of penetration (stomatal and/or cuticular, then cellular), is described; since substances that enter the substomatal chambers by mass movement must still cross a lipoidal barrier, the internal cuticle, entry via the cuticle is stressed. The physical and chemical nature of plant surfaces is discussed in relation to the penetration of herbicides via polar, apolar, or combination routes through the cuticle. The individual properties of cutin, waxes, pectin, and cellulose, the four major components of cutinized cell walls, are reviewed in relation to other contributing influences on cuticular penetration. Ontogenetic changes in plant surfaces, environmentally induced and otherwise, are also pointed out. Influences of cuticular structure and function upon the deposit, accumulation, and disappearance of pesticide and surfactant residues are recognized. Further research on cuticular synthesis and ultrastructure, mechanisms of surfactant action, and the pathways and mechanics of cuticular penetration is encouraged.

With further study, the complexity and interaction of factors governing the effectiveness of foliage applied herbicides becomes increasingly apparent. Herbicidal foliar sprays may be selective or nonselective depending upon many factors (*1*). Many of the bases of herbicidal selectivity relate directly or indirectly to penetration.

A herbicidal foliar spray may act as a contact toxicant or be translocated systemically following absorption. Several requisite processes are involved in the satisfactory performance of a foliage-applied herbicide. First, the chemical spray must contact and be retained by the leaf surface. The herbicide must then be sorbed (adsorbed to the surface, absorbed into the cuticle or substomatal chambers, and finally desorbed). Actual penetration of the cell, which follows, may involve either the destruction or relatively harmless crossing of the outer cell membrane (the ectoplast). A systemic herbicide must also move innocuously from cell to cell and out of the treated region via the conductive tissues then finally "accumulate" to toxic concentrations in remote tissues. With both contact toxicants and translocated herbicides, the morphological and biochemical "sites of action" are often nebulous.

Factors Influencing Foliar Penetration

Currier and Dybing (*5*), in 1959, classified and reviewed the major factors influencing foliar penetration of herbicides. More recently, Crafts and Foy (*4*) discussed, in some detail, the importance of the chemical and physical nature of plant surfaces in relation to the use of pesticides. Much of the following discussion is summarized from that review, which is available in book form only.

The literature dealing with plant surfaces is very extensive. Comprehensive review of all facets is beyond the scope of the present discussion, which is intended mainly as a summary of the status of knowledge concerning the physical and chemical nature of plant surfaces as related to foliar penetration. Thus, for the most part, original research references are not cited directly.

Various aspects of the broad subject have been treated in texts, theses, and other reviews as follows: waxes (*14*, *26*); cuticle (*20*, *22*, *25*); retention and penetration of pesticides and growth regulators (*5*, *17*, *28*, *29*); plasmodesmata (*16*). The occurrence and possible importance of cuticular pores and ectodesmata (protoplasmic strands protruding for various distances into the exterior walls of epidermal cells) have been of recent interest (*9*, *10*, *27*; see also earlier references cited in *4*). Currently, these are areas of active research and controversy, particularly in Germany.

Both surfaces of leaves function in the absorption of chemicals. Usually the lower epidermis is more penetrable than the upper. Not all areas of the leaf are equally permeable. Preferential areas of foliar absorption named in the literature for various substances (herbicides, nutrients, fluorochromes, etc.) are the veins, anticlinal epidermal walls, glandular trichomes, open stomata, fissures, insect punctures, or other imperfections in the cuticle.

Penetration may be classified as cuticular and/or stomatal, then cellular. Whether cuticular or stomatal entry is most important as a generalization is a moot question. Both are known to occur under appropriate circumstances, and which predominates in a given situation may be determined by many interacting factors. Entry of oils, and aqueous sprays with lowered surface tensions, into stomata is apparently by mass movement; entry through the cuticle is by diffusion, at least initially.

Oils and aqueous solutions having lowered surface tensions due to the addition of suitable surfactants penetrate large open stomata readily. Completely closed stomata, however, tend to exclude all liquids. Even substances entering through stomata must next penetrate the internal cuticle. Although perhaps reduced in amount, and different in composition from the external cuticle, the internal cuticle seemingly constitutes a lipoidal barrier, nonetheless. Since the cuticle appears to be deserving of primary consideration, the remaining comments will be confined to its physical and chemical nature in relation to penetration.

Polar vs. Apolar Routes across the Cuticle

Both oils and aqueous solutions apparently penetrate the cuticle slowly, i.e., from evidence on cuticular transpiration and herbicide penetration, the cuticle is presumed to be difficultly permeable. Since both polar ions and nonpolar fat-soluble molecules penetrate cuticle, both a foliar or aqueous route and a nonpolar or lipoid route are assumed to exist. Various authors (*3, 4, 23*) have considered the possibility that water-soluble compounds may follow an aqueous path and the more oil-soluble substances a lipid path. Pallas and Williams (*21*) also suggest that possibly an organic molecule, such as 2,4-D, follows a lipoidal pathway into the leaf, and an inorganic ion, such as phosphate, follows an aqueous pathway. However, this hypothesis is not yet supported by adequate research. For example, it is not yet possible with ultramicroscopic studies to describe these routes, morphologically, within the cuticle. However, some understanding of how the two routes could conceivably exist is gained by a consideration of the chemical as well as the physical properties of the components which go to make up the cuticular layers. (Since roots are not normally covered by a cuticle, the following remarks apply to only foliar penetration.)

Properties of Cuticular Components

The cuticle is a more or less continuous but nonuniform and, in old leaves often imperfect layer laid down of products of metabolism of cells of laminar organs.

In 1948, Frey-Wyssling (*12*) summarized the chemical nature of cutinized plant cell walls; they are composed of four rather distinct substances (or groups of compounds) all of which may vary in distribution within the wall. Probably still our best concept of the submicroscopic anatomical relationships of the cutinized epidermal wall is that represented diagrammatically by Orgell (*4, 20*), as modified from Frey-Wyssling (*12*), Mueller *et al.* (*18*), and Roelofsen (*24*). These substances are cutin, waxes, pectin, and cellulose. The term cuticular layer refers to the semilipoidal lamellae of the surface covering that have become impregnated with wax and cutin. It may be referred to as cutinized cell wall and includes pectin and the cellulose of epidermal walls when it has become impregnated with lipid substances.

Each constituent imparts its own physical and chemical properties to the plant surface layer. Each of these four major components will be discussed briefly.

Cutin. This substance is described as a semilipoidal oxidative polymer of long-chain fatty acids and alcohols. Such compounds, "unused" products of metabolism, migrate to the ectoplasmic surface and then to the outer epidermal wall. Here at the air-water interface they tend to become oriented with their polar groups in the water phase and their hydrocarbon chains toward the outside. With age, as water is lost and reaction with oxygen proceeds, the more or less continuous "varnish-like" layer is formed. Cutin, then, results from the oxidation and polymerization of various unsaturated lipid compounds. Once formed it is insoluble in most organic solvents and apparently constitutes the matrix of the cuticle.

Suberized and cutinized wall layers contain unsaturated high molecular weight ketones, alcohols, or esters of unsaturated alcohols; polymerization to form chains of complex structure is made possible by their reactive end groups which enable these compounds to form esters. Since cutin possesses a negative charge in water, has selective cation permeability, and is stainable with basic dyes, it must be only partially esterified, with free carboxyl groups exposed at the surface. Also, since it is optically isotropic, cutin is assumed to possess a reticular linkage structure like lignin. Because of its chemistry, cutin has both polar and apolar properties; it is semilipoidal but also semipolar.

Waxes. Cuticular wax refers to the petroleum ether-soluble mixture of more saturated lipid substances embedded in the cuticular layers. Surface wax refers to the usually irregular deposits of similar material found on the cuticle surface of some species. Part of the cuticular wax impregnating the framework of cutin is believed to be in chemical combination with the cuticle. Cuticular waxes apparently consist of short, rod-shaped molecules having no reactive end groups; being relatively inert, they are unable to polymerize and are of low molecular weight. Cuticular waxes are optically negative, stainable in lipid dyes, and melt above 220° C.

Waxes are hydrophobic and resistant to wetting with pure aqueous sprays. The waxy rodlets of leaves forming a "bloom" may prevent contact of a spray droplet with the leaf surface. The selectivity of certain formulations of 4,6 - dinitro - *o* - *sec* - butylphenol (DNBP) contact sprays in peas, for example, is dependent upon this mechanism. Pretreatment with certain herbicides, such as trichloroacetic acid and dalapon, which interfere with or alter wax deposition and thereby increase the wettability of peas to aqueous sprays of DNBP, may cause a loss of herbicidal selectivity based on differential wetting (*29*). More recently, ethyl - *N*,*N* - di - *n* - propylthiolcarbamate (EPTC) and related compounds have also been shown to interfere with the disposition of surface wax in *Brassica* spp. (*13*).

Waxy leaf surfaces are readily wet normally by oils or aqueous sprays containing a suitable surfactant. In the latter case, however, enhanced wetting is not always synonymous with enhanced penetration. Surface wax deposits may interfere appreciably with wetting but little with penetration provided that good surface contact is ensured; despite adequate external wetting, however, internal cuticular wax can still constitute a serious barrier to penetration of aqueous substances. Once laid down, the waxes are rather inert.

Pectins. These compounds consist of long-chain polygalacturonic acid molecules having side carboxyl groups. They are capable of forming salts; they impart to pectins base exchange properties. Polygalacturonic acid, as well as its methylated derivative, is soluble in water, however, calcium pectate is insoluble. Pectic substances have little tendency to crystallize; they occur in an amorphous state and are responsible in large measure for the strong water-holding capacity of cell walls. Thus, the pectic layer and the cellulose which it bounds are hydrophilic or polar.

Cellulose. This structural component is composed of long-chain molecules that are relatively stable. These molecules are arranged in micelles, and the micelles are associated into microfibrils. Thus because of its microfibrillar organization, cellulose imparts tensile strength and elasticity. It is not considered an important obstacle to the penetration of aqueous sprays as are cutin and cutin waxes, the components of the outermost layer (the cuticle proper).

Since the hydrophobic wax molecules repel the hydrophilic cellulose, the polar (but semilipoidal) cutin molecules are probably interposed between them predominantly with their hydrophilic OH, COOH, and OCH_3 groups bounding the cellulose, and their hydrophobic hydrocarbon chains in contact with the waxes.

Cutin may contain appreciable quantities of polymerized carboxylic acids. Having many polar groups, such cutin may absorb water and swell appreciably. This hydration spreads apart the wax components and tends to increase the permeability of the cuticle to polar molecules and promotes the absorption of water soluble herbicides.

This proposed "value system" may have real significance with respect to the absorption of herbicides via an aqueous (polar) route as contrasted to a lipoidal (apolar) route. High turgidity of underlying tissue, high relative humidity (near saturation), and, locally, the spray droplet itself may influence this phenomenon.

Generally speaking, there exists a gradient from low polarity at the exterior of the cuticle to relatively high polarity in the layers bordering the epidermal cell wall. Lipophilic waxes predominate toward the outside; the outer layers contain only wax and semilipoidal, semipolar cutin. Hydrophilic substances, cellulose and pectins, are in predominance in the inner regions, where they lend strength and water-retaining properties.

Coefficients of asymmetry of penetration of water through cuticle have been calculated. Schieferstein (*25*) found ivy cuticle to have a greater permeability to water in the inward direction than the outward direction.

The polarity of herbicide molecules determines their solubility in the carrier solution, the cuticle, cell wall, and membrane. The less polar the molecules, the more lipid-soluble they are. Undissociated solutes are relatively nonpolar, and therefore are oil-like and penetrate lipoid barriers more readily. Thus one may speak of the apolar or oil-like properties of even a polar compound like 2,2-dichloropropionic acid (dalapon) if it is at a low pH in water (i.e., relatively undissociated) or modified by the addition of surfactants which combine both polar and apolar properties (*6*).

Postulated Absorption Pathways

Penetration then may generally involve the following:

For aqueous solutions of inorganic salts, acids, bases, and polar organic compounds—entry through cracks, punctures, or areas of leaves not completely covered by waxy lamellae, then following a polar (aqueous) route presumably by the hydrated cutin and/or the hydrophilic pectic and cellulose portions of the wall; for oils or apolar solutes—absorption directly through the waxy portions of the cuticle via an apolar (lipoid route), at least initially; and for substances exhibiting both polar and apolar properties (e.g., many formulated organic herbicides and most surfactants) which tend to render compatible, in the spray mix–plant surface complex, the opposing properties of the other two groups—entry and transport via a combined aqueous and lipoid route through the cuticle proper as well as through imperfections. Later ectodesmata may or may not play an important role in further penetration.

Hydrocarbons and surfactants may "solubilize" into the cuticle and/or the plasma membrane displacing the lipoid molecules and increasing permeability. Oils penetrate lipid surfaces readily; aqueous sprays are given some of the properties of oils by addition of suitable surfactants. In fact, surfactants may be simply defined as substances which are capable of altering the energy relationships at surfaces or interfaces, thereby reducing surface or interfacial tension.

Since penetration of herbicides is ultimately controlled by adhesion between molecules, the composition and surface chemistry of the cuticle are important. Both chemical and physical properties are involved and the two are not easily separable.

As quoted in a recent review (*4*), movement of substances through cuticle involves diffusion which is conditioned by particle size, pH, molecular structure of the penetrant, prevalence of water, and possibly other factors. Interactions between cuticle and applied substances may be mechanical (relation of penetrant particle to pore size), physicochemical (competition for adsorption sites, etc.) or chemical (chemical or electrical reactions). The nature of the cuticle and penetrating substances, and their physical and chemical environments determine the extent of these interactions and whether they will help or hinder penetration. The steric, polar, electrical (ion charge), and chemical properties of the penetrant molecule will influence its action with cuticle (*17, 20*).

Surfactant Action

Finally, it has long been recognized that surfactants may facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing, and/or other surface-modifying properties of herbicidal formulations to bring about enhancement of penetration and herbicidal action. An increasing number of chemically diverse types of surface active agents (*15*) have found application in various phases of biological research in recent years. However, the nature of total surfactant action is complex and not yet fully explained.

Surfactants, by their nature, normally reduce surface tensions of aqueous systems and improve wetting. Under some conditions, this may be their only enhancing effect. However, for many others, surfactants improve herbicidal penetration (and perhaps even translocation) at levels far above the critical micelle concentration range (*2, 8, 11*). For the present discussion, it is sufficient to say that surfactants act, perhaps primarily, by virtue of their combined polar and apolar properties in the same molecule, rendering compatible two phases (e.g, lipoidal and nonlipoidal substances) which were otherwise incompatible. Some surfactants whose properties are known probably orient polarly, and become solubilized in the cuticle, thus causing a loosening or swelling of the cuticular architecture and thereby enhancing penetration. Other functions are also possible.

Perhaps radiotracer studies now in progress, employing both labeled and nonlabeled surfactants and herbicides, will provide some of the much needed answers concerning the sites and mechanisms of herbicidal enhancement by surfactants (*7, 19*).

Pesticide Residues

The properties and forces discussed above may also be expected to influence, strongly, patterns of deposit, retention, accumulation, and release of herbicides and spray adjuvants—i.e., to affect residue levels of both. The cuticle, a semilipoidal layer, may greatly influence not only the penetration but the ultimate fate of foliage-applied herbicides and, on occasion, may itself serve to accumulate pesticide and/or surfactant residues. Conceivably, the waxy and fatty constituents may constitute an important pool for holding fat-soluble compounds such as 4,6-dinitro-*o-sec*-butylphenol (DNBP), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and esters of 2,4-dichlorophenoxyacetic acid (2,4-D) in solution. Such solution may effectively prevent or retard partition into the symplast and transport to roots of systemic pesticides. It may also hold such compounds intact for long periods and thus create residue problems where hazardous chemicals are involved.

Finally, despite the foregoing attempts to generalize, it must be borne in mind that the cuticle of plants is highly variable both in composition and in physical structure. Also, like other plant structures, it undergoes ontogenetic changes. Hydration, weathering and degradation, insect punctures, abrasion, and other physical stresses may also render the cuticle more permeable. Much of the above is subject to and deserving of further experimentation.

Some Research Needs

Although specific information is still lacking on many points relative to herbicide penetration, further research is especially needed, and therefore encouraged in the following areas: the structural and functional relationships of plant cuticle in the foliar absorption of pesticides, specifically herbicides and spray adjuvants; cuticular synthesis and ultrastructure, under various conditions of the physical and chemical environment, as determined by electron microscopy using both surface replica and thin sectioning techniques; specific pathways and mechanisms of cuticular penetration by various polar and apolar compounds, as well as substances which exhibit both polar and apolar properties; the role(s) of the cuticle in magnifying or minimizing residue problems under varying treatment and environmental conditions; and the sites and physicochemical mechanisms involved in the total enhancement of herbicidal action by surfactants.

Literature Cited

(1) Ashton, F. M., Harvey, W. A., Foy, C. L., *Calif. Agr. Expt. Sta. and Ext. Serv. Circ.* **505,** December 1961.

(2) Colwell, C. E., Kixon, W. E., *Am. Dyestuff Reptr.* **50,** 39 (1961).

(3) Crafts, A. S., *Am. J. Botany* **43,** 548 (1956).

(4) Crafts, A. S., Foy, C. L., *Residue Reviews* **1,** 112 (1962).

(5) Currier, H. B., Dybing, C. D., *Weeds* **7,** 195 (1959).

(6) Foy, C. L., *Hilgardia* **35,** 125 (1963).

(7) Foy, C. L., Smith, L. W., *Research Progress Rept., Western Weed Control Conf.*, p. 88 (1963).

(8) Foy, C. L., Smith, L. W., *Weed Soc. Am. (Abstracts)* p. 77 (1964).

(9) Franke, W., *Ber. Deut. Botan. Ges.* **75,** 295 (1962).

(10) Franke, W., *Planta* **59,** 222 (1962).

(11) Freed, V. H., Montgomery, M., *Weeds* **6,** 386 (1958).

(12) Frey - Wyssling, A., "Submicroscopic Morphology of Protoplasm and its Derivatives," Elsevier, New York, 1948.

(13) Gentner, W. A., *Weed Soc. Am. (Abstracts)*, p. 53 (1961).

(14) Kreger, D. R., *Rec. Trav. Bot. Neerl.* **41,** 603 (1948).

(15) McCutcheon, J. W., "Detergents and Emulsifiers," New York, N. Y., 1962.

(16) Meeuse, A. D. J., *Protoplasmatologia* **2,** 1 (1957).

(17) Mitchell, I. W., Smale, B. C., Metcalf, R. L., *Advan. Pest Control Res.* **3,** 359 (1960).

(18) Mueller, L. E., Carr, P. H., Loomis, W. E., *Am. J. Botany* **41,** 593 (1954).

(19) Norris, L. A., Freed, V. H., *Research Progress Rept., Western Weed Control Conf.*, p. 92 (1962).

(20) Orgell, W. H., "The Isolation and Permeability of Plant Cuticle," Ph.D. dissertation, Univ. of Calif., Davis, Calif., 1954.

(21) Pallas, J. E., Williams, G. G., *Botan. Gaz.* **123,** 175 (1962).

(22) Priestley, J. H., *Botan. Rev.* **9,** 593 (1943).

(23) Roberts, E. A., Southwick, M. D., Palmiter, D. H., *Plant Physiol.* **23,** 557 (1948).

(24) Roelofsen, P. A., *Acta Botan. Neerl.* **1,** 99 (1952).

(25) Schieferstein, R. H., "Development of Protective Structures of the Plant Cuticle," Ph.D. dissertation, Iowa State College, Ames, Iowa, 1957.

(26) Schieferstein, R. H., "Formation of Wax Deposits on Leaves," M.S. thesis, Iowa State College, Ames, Iowa, 1955.

(27) Schnepf, E., *Planta* **52,** 644 (1959).

(28) Van Overbeek, J., *Ann. Rev. Plant Physiol.* **7,** 355 (1956).

(29) Woodford, E. K., Holly, K., McCready, C. C., *Ann. Rev. Plant Physiol.* **9,** 331 (1958).

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