Penetration of $^{14}$C-labeled plant growth regulators into the surface of apple (Malus pumila M.) leaves pre-soaked in 10 ml l$^{-1}$ solutions of surfactant was measured by detecting changes in radioactivity of the solution in glass cylinders sealed to the stomatal, adaxial surface of the leaf discs. Penetration of 2,4-D [(2,4-dichlorophenoxy)acetic acid] and β-NAA (2-naphthaleneacetic acid) was significantly increased by surfactant Pegosperse 200-ML (polyethylene glycol monolaurate) but unaffected by four other surfactants with the same hydrophilic-lipophilic balance (HLB) as Pegosperse 200-ML. Penetration of 2,4-D and β-NAA decreased as the HLBS of Pegosperse (polyethylene glycol or PEG) surfactants increased. Penetration of maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) was unaffected by any surfactant tested.

To understand surfactant-cuticle interactions, enzymatically-isolated cuticles of apple leaves were soaked in PEG 100-O (diethylene glycol oleate) solution and the weight change of cuticles or the surface tension change of the solutions
was measured to determine the amount of surfactant sorbed or desorbed by the cuticles. PEG 100-O sorption and desorption of both cuticular membranes (CMs) and dewaxed cuticular membranes (DCMs) occurred primarily in the first three hours. The sorption was concentration-dependent and the DCMs always sorbed more surfactant than the CMs. PEG 100-O could be completely desorbed from both CMs and DCMs.

The impacts of surfactant on cuticular penetration of 2,4-D were studied by placing $^{14}$C-2,4-D (0.78 mM) in cylinders affixed to the external surface of cuticles after the cuticle segments had been treated with 10 ml l$^{-1}$ of various PEG surfactant. The same surfactant pretreatments were included in sorption studies in which cuticle segments were immersed in $^{14}$C-2,4-D solution. Penetration of 2,4-D were inversely related to the HLB of the surfactant. Sorption of 2,4-D by CMs was unaffected by surfactant pretreatment. PEG 100-O significantly increased cuticular penetration of dissociated 2,4-D at pH 4.5. The enhanced penetration occurred only when surfactant was in the cuticles. Results of this study support the 'hydrophilic channel' hypothesis, viz. that surfactant may induce ‘hydrophilic channels’ formation in the cuticle, consequently enhancing the passing of polar molecules of pesticides through the cuticle.
Role of Surfactants in Foliar Uptake of Plant Growth Regulators

By

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A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Completed July 2, 1992

Commencement June 1993
ACKNOWLEDGMENTS

Sincere appreciation is expressed to Dr. Garvin D. Crabtree, my major professor, for his understanding, guidance, encouragement, and constant help during my study at Oregon State University.

Special thanks go to the members of my graduate committee: Drs. Arnold P. Appleby, Anita N. Azarenko, Leslie H. Fuchigami, Michael V. Martin, and Thomas C. Moore for their advice and support.

I am also grateful to Drs. William S. Braunworth, Jr. and Ray D. William for their help in many ways during my study at OSU and to Drs. Patrick J. Breen and David W. S. Mok who provided laboratory facilities for the work with radioactive chemicals.

Thanks to many faculty and graduate students in Horticultural Department and the Campus Weed Group for their friendship, encouragement, and help during my graduate study at OSU.

Thanks to my family and parents for their understanding, love and constant support.
NOTES

Chapter 3 was written in the format of CRC Press Inc., Boca Raton, Florida.

Chapter 4-6 were written in the format of Pesticide Science.
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ROLE OF SURFACTANTS IN FOLIAR UPTAKE OF PLANT GROWTH REGULATORS

CHAPTER 1
INTRODUCTION

Agrichemicals, including pesticides, play very important roles in the agricultural industry. They counted for 8% of farm production expenses in the USA in 1988 (USDA, 1990). Inadequate penetration of pesticides into plants represents a direct financial loss to farmers and a potential contribution to environmental pollution (Price, 1982). Maximizing pesticide efficacy potential and minimizing pesticide costs and residues in the environment are great challenges for scientists and the agricultural industry.

