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Sorptive Properties of Plant Cuticle

Wallace H. Orgell
Iowa State College

Sorptive Properties of Plant Cuticle[1]

By WALLACE H. ORGELL

Before a foliar applied chemical can contact plant protoplasm, it must cross a barrier of cuticle, present on either the external (14) or internal (18) surfaces of the plant. However, little is known about the nature of the cuticle or its interactions with various chemicals.

Cuticle is generally considered to be lipoidal in nature. Crafts (2) has suggested that substances which can exist in an ionized (more polar) and in an unionized (less polar) form penetrate cuticle more readily in the less polar form. Several investigations have shown that acidic chemicals, e.g. the substituted dinitrophenols and carboxylic growth regulators, are most active at low pH values (3, 4, 6, 9, 20). Skoss (19) has postulated that water soluble substances do not readily penetrate intact external cuticle but must enter stomata before uptake will occur. It has been observed that certain spray solution additives, e.g. surfactants, acid salts, co-solvents, etc., can greatly alter plant response to a given chemical (3, 5, 6, 9, 10). These results and others have indicated that the cuticle may play an important role in the penetration of foliar applied chemicals. The present work was undertaken in order to learn something of the mechanisms by which chemicals interact with plant cuticle.

In this paper, the cuticle is considered to consist of the semi-lipoidal material exterior to the outer epidermal cell wall. Studies on the microscopic and submicroscopic structure of cuticle have revealed several interesting features (Fig. 1). Electron microscopy of the outer surface by a replica technique (11, 17) has demonstrated the presence of rodlets and granules of wax-like substances. These may play an important part in phenomena involving wetting of the plant surface. Macroscopic surface deposits of wax in the form of layers, plates, granules, and rods, e.g. bloom, are commonly observed. Lamellae of wax also occur within the body of the cutin layer (1, 7, 16). The chemical properties of cutin itself are not well known but it is believed to be a polymer consisting of oxidized unsaturated lipids (14), or a poly-ester (7). A layer of pectates is often noted at the boundary between the cuticle and the outer epidermal cell wall. Plasmodesma have been observed in the outer epidermal cell

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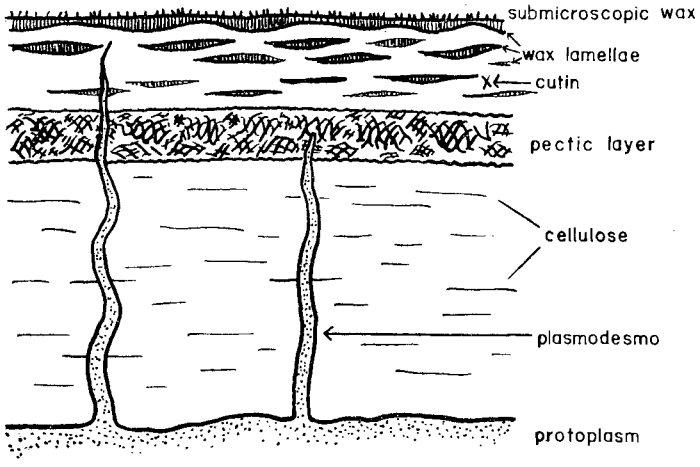

LEAF EPIDERMAL CELL, OUTER WALL (schematic)

Figure 1. Diagrammatic representation of a leaf outer epidermal cell wall and cuticle. Based on studies by Anderson (1), Frey-Wyssling (7), Lambertz (8), Mueller *et al.* (11), Roelofsen (16), and Schieferstein and Loomis (17). Not to scale.

wall (8), but it is not definite whether they extend into or influence the properties of the cuticle. Through the use of pectic enzyme solutions, the cuticle can be isolated in sheets large enough for permeability or sorption studies (12, 13). Substances may concentrate and become oriented at the interface between the cuticle and a solution by the process of adsorption, or they may enter into the bulk of the cuticle by the process of absorption. The term sorption includes both of these processes. Penetration refers to the movement of a substance completely through the cuticle.

Materials and Methods

Two experimental approaches were used in studying the interaction of plant cuticle and various chemicals. The first consisted of observing the penetration of aqueous solutes through isolated cuticle disks in a simple diffusion cell. The second consisted of measuring the sorption of various chemicals by isolated cuticle disks.

The diffusion cell was composed of isolated cuticle disks spread on filter paper supported by a 5 mm thick layer of 2% agar. Small droplets containing various chemicals in solution were placed on the cuticles, and after a period of time the filter paper and agar were examined for indications of penetration. Penetration was detected by the use of colored solutes, or by the formation of precipitates or

colors in the agar. Only aqueous solutions with a high interfacial tension against cuticle were tested by this method.

Two types of sorption experiments were carried out. In the first, or qualitative type, the effects of various conditions and chemicals upon the sorption of dyes, fluorescent compounds, and radioactive substances were noted by observing cuticles which had been gently agitated for a given period of time in solutions of the substance under study. The average amount of substance present on or within the cuticles was estimated by a subjective rating system in which no staining was indicated by zero, and intense staining by ten. In the second, or semi-quantitative type, the rate at which isolated cuticles

Figure 2. Sorption of acidic and basic dyes at various pH values by isolated apricot cuticles.

sorbed a substance was determined by measuring the rate at which the substance was removed from the solution surrounding the cuticles.

In the qualitative experiments, a certain number of cuticles were placed in vials, each containing a known concentration of substance to be sorbed, and adjuvants of various types (buffers, surfactants, etc.). The vials were placed on a slow rotary shaker (100 rpm—2.5 cm radius of rotation), and 24 to 48 hours were allowed for sorption to take place. The cuticles were then removed from solution, uniformly rinsed free of excess substance (dye 2,4-D-C^{14},

etc.) and placed with a glass loop on filter paper supported by 2% agar. After moisture equilibrium was obtained, the paper and adhering cuticles were stripped from the agar and dried at room temperature for examination or radioautography.

In the semi-quantitative sorption experiments, a certain number of cuticles were placed in vials containing a known concentration of dye. The decrease in dye bath concentration with time was followed colorimetrically and the approximate amount of dye sorbed by the cuticles was calculated. As an example, 6 isolated cuticle disks might be added to 5 ml of an aqueous 0.01% dye solution. A corresponding vial without cuticles would also be prepared. After 24 hours at 25° C. colorimeter measurements would be made on both vials. The difference in readings could be related to the amount of dye sorbed by the cuticles. Treatments were run in duplicate or triplicate.

Cuticle disks, 1.8 centimeter in diameter, from the upper leaf epidermis of mature apricot leaves were used in most of these experiments because (a) stomata are not present, (b) the cuticle is easily isolated with pectic enzymes, and (c) isolated cuticles from the upper and lower leaf epidermis can be easily distinguished without microscopic inspection.

Carbon-14 labeled urea and 2,4-dichlorophenoxyacetic acid (2,4-D) labeled with C^{14} in the carboxyl group were employed in some of the experiments. Concentrations used were comparable to those of the dyes. After the period of sorption, the rinsed and dried cuticles were radioautographed against Kodak No-Screen X-ray film. The density of the resulting cuticle image was then rated in the same manner as the dyes, i.e., zero for no image to 10 for a dark black image. Acid substances were generally used in the form of their sodium salts.

Experimental Results

In experiments with the penetration cells, it became apparent that about 90% of the isolated cuticle disks employed contained imperfections such as insect punctures, cracks, etc., which permitted rapid penetration of the applied droplet. Hence conclusions were necessarily limited to those cases where penetration of a given cuticle disk or group of disks did not occur. Cuticles from the lower epidermis contained stomatal pores which also permitted rapid penetration. Polar solutes in aqueous solution, e.g. acids, bases, salts, dyes and miscellaneous other compounds, did not penetrate uninjured upper apricot cuticles in visually detectable quantities in 48 hours or longer.

Qualitative sorption experiments indicated that the sorption of 2,4-D acid as well as anionic and cationic dyes was sufficient, within

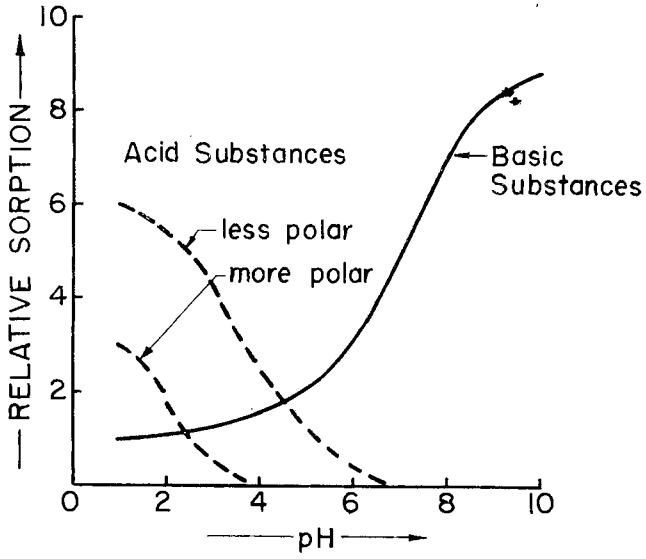

Figure 3. Generalized qualitative relation between the sorption of acidic and basic substances by isolated plant cuticle and the pH of the sorption bath.

approximately 24 hours at room temperature, to clearly demonstrate the effects of various additives. Brief rinsing of treated cuticle disks with distilled water removed adhering sorption bath liquid without permitting significant desorption.

Acid substances, e.g. eosin Y, 2,4-dinitrophenol, orange G, fast green FCF, aniline blue and 2,4-D acid, were sorbed to the greatest extent at acid pH values. Basic substances, e.g. rhodamine B, crystal violet, safranin A, nile blue sulfate, acridine orange and Amine 220 (Carbide and Carbon Co.—a basic interfacially active substance) were sorbed particularly well at alkaline pH values (Fig. 3). At pH values of 8 to 10, six isolated apricot cuticle disks removed practically all of certain basic dyes (e.g. crystal violet and acridine orange) from 5 ml of 0.01% dye solution. Other basic dyes, e.g. safranin A, were not sorbed so strongly (table 1). The particular shape and position of the sorption vs. pH curve depended upon the polarity of the substance involved. For example, appreciable sorption of relatively polar dyes (e.g. orange G) occurred only at pH values 2 or 3 units below that at which sorption of less polar dyes (e.g. eosin Y) was evident. (Fig. 3.) The behavior of eosin Y in sorption experiments very closely paralleled that of 2,4-D acid. With both substances, there was no visible sorption at pH 7 or higher, slight sorption at pH 6, appreciable sorption at pH 5, and strong sorption at lower pH values (table 2). Both compounds contain a carboxyl group (Fig. 4) which probably determined the effect of pH on the polarity of the molecules in these experiments.

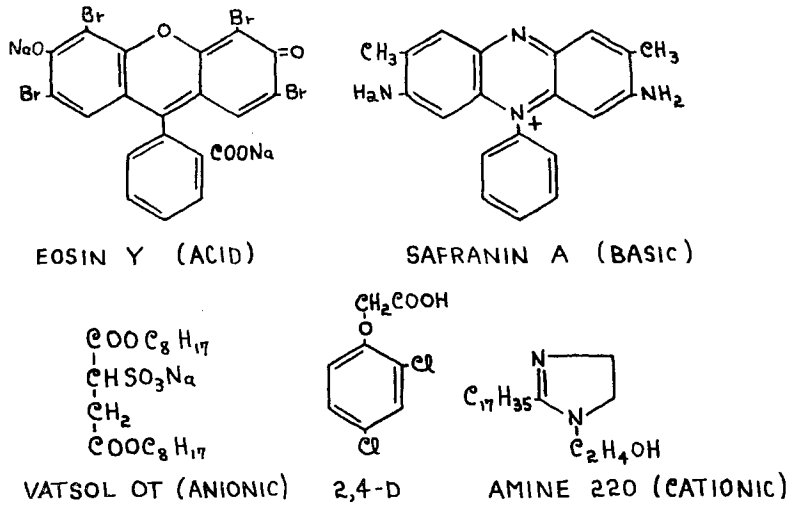

Figure 4. Structural formulae of some of the sorbants and adjuvants mentioned in the text.

The sorption of urea was negligible in most instances, and was not markedly affected by pH (table 2). Acetate buffer at pH 3 and at sufficiently high concentrations (0.5 M) slightly inhibited the sorption of 0.01% eosin Y or saturated 2,4-D acid in aqueous solution, perhaps by competition for sorption sites. Cuticles from the lower epidermis of several species sorbed slightly more eosin Y than those from the upper epidermis. Xylene-extracted apricot cuticle sorbed much more eosin Y than non-extracted cuticle. Neither waxed paper or filter paper sorbed eosin Y to the same extent as isolated cuticle.

A number of miscellaneous additives were tested at 1 to 10% (w/v) in acetate buffer at pH 5 to determine their effects on the sorption of eosin Y and safranin A by apricot cuticle. Certain organic bases, e.g. 2-aminoethanol, ethylenediamine, 3-aminopropanol, and 2-dimethylaminoethanol, ethylendiamine, 3-aminopranol, sorption of eosion Y but greatly increased the sorption of safranin A. Furfurylamine apparently formed a salt with eosin Y which then precipitated on the cuticles. Lauryl isoquinolium bromide and a surface active imidazoline (Amine-O, Alrose Chemical Co.) pre-

Table 1

Effect of pH on the Per Cent of Safranin A Removed from 22 ml of a 0.00150% Solution by 5 Isolated Apricot Cuticle Disks

Sorption Time	pH									
	1	2	3	4	5	6	7	8	9	10
	per cent									
24 hrs.	1.0	2.7	4.2	4.5	9.9	15.3	24.9	35.0	52.4	49.5
210 hrs.	2.1	4.1	4.9	4.6	11.0	22.0	35.6	61.5	69.8	73.7

Table 2
Effect of pH and Surfactants on Sorption by Isolated Apricot Leaf Cuticle Disks*

	Eosin Y**			Safranin A				
pH	alone	+Amine 220†	+Vatsol OT	alone	+Amine 220†	+Vatsol OT	2,4-D-C^{14}	Urea-C^{14}
1	5	5	1	2	1	7	..	..
2	6	1	1	3	1	5	..	..
3	6	2	1	4	1	5	7	1
4	8	2	2	5	1	5	6	1
5	5	2	5	6	2	5	5	1
6	2	2	0	7	3	5	2	1
7	0	2	0	8	..	6	0	1
8	0	..	0	8	9	6	0	1
9	0	2	0	8	10	6	0	1
10	0	2	0	8	10	6	..	..

*Initial sorbant concentration 0.01% (w/v); initial surfactant concentration 0.1%; staining time for dyes 48 hrs., for 2,4-D and urea, 2 wks.; temperature 25° C.; buffer at pH 3-6 acetate, pH 7-8 phosphate, pH 9-10 borate. Intensity of staining (sorption) estimated from 0 (no staining) to 10 (most intense staining).

**Eosin Y precipitated from solution at pH values less than 4.

†Amine 220 and phosphate buffer formed a precipitate at pH 7 and 8.

vented the sorption of safranin A and inhibited the sorption of eosin Y.

The interaction of various types of surfactants and pH resulted in striking effects upon the sorption of acid and basic dyes, and 2,4-D acid (table 2). An anionic surfactant, Vatsol OT (Carbide and Carbon Co.), at 0.1% (w/v) increased the sorption of 0.01% safranin A at pH values 1 to 3 and decreased sorption from pH 5 to 10 (relative to sorption in the absence of the surfactant). Vatsol OT decreased the sorption of eosin Y and 2,4-D acid at pH values lower than 4. A cationic surfactant, Amine 220 (Carbide and Carbon Co.), at 0.1% decreased the sorption of safranin A at pH values lower than 8 and increased sorption at higher values. Below pH 6, Amine 220 decreased the sorption of eosin Y and 2,4-D acid whereas above pH 6, sorption of these substances was increased. It was noted that 0.1% Amine 220 increased markedly the sorption of 2,4-dinitro-6-sec-butylphenol at pH values higher than 7. Without the Amine 220, sorption of this substance at pH 7 was slight. A nonionic surfactant, Tween 81 (Atlas Powder Co.), at 0.1% decreased slightly the sorption of 2,4-D acid at pH 3 and had very little effect at pH 9. The only instance in which urea was sorbed to any appreciable extent was at pH 3 with the addition of 0.1% Vatsol OT. As might be expected, desorption of substances from the isolated cuticles occurred most rapidly under conditions opposite to those which favored their sorption, e.g. eosin Y (sorbed at pH 3) was desorbed rapidly by 95% ethanol or in alkaline solution. Eosin Y sorbed at pH 7 with the aid

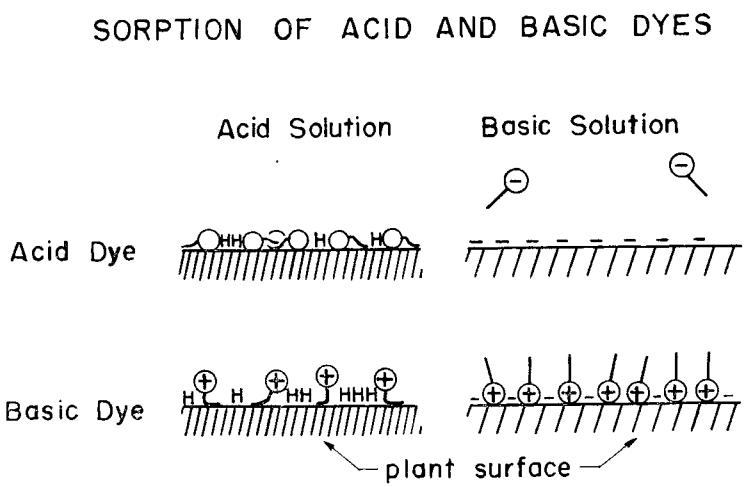

Figure 5. Schematic representation of the interaction of molecules of acidic and basic substances and the plant or isolated cuticle surface when in acid (below pH 5) or alkaline (above pH 7) solutions. The sorbant molecules are pictured as having an ionized or ionizable hydrophilic "head" and a hydrophobic "tail." The cuticle is shown as contracted and neutral in acid solution, and expanded and negatively charged in aklaline solution.

of 0.1% Amine 220 was desorbed rapidly at the same pH in a solution of 0.1% Vatsol OT.

Discussion and Conclusions

Experiments with penetration cells (containing 2% agar as the receiving medium) demonstrated that substances in aqueous solution either did not penetrate intact isolated apricot cuticle disks, or penetrated very slowly. This does not indicate that such substances do not penetrate intact cuticle on the plant, since the nature of the receiving medium of the plant must be considered. Another factor is that the cuticles of some species may be perforated to such an extent with insect injuries or cracks that the permeability of the intact portions of the cuticle is of little practical consequence. It is also possible that hydrophilic lamellae may be present in the cuticle of certain plants (15). These might provide a pathway for the penetration of polar materials.

The effects of pH on the sorption of acid and basic substances can be interpreted by assuming that the cuticle surface is semi-lipoidal and weakly acidic (Fig. 5). At low pH values (below pH 5) the cuticle would be relatively uncharged. Acid substances (e.g. acid dyes, 2,4-D acid, and the dinitrophenols) would be relatively undissociated and their sorption would depend upon their interfacial activity, or capacity to form proton or van der Waals' bonds with the cuticle surface, as well as their solubility in certain phases of the

cuticle. A basic substance (e.g. a quaternary ammonium dye) would be positively charged and relatively polar at a low pH, and hence sorption to the relatively unionized cuticle would not be great. At higher pH values (above pH 6) both cuticle and acid substances would be negatively charged and electrostatic repulsion could hinder or prevent sorption. On the other hand, a basic quaternary ammonium dye would remain positively charged and electrostatic attraction would result in very pronounced sorption similar to salt formation. With respect to these properties, the cuticle resembles a semi-lipoidal cation exchange membrane.

Mechanisms based on concepts of competition and solubilization can be postulated to explain the interacting effects of pH and surfactant type on the sorption of acid and basic compounds (12). However, more experimental evidence is required before any of these can be demonstrated with certainty. It is apparent that each new substance introduced into a spray mixture increases the complexity of sorption and penetration relationships many fold. One must also consider that the effects of various factors on sorption do not necessarily parallel their effects on penetration.

Summary

Preliminary studies have been made on the interaction of isolated plant cuticles and foliar applied chemicals. Experiments with penetration cells indicated that polar substances in aqueous solution move very slowly, if at all, through intact apricot cuticle and into a layer of agar gel. It was noted that isolated cuticles of several species exhibited numerous minute perforations and cracks.

Qualitative and semi-quantitative studies were made on the sorption of acid and basic dyes, radioactive 2,4-D and radioactive urea, by disks of isolated apricot leaf cuticle. The pH of solution and type of surfactant were outstanding factors modifying sorption of acid and basic substances. Acid substances were sorbed best at acid pH values, and basic substances best at basic pH values. Both an anionic and a cationic surfactant hindered sorption of acid substances at low pH values. The cationic surfactant increased sorption of acid substances at high pH values. An anionic surfactant increased sorption of a basic dye at low pH values and decreased its sorption at high pH values. A cationic surfactant had the opposite effect.

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Department of Zoology and Entomology
Iowa State College
Ames, Iowa

