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FOLIAR APPLICATION

Effects of Chloroform and Surfactants on Permeability of Apricot Leaf Cuticle

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A penetration test with plant leaf cuticle membranes was used to measure the effect of surfactants (anionic, cationic, and nonionic) on the permeability of leaf cuticle to a slowly penetrating compound (sucrose) and a rapidly penetrating compound (*N*-isopropyl-α-chloroacetamide). Mixing surfactants with these compounds altered their penetration little, if at all, and even prior soaking of the cuticle disks in 1% solutions of the surfactants or sodium hydroxide increased the penetration of the α-chloroacetamide slightly in only one case. Prior soaking of the cuticle disks in chloroform, however, increased the permeability of leaf cuticle to the acetamide markedly. It is concluded that although cuticle permeability can be modified, the surfactants currently used commercially in pesticide formulations do not exploit the opportunity to alter the permeability of hydrated leaf cuticle.

THE successful marketing of agricultural chemicals administered by foliar application depends on effective formulation. Subsequent to the requirements for storage stability, dispersion, and surface coverage, a requirement for optimal interaction with the plant surface is reached. For herbicides, defoliants, and systemic pesticides, maximum penetration of the toxicant is desired, whereas for surface protectants, poor penetration can minimize pesticide dilution, phytotoxicity, and residue problems.

Surfactants are widely used in pesticide formulating since enhancement of herbicidal effectiveness by surfactants has been amply demonstrated (*1, 3*), but the reason for the effect is not clear. In an extensive comparison of surfactant effects, Jansen, Gentner, and Shaw (*9*) observed three types of surfactant-herbicide interaction. Enhancement of herbicidal activity was most common, but in some cases the surfactants were ineffective, and in others suppressed activity. In a study of the phytotoxicity of surfactants, Furmidge (*5–7*) suggests that penetration of the wax and cutin layers of the leaf is a prerequisite, and Freed and coworkers (*4, 8*) emphasize the specificity of surfactant-herbicide interaction required for maximum effectiveness.

In this work, a previously described (*2*) penetration test was used to determine if surfactants altered the permeability of leaf cuticle.

Experimental

The methods used have been described in detail (*2*). Apricot (*Prunus armeniaca* L.) leaves were procured locally and 1000 upper apricot leaf cuticle disks produced. Approximately 750 were expended in this work.

The radioactive compounds were reserves of those used in the earlier work (*2*).

The surfactants used, described in Table I, included anionic, cationic, and nonionic types. A comparison of their effects on surface tension (Table I) shows no large differences in this property. Anionics included an alkyl sulfonate, an alkyl sulfate, and an alkyl benzene sulfonate, and the cationics were represented by the quaternary ammonium compound. The nonionics are the surfactants of choice for commercial pesticide use because of their low phytotoxicity (*7*), and the five tested included a commercial alkylaryl polyoxyethylene glycol formulation (Colloidal Products X-77); Sterox NJ (nonylphenyl polyoxyethylene ether) which Monsanto sells for pesticide formulation and use; and Sterox SK (dodecyl polyoxyethylene thioether) which has shown promise in USDA greenhouse evaluations (*9*).

The tests were made with two C^{14}-labeled compounds, sucrose being representative of slowly penetrating compounds, and *N*-isopropyl-α-chloroacet-

Table I. Surfactants Used and Surface Tension of 0.1% Solutions

Surfactant	Manufacturer[a]	Trade Name	Dynes/Cm. R. T.[b]
Sodium *n*-dodecane sulfonate	1		26.1
Sodium *n*-dodecylbenzene sulfonate	1		33.9
n-Dodecyl polyoxyethylene ether	1		34.0
Sodium dodecyl sulfate	2	Sipon WD	33.3
Cetyltrimethylammonium bromide	3	T-5650	31.0
Blend of alkylaryl polyoxyethylene glycols, free fatty acids, 2-propanol	4	X-77	30.0
Dodecyl polyoxyethylene thioether	1	Sterox SK	29.1
Dodecylphenyl polyoxyethylene ether	1	Sterox DJ	29.7
Nonylphenyl polyoxyethylene ether	1	Sterox NJ	32.0

[a] 1. Monsanto Co., St. Louis, Mo. 2. Alcolac Chemical Corp., Baltimore, Md. 3. Distillation Products Industries, Rochester, N. Y. 4. Colloidal Products Corp., Sausalito, Calif.
[b] Data supplied by T. B. Hilton, Inorganic Division, Monsanto Co.

Table II. Surfactant Effect on Penetration of Sucrose through Apricot Leaf Cuticle

Surfactant Addition[a]	Intact Cuticles	Sucrose Penetration, %
None	6	2.12 ± 0.71[b]
	3	2.91 ± 0.81
Sodium *n*-dodecane sulfonate, 0.1%	8	1.88 ± 0.48
n-Dodecyl polyoxyethylene ether 0.1%	9	2.52 ± 0.92
1.0%	1	2.79
Sodium dodecyl sulfate 0.1%	7	2.12 ± 0.72
0.4%	3	2.07 ± 0.52
0.8%	2	3.15 ± 0.07
1.2%	3	2.02 ± 0.94
4.0%	2	2.48 ± 0.27
1% X-77 blend (see Table I)	4	2.00 ± 0.44
Dodecyl polyoxyethylene thioether, 1%	1	1.57
Dodecylphenyl polyoxyethylene ether, 1%	8	1.67 ± 0.89
Nonylphenyl polyoxyethylene ether, 1%	2	2.00 ± 0.17

[a] Surfactant added to upper agar cylinder with $10^{-3}M$ sucrose and 50 mg. per liter disodium fluorescein. Diffusion time 48 hours at 30° C.
[b] Standard deviation.

Table III. Surfactant Effect on Penetration of *N*-Isopropyl-α-chloroacetamide through Apricot Leaf Cuticle

Surfactant[a]	Intact Cuticles	Amide Penetration, %
None	17	16.35 ± 0.94
	10	15.51 ± 1.04
1.0% Sodium *n*-dodecane sulfonate	20	14.48 ± 1.02
1.0% *n*-Dodecyl polyoxyethylene ether	20	14.50 ± 1.26
1.0% Sodium dodecyl sulfate	20	12.47 ± 0.79
1.0% X-77 blend (see Table I)	10	15.18 ± 0.97
1.0% Dodecyl polyoxyethylene thioether	10	17.30 ± 1.98
1.0% Dodecylphenyl polyoxyethylene ether	10	17.45 ± 0.90
1.0% Nonylphenyl polyoxyethylene ether	10	16.63 ± 1.92

[a] Surfactant added to upper agar cylinder with $10^{-3}M$ *N*-isopropyl-α-chloroacetamide and 50 mg. per liter of disodium fluorescein. Diffusion time 4 hours at 30° C.

Table IV. *N*-Isopropyl-α-chloroacetamide Penetration through Soaked Apricot Leaf Cuticle[a]

Surfactant	Intact Cuticles	Penetration, %
None (Table III)		
1% Sodium *n*-dodecane sulfonate	10	19.97 ± 1.87
1% Sodium *n*-dodecylbenzene sulfonate	10	15.91 ± 0.78
1% *n*-Dodecyl polyoxyethylene ether	10	16.51 ± 0.95
1% Sodium dodecyl sulfate	10	16.34 ± 0.55
1% Cetyltrimethylammonium bromide	9	16.92 ± 1.49
1% X-77 blend (Table I)	10	15.41 ± 1.35
1% Dodecyl polyoxyethylene thioether	9	16.61 ± 1.11
1% Dodecylphenyl polyoxyethylene ether	8	15.76 ± 1.03
1% Nonylphenyl polyoxyethylene ether	10	15.77 ± 0.53

[a] Leaf cuticle disks soaked 4 days, washed, and tested. Each upper agar cylinder contained $10^{-3}M$ *N*-isopropyl-α-chloroacetamide and 50 mg. per liter of disodium fluorescein. Diffusion time 4 hours at 30° C.

amide being one of the fastest penetrating of the series of α-chloroacetamides tested previously (*2*). All other chemicals used were as specified previously (*2*).

Assay of Radioactivity. Radioactivity was assayed in a Packard-Tricarb liquid scintillation spectrometer (*2*).

Preparation of leaf cuticle was as previously described (*2*).

Cuticle Penetration Test. When surfactant was present, a number of cuticle disks were placed on agar cylinders. On top of each cuticle was then placed a smaller agar cylinder containing the radioactive penetrant, the additive being tested, and the fluorescein dye. After a suitable incubation period (48 hours for sucrose; 4 hours for *N*-isopropyl-α-chloroacetamide) in an incubator at 30° C., the upper agar cylinders, cuticle disks, and lower agar cylinders were separated. The lower agar cylinders were examined under ultraviolet light for dye penetration, and the acceptable (less than 0.2 mg. per liter of dye penetration) upper and lower agar cylinders were placed in dioxane scintillation solvent for radioactivity assay. The per cent of test compound that penetrated to the lower cylinder was then calculated.

In the pretreatment experiments the cuticle disks were soaked in the appropriate solution for 4 days before testing, then removed from the pretreatment solution, rinsed with water, and tested as previously described (*2*).

The size of the two agar cylinders was such (upper, 14-mm. diameter, 2 mm. thick; lower, 21 × 2 mm.) that equilibrium is reached at about 70% penetration. Recoveries were again calculated.

Results and Discussion

The activity of surfactants in increasing herbicidal effectiveness on whole plants does not correlate with any single property of surfactants (*9*). Currier and Dybing (*1*) have illustrated the complexity of the problem by listing the suggested ways through which a surfactant effect could be expressed. It could be the result of: inducing stomatal entry; facilitating cell wall movement in the region of the wall-cytoplasm interface; increasing the permeability of the plasma membrane through stimulation or incipient toxicity; improving coverage; removing the air film between spray and leaf surface; acting as humectants secondarily; acting as cosolvents; reducing interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle; or interacting directly with the herbicide in some manner.

The first seven of these variables in the complex plant system are eliminated in the cuticle disk test, which also has the following advantages. It is a direct test giving a quantitative result; the penetration area is constant; humidity and temperature are controlled; the upper apricot leaf cuticle is free of stomata; the system is inanimate; and replicates with defective cuticles or leaks over the cuticle edges are rejected by the dye test.

In other words, the cuticle disk test with upper apricot cuticle should be a direct measure of the effect of surfactants on cuticle penetration.

Surfactant Present during Test. In Table II the effect of surfactant incorporation in the upper agar cylinder is shown on the penetration of sucrose. The *n*-dodecylbenzene sulfonate was not tested because of limited solubility, and cetyltrimethylammonium bromide could not be tested because of interaction with the agar. Although the surfactants tested included several types and one (sodium dodecyl sulfate) was tested over a 40× range of concentrations, none altered the penetration of the sugar through the leaf cuticle disks. Similarly, Table III records only slight changes in the penetration of *N*-isopropyl-α-chloroacetamide. Under these conditions the surfactants did not alter the penetration of the slowly penetrating sucrose, and had little effect on the rapidly penetrating α-chloroacetamide.

The low incidence of acceptable replicates in the sucrose tests (Table II) contrasts with the high numbers in the α-chloroacetamide tests (Table III), and is a result of the difference in test time. Fluorescent dye penetration is too slow for any but gross leaks to be perceptible in the 4-hour test, even though surface creeping becomes a problem at high

Table V. Penetration of *N*-Isopropyl-α-chloroacetamide through Chloroform- or Alkali-Soaked Apricot Leaf Cuticle

Cuticle Treatment	Intact Cuticles	Penetration, %
None	10	15.5 ± 1.05
$CHCl_3$[a]	5	63.5 ± 5.24
1% NaOH[b]	5	16.6 ± 1.12

[a] Presoaked 4 days in $CHCl_3$.
[b] Presoaked 4 days in 1% NaOH.

surfactant concentrations. The dye test, however, also eliminates this variable.

Pretreatment with Surfactant. Since little change in permeability was observed in the conventional test, leaf cuticle disks were soaked for 4 days in 1% solutions (or suspensions) of the surfactants at 25° C. After removal and rinsing with water, the cuticle disks were tested in the normal manner. The low incidence of defective disks by the dye test showed that gross damage to the cuticle had not occurred. Table IV also shows that only the treatment with sodium *n*-dodecane sulfonate significantly altered the permeability of the cuticle disks to the test compound *N*-isopropyl-α-chloroacetamide, and the increase was not large.

Pretreatment with $CHCl_3$ and NaOH. To show that removal of cuticular waxes would increase permeability, cuticle disks were soaked in chloroform, a good solvent for cuticular waxes (*10*), and in 1% NaOH. Although the latter treatment was without effect, soaking in chloroform increased the penetration of *N* - isopropyl - α - chloroacetamide from 16 to 64%—almost the equilibrium distribution—in the 4-hour test period (Table V). The permeability to the dye, however, was increased only slightly.

These results show that the surfactants tested, including commercial types, do not increase the permeability of hydrated leaf cuticle, and are not good solubilizers of leaf waxes. The result with chloroform shows that inclusion of a solubilizer in pesticide formulations where penetration is desired could increase at least tenfold the penetration rate and activity of nonpolar compounds. The lack of excessive penetration of the fluorescein dye after chloroform treatment of the cuticle suggests that cutin is a major barrier to the penetration of cuticle by polar compounds. However, it is not established that the chloroform treatment will completely remove nonpolar constituents embedded or enmeshed intimately in the cutin framework.

A possible disadvantage of the cuticle disk test as used here is that the cuticle disks are maintained and tested in aqueous media and therefore represent fully hydrated cuticle. Since hydrated cuticle is considered more permeable than that of plants under water stress (*1*), surfactants could affect cuticle permeability in the field by influencing the degree of hydration of cuticular tissue. Similarly, the 40× concentration range of sodium dodecyl sulfate tested in Table II is only an approximation of the field situation where evaporation exposes the cuticle to increasing concentrations of surfactant.

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TOBACCO SUCKER CONTROL

Inhibition of Tobacco Axillary Bud Growth with Fatty Acid Methyl Esters

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Experiments were conducted employing methyl esters of fatty acids with chain lengths from C_6 to C_{18} for the inhibition of axillary bud growth of tobacco. Results from four tobacco types showed that the methyl esters of fatty acids with eight to 14 carbon atoms gave a high degree of inhibition. Methyl pelargonate and undecenoic acid were also effective. Generally, the methyl ester of the C_{10} acid was the most effective, effectiveness gradually being reduced with either increased or decreased carbon chain lengths. The exact mode of action of these fatty acid esters is not yet known. Only the meristematic and differentiating cells of axillary buds were destroyed when they came in contact with these fatty acid esters.

In tobacco production, decapitation is a necessary process in obtaining leaves with desired physical properties and chemical composition. The consequent growth of axillary buds, or "suckers," and the problem of inhibiting such growth are of academic interest and economic importance. A number of compounds, synthetic or naturally occurring, are known to be effective inhibitors of tobacco sucker growth (*3, 8, 9*). Some compounds, however, induce further metabolic changes which are considered undesirable to leaf quality (*2*). Since many fatty acids and esters have been found in tobacco plants (*4, 6*), compounds of this group, if effective as sucker control agents, would be more desirable than other compounds which are entirely foreign to the tobacco plants. Methyl esters of fatty acids with various carbon chain lengths were used in this experiment to study their effectiveness in sucker growth inhibition.

Materials and Methods

Four major types of tobacco (*Nicotiana tabacum* L.) including Hicks, Catterton, Burley 21, and Connecticut Broadleaf were used. These plants were grown