One approach is to use surfactants as adjuvants to improve pesticide efficiency and efficacy, consequently reducing pesticide residues and costs without sacrificing pesticidal potential (Bayer, 1965). Surfactants are capable of not only enhancing efficacy of pesticides but may also be instrumental in the development of new uses for existing pesticides (Bayer, 1965; Gillard, 1987; Foy, 1989a). For example, some herbicides such as diuron that formerly showed little or no foliar activity and were applied to the soil for root uptake have been used effectively with surfactants as postemergence, foliar applied herbicides (Foy, 1989a). Consistency in responses for some pesticides such as plant growth regulators has been a limiting factor for their commercial use (Bukovac, 1985).
Surfactants are shown to reduce the impact of interacting environmental factors and increase the consistency of response of foliar applied chemicals (Hughes and Freed, 1961; Stickle, 1989). Surfactants are also able to broaden the spectrum of control with pesticides (Bayer, 1965).

Foy (1989b) pointed out that chemical additives including surfactants hold the promise for a virtual revolution in the way in which agricultural chemicals, particularly pesticides, are used in plant protection. Studying roles of surfactants in pesticide application will enable us to use existing pesticides more effectively and economically, with fewer problems created by residues. Moreover, studying the interaction of surfactants and pesticides will help us achieve a better understanding of the general interactions between agrichemicals.

Surfactants, also called surface active agents, are a group of chemicals with a hydrophilic moiety and a lipophilic moiety in their molecular structure. Surfactants reduce the surface tension of a pesticide solution, thereby improving the foliar coverage by the solution and consequently increasing the opportunity for pesticides to penetrate into leaves. However, this is not the only role of surfactants in the absorption process (Westwood and Batjer, 1958; Smith et al., 1966). There is evidence that the surfactant concentration required to achieve maximum foliar absorption of a pesticide is higher than that required to achieve minimum surface tension (Foy and Smith, 1964). This finding indicates that effects of surfactants other than reducing surface tension must be involved in penetration enhancement.
Several other roles of surfactants in foliar uptake of pesticides, besides improving spray coverage, have been proposed: (1) react directly with pesticides, (2) act as solubilizing agents (3) serve as humectants, (4) prevent or increase degradation of pesticides, (5) act as stickers, (6) act as co-penetrators, (7) increase stomatal entry, (8) increase the permeability of leaf cuticles, (9) facilitate pesticide movement in cell walls, (10) increase the permeability of plasma membranes, and (11) modify pesticide translocation inside plants (Currier and Dybing, 1959; Parr and Norman, 1965; Sargent, 1965; Foy, 1969; Seaman, 1982; Anderson and Girling, 1983). There is some overlapping in these roles as listed. Among the layers (cuticle, cell wall, and cell membrane) of the leaf surface, the cuticle is not only the first barrier for foliar penetration but also the only layer to be considered important (Schonherr and Riederer, 1989).

Chow and Grant (1989) pointed out that it is impossible to evaluate every surfactant-pesticide combination. Understanding the mechanism of surfactant effects on pesticide penetration will be able to predict the optimum surfactant-pesticide combinations and reduce costs of developing pesticide formulations.

However, the mechanism by which surfactants facilitate foliar entry of pesticides has received little attention (Kirkwood, 1987), and research on the mechanism of surfactant-enhanced penetration or on the nature of surfactant interaction with the cuticular membranes is limited (Shafer and Bukovac, 1987). To better understand how surfactants enhance cuticular penetration of plant growth regulators is the objective of this dissertation.
1. FOLIAR UPTAKE OF PESTICIDES AND MAJOR INFLUENCING FACTORS

1.1. Leaf surface structures and routes for pesticide penetration

The plant leaf surface is covered by a cuticle layer consisting of waxes and cutin. The cuticle is separated from the outer walls of underlying epidermal cells with a pectin layer (Martin and Juniper, 1970). A pesticide must penetrate the cuticle layer and then be translocated in either the apoplast, symplast or both to reach its site of action inside the plant (Robertson and Kirkwood, 1969; Haynes and Goh, 1977; Ashton and Crafts, 1981).

There are two proposed routes through the leaf surface layers by which exogenous molecules move into the living inner cells, a lipoid route and an aqueous route (Crafts and Foy, 1962; Foy, 1964; Ashton and Crafts, 1981; Hull et al., 1982). The lipoid route consists epicuticular waxes and cuticular wax lamellae in the cuticle, while the aqueous route consists of cracks and semi-polar cutin of the cuticle and the cell walls.

The aqueous pathway has been confirmed by several researchers (Schonherr and Bukovac, 1970; Hoch, 1979). Ectodesmata, a cuticle-originated structure as Bukovac (1970) believed or a cell-wall-determined structure as Franke (1967)
suggested, serves as a polar bridge across the chiefly lipoidal cuticular membrane (Schonherr and Bukovac, 1970). Hoch (1979) observed polysaccharide microfibrils in apple leaf cuticle. Their origin is at the outer boundary of the epidermal cell wall. They occasionally extend to the cuticle surface beneath epicuticular wax and serve as a pathway for polar transport.

Lipophilic chemicals follow the lipoidal route and can easily penetrate the waxy barrier and move through the lipophilic portions of the cutin and cuticular waxes into leaves. Hydrophilic molecules must depend upon cracks, insect punctures, etc. and then traverse the hydrophilic part of the cutin to reach the cell wall. Chemicals exhibiting both hydrophilic and lipophilic properties such as surfactants may enter and move via a combined aqueous and lipid route through cuticles (Ashton and Crafts, 1981; Norris, 1982). Molecules of weak acid in solution exist in two forms determined by pH. One is the undissociated form and nonpolar and the other is the dissociated form and polar (Crafts and Foy, 1962). Phenoxy acid compounds such as 2,4-D penetrate the cuticle mainly in the nonpolar, undissociated form (Ashton and Crafts, 1981).

In order to move to the nearest cytoplasm, pesticides have to penetrate the cuticle, cell wall, and plasmalemma (Haynes and Goh, 1977). The resistance of these layers to pesticide penetration varies (Schonherr and Riederer, 1989). For cuticles it ranges from $10^6$ to $10^{10}$ second/m. This wide range covers both variability among species and among compounds. For the cell wall it is about $5\times10^9$ second/m and is independent of water or lipid solubility of the chemicals. The resistance of plasma membranes has been calculated to have a range from
50 to $5 \times 10^5$ second/m. The resistances of both cuticle and plasma membranes decreased with increasing lipid solubility of the chemicals. The cuticular resistance is much higher than that of either the cell wall or the cell membranes. Schonherr and Riederer (1989) pointed out that the cuticle is not only the first barrier in foliar penetration of chemicals but also the only barrier to be considered important to the penetration.

1.2. Major factors affecting foliar uptake of pesticides

**Plant characters.** Morphologic and anatomic structures of plants vary from species to species. For example, leaf cuticles range from none in aquatic leaves to a heavy covering in such succulent leaves as those of *Sedum* (Crafts and Foy, 1962; Ritchie and Carola, 1983). Some species such as apples have stomata on only one side of their leaves (Leece, 1976) and others have stomata on both sides. These differences will influence foliar uptake of pesticides by the plants.

Price and Anderson (1985) reported that uptake of 10 compounds varied greatly between the ten species studied.

Leaf age is another important factor influencing foliar absorption. In his study on absorption of 3-CPA by peach leaves, Bukovac (1965) found that young, unfolding leaves showed a greater absorption than did fully expanded or mature leaves. Greene and Bukovac (1971) observed that penetration of NAAm into pear leaves was greater through the upper than lower surface in expanding leaves, but the reverse was true when leaves were fully expanded.
Stomata of plant leaves play a very important role in transpiration, but their role in foliar absorption of pesticides is limited because the surface tension of the spray solution prevents its infiltration into stomatal cavities (Greene and Bukovac, 1974). In addition, cuticles are also formed on the surfaces inside stomatal cavities. To be systemic, pesticides would have to penetrate these internal cuticles even if they have infiltrated the stomatal cavities (Crafts and Foy, 1962). Although stomatal pores may not be important for pesticide penetration, stomata guard cells and accessory cells are believed to be preferred sites of entry because of their thin wax cover (Bukovac, 1970; Leece, 1976).

Some plants have trichomes on the lower or both surfaces of their leaves. An abundance of trichomes makes foliage difficult to wet; on the other hand, some trichomes may serve as portals of entry for spray solutions, especially solutions of polar compounds (Bukovac, 1970; Hull, 1970).

Properties of pesticides. Molecular structure of pesticides as it relates to the solubility of the parent molecule will influence foliar penetration. Bukovac et al. (1971) found chlorination of phenoxyacetic acid increased its lipid solubility and penetration through isolated tomato fruit cuticles and into bean leaves. In general, foliar absorption is maximized with molecules of intermediate lipophilicity (Baker and Hunt, 1988). As mentioned before, weak acid molecules like 2,4-D can be dissociated at high pH and as a result their lipid solubility and foliar penetration will be decreased.

Partition of a pesticide between cuticle and water is an important parameter affecting penetration since the flow rate across a membrane is proportional to
the concentrations of the solute in the membrane at the two water/membrane interfaces, rather than to the concentration in the bulk solution (Schonherr and Riederer, 1989). This partition is usually expressed as a coefficient (K) calculated as the ratio of equilibrium concentrations of the pesticide in the cuticle (C_c) and in water (C_w), or K = C_c/C_w (Chamel, 1986; Schonherr and Riederer, 1989). Penetration of a solute is not always positively related to the partition coefficient. Bukovac (1970) pointed out that molecules with a high K become so closely associated with the cuticle that they may not desorb from it.

The molecular size of the pesticide may also influence its penetration. Schonherr and Riederer (1989) indicated that dewaxed citrus leaf cuticles have a pore size of about 0.47 nm in radius. These cuticles were permeable to molecules with radii smaller than 0.47 nm such as water (r = 0.197 nm) and impermeable to sucrose (r = 0.555 nm).

Foliar absorption generally increases as pesticide concentrations increase (Bukovac, 1970). This is because the chemical concentration gradient between the outer surface of leaves and the cytoplasm of epidermal cells, a driving force for penetration, becomes greater as the concentration increases.

**Environmental conditions.** Environmental conditions can influence foliar absorption of pesticides directly by affecting uptake processes and indirectly by altering properties of plant leaves and pesticides. Among environmental factors, temperature, humidity, pH, and light are most important.

It is generally believed that absorption of most foliar-applied compounds is governed by both metabolic and non-metabolic components, and that the overall
process of absorption would be accompanied by fairly high temperature coefficients (Hull, 1970). Sargent and Blackman (1962) found that the rate of 2,4-D penetration into leaf disks of *Phaseolus* increased with temperature and with $Q_{10}$ values of 2.3-2.8. Greene and Bukovac (1971) reported that increasing temperature from 5 to 35 °C caused a marked increase in NAAm penetration into pear leaves with $Q_{10}$ values ranging between 1.6 to 5.5. Temperature change could alter cuticle permeability (Bukovac, 1970) and induce phase transitions in plant cuticles (Eckl and Gruler, 1980).

Humidity affects the evaporation rate of spray droplets. High humidity will make spray droplets evaporate less rapidly, allowing more time for the pesticide to be absorbed by leaves. Also, the hydrophilic substances in the cuticular layer, after absorbing moisture under high humidity conditions, will cause the cuticular layer to swell. This gives the spray droplets in contact with the water continuum of the leaf better access to the aqueous route of penetration (Ashton and Crafts, 1981). High humidity also enhances the opening of stomata and changes pesticide penetration via this route.

Spray solution pH has a pronounced effect on penetration of weak organic acid type pesticides such as NAA (Norris and Bukovac, 1972). The pH effect is primarily the result of the dissociation of the weak acid molecule with the undissociated molecule penetrating more readily than the dissociated one (anion) (Sargent and Blackman, 1962). The pH also influences the electrical charges on cuticles. Schonherr and Huber (1977) found that the cuticles of citrus, apricot, and pears have isoelectric points of around 3.0. Below the
isoelectric point, the cuticular membranes carry a net positive charge and are permselective to anions; while above the point, they carry a net negative charge and are permselective to cations. Therefore, pH could affect the foliar absorption of an ionic pesticide by altering cuticular charges.

Light may influence foliar absorption in several ways. It is able to regulate stomatal opening, change the temperature of the leaf surface, and enhance photosynthesis which may increase active uptake (Hull, 1970). Photodegradation of pesticides is another effect of light that may influence measured uptake (Tanaka, 1989). Light, as well as other environmental factors such as temperature and humidity, is also able to affect foliar penetration of pesticides indirectly by influencing the growth and development of the plant leaves (Baker and Hunt, 1988).

Adjuvants. An adjuvant is an ingredient in a pesticide formulation that aids or modifies the action of the pesticide. It can influence foliar uptake of pesticides directly or indirectly through modifying mixing in the sprayer tank, deposition, retention, penetration, and translocation of the pesticides. The following are just a few examples of the many uses of adjuvants in the application of pesticides. Adjuvants can be used as spreaders, stickers, buffers, penetrants, drift control agents, deposition and retention agents, wetting agents, and compatibility agents (Harvey, 1989). Among the various types of adjuvants surfactants are most popularly used with pesticides and most of the published information on spray adjuvants deals with surfactants (Foy, 1989a).
2. SURFACTANTS AND THEIR ROLES IN PESTICIDE APPLICATION

2.1. Important physical and chemical properties of surfactants

Surfactants or surface active agents are a group of chemicals with a lipophilic and a hydrophilic part in their molecules. The combination of these two dissimilarly structural parts in a single molecule is responsible for their surface active nature. Accordingly, surfactants tend to be compatible with both oily materials and water (Behrens, 1964).

Classification of surfactants. In general, surfactants are classified into four groups based on the electric charge of the polar portion of the molecule. The groups are nonionic, cationic, anionic, and ampholytic surfactants (Rosen, 1989).

The nonionic class includes a large number of products that are sometimes further divided into sub-groups according to raw materials employed, types of chemical bonds in the molecule, or chemical structures involved. The molecule obtains surface active properties without forming ions and relies on ether linkages and hydroxyl groups for its hydrophilic character, and hydrocarbon groups for its lipophilic nature (Behrens, 1964). An example of this surfactant group is polyethylene glycol monolaurate \([C_{11}H_{23}-CO-O-(CH_2CH_2O)_nH]\). The most common hydrophilic group of nonionic surfactants is composed of polyoxyethylenes, also called polyethylene oxides or polyethylene glycol \((CH_2-CH_2-O)_n\). The abbreviation for this component is PEG or PEO (Schick, 1987).

The hydrophilicity of a surfactant molecule increases as \(n\) (average value of
the chain length) increases. The nonionic character endows this group of surfactants with desirable properties including compatibility with many chemicals, and considerable insensitivity to the presence of hard water. Therefore, nonionic surfactants are often the first choice for use in pesticide applications (Bayer, 1965; Van Valkenburg, 1982).

Anionic and cationic surfactant molecules ionize in water, and the active portion of the molecule (the portion containing the hydrophilic and lipophilic segments) is an anion in the case of anionic surfactant, and a cation in the case of cationic surfactant (Parr and Norman, 1965). An example of an anionic surfactant is sodium dodecyl sulfate (SDS) with a structure of \( \text{C}_{12}\text{H}_{25}\text{SO}_4\text{Na} \). This compound is popularly used in SDS gel electrophoresis (Cooper, 1977). An example of a cationic surfactant is cetyl trimethyl ammonium bromide \( [\text{CH}_{33}\text{H}(\text{CH}_3)_3\text{Br}] \) that yields \( \text{C}_{16}\text{H}_{33}\text{N}^+(\text{CH}_3)_3 \) and \( \text{Br}^- \) in water. The anionic and cationic surfactants depend on ionization to develop their surface active properties. The degree of ionization regulates overall behavior, and it is easily influenced by hard water or ionic chemicals (Van Valkenburg, 1982).

Ampholytic surfactants have both positively- and negatively-charged moieties in the molecular structure as in, for instance, N-alkylbetaines \( [\text{RN}^+(\text{CH}_3)_2\text{CH}_2\text{COO}^-] \) (Rosen, 1989). This group of surfactants is seldom used in herbicidal formulations (Van Valkenburg, 1982).

**Hydrophilic-lipophilic balance (HLB)** of surfactants. Since the most significant character of all surfactants is a molecular structure with both hydrophilic and lipophilic segments, the ratio of these two segments could be
important in determining their surface activity. This ratio has been defined as hydrophilic-lipophilic balance or HLB. \[ HLB = \frac{W_h \times 20}{(W_h + W_l)} \], where \( W_h \) and \( W_l \) are weights of the hydrophilic and lipophilic portions of the molecule, respectively (Steed, 1973; Rosen, 1989). The HLB has a range of 0-20.

Surfactants with lower HLBs have a predominantly lipophilic character and tend to promote water in oil emulsions, while those with higher HLBs have a stronger hydrophilic nature and promote oil in water emulsions.

Studies indicate that the HLB is important in characterizing surfactant enhancement of foliar uptake of pesticides. Stevens and Bukovac (1985), in their study of octylphenoxy surfactant (Triton X series) effects on atrazine and DDT uptake by maize leaves, observed that uptake of the two pesticides was inversely related to their HLB.

**Micellization of surfactants.** Surfactants in an aqueous solvent will form into colloidal-sized clusters or small aggregates when the surfactant concentration increases to a certain point (Attwood and Florence, 1983). The small aggregates are called micelles and the formation of micelles is called micellization. In micellization, the lipophilic moieties of surfactant molecules orientate toward the inside of the micelle and compose its core, while the hydrophilic moieties form the outside of the micelle. The concentration at which micelles first appear in solution is termed critical micelle concentration or CMC (Attwood and Florence, 1983). Examples of structures similar to the micelle in nature are cell membranes and globular proteins (Rosen, 1989).

The CMC is important because it is a point at which many properties of
surfactant solutions, such as surface tension and osmotic pressure, show large changes in their general responses to surfactant concentrations (Parr and Norman, 1965; Rosen, 1989).

Reduction of surface tension by surfactants. Molecules at the surface of a liquid have potential energies greater than those of similar molecules in the interior of the liquid. This is because attractive interactions of molecules at the surface with those in the interior of the liquid are greater than those with the widely separated molecules in the gas phase. Surface tension is a measure of the minimum amount of work required to bring sufficient molecules to the surface from the interior to expand it by unit area. The surface tension is often conceptualized as a force per unit length at a right angle on the surface to the force required to pull apart the surface molecules (MacDonald and Burns, 1975). Surfactant molecules have a tendency to concentrate at an interface and reduce the surface tension. The reduction in surface tension is positively related to surfactant concentrations but with a breaking point at the CMC. Above this concentration the surface tension of the solution remains essentially constant since only the monomer form of surfactants contributes to the reduction of the surface or interfacial tension. Near the CMC the interface is considered to be saturated with surfactant and the slightly continued reduction in the surface tension is due mainly to the increased activity of the surfactant in the bulk phase rather than at the interface (Rosen, 1989).

Because surfactants can reduce surface tension of a solution, they are popularly used in pesticide formulation. Reduction in surface tension of a spray
solution will lead to better wetting and coverage of the solution on targets and, consequently, increased penetration of the pesticides through leaf surfaces (Seaman, 1982).

If the minimum surface tension is achieved at the CMC, increasing surfactant concentration above the CMC will not reduce the surface tension further. However, studies show that the surfactant concentration required to achieve maximum pesticide uptake by leaves is higher than the CMC, implying that surfactants increase foliar uptake of pesticides not only by reducing surface tension of spray solution but also by other mechanisms (Staniforth and Loomis, 1949; Westwood, 1958; Smith et al., 1966).

2.2. Roles of surfactants in pesticide application

A surfactant as a pesticide adjuvant could influence the pesticide's activity at one or more stages from the spray tank to the site of action. The surfactant could affect pesticide tank mixing, deposition and retention on the targets, penetration into leaves, and translocation from the applied area to the site of action.

Tank mixing of pesticides. Pesticides may interact with surfactants in the spray tank. For example, cationic pesticides like paraquat often react with anionic surfactants (Bayer, 1965). Surfactants as components of pesticide formulations tend to reduce ionization of weak electrolytes such as 2,4-D by decreasing the dielectric constant of the spray liquid (Schonherr and Riederer,
Deposition of pesticide solution. Surfactants can reduce surface tension and consequently improve wetting and coverage of the solution on targets (Seaman, 1982). They could be also used as foaming agents to reduce drift of spray solutions since larger particles of spray solution can be generated with a foaming agent, utilizing air as the principal conveying medium (Van Valkenburg, 1982). However Seaman (1982) argued that lowering surface tension with surfactants should lead to the formation of a longer lived and thinner sheet of the solution emerging from the nozzle, and subsequently the finer droplets produced may result in drift.

Retention of pesticides. O' Donovan et al. (1985) observed that surfactant Renex 36 enhanced the retention of a glyphosate-dye spray solution by barley leaves. Surfactants could either increase or decrease photodegradation of herbicides depending on the type of herbicide (Tanaka et al., 1981, 1989). Stevens and Bukovac (1987) reported that the volatilization of atrazine was increased in the presence of surfactant, while that of DDT was reduced. They (1985, 1987) also found that surfactants absorbed moisture in the air and could play a role as humectants on leaf surfaces. Uptake of glucose by maize leaves was positively correlated with the hygroscopicity of the surfactants. On the other hand, Seaman (1982) argued that droplets containing surfactants and spreading to a thin film can dry more quickly than drops without surfactant.

Pesticide penetration into leaf surfaces. Greene and Bukovac (1974) found that surfactants induced stomatal penetration of aqueous solutions (NAA) into
pear leaves. However Schonherr and Bukovac (1972) showed that only liquids having a surface tension less than 30 dyne/cm and zero contact angle on the leaf surface of Zebrina purpusii Bruckn infiltrated stomata spontaneously, while liquids with surface tensions greater than 30 dyne/cm failed to infiltrate stomata. They pointed out that few commercially available surfactants will reduce the surface tension of aqueous solutions below 30 dyne/cm.

Surfactants were reported to increase cuticular penetration of pesticides (Norris, 1973; Erickson, 1980) and alter permeability of cell membranes (Sutton and Foy, 1971; John et al., 1974; Watson et al., 1980). Co-penetration of surfactants with pesticides also has been observed (Stevens and Bukovac, 1985; Silcox and Holloway, 1989).

Translocation of pesticides in plants. McWhorter et al. (1980), in their study of glyphosate translocation in soybeans and johnsongrass, found that surfactants increased absorption of glyphosate by leaves but decreased translocation out of soybean leaves. Similar results were observed by Brian (1972) with paraquat; and he concluded that surfactant penetrating into the leaf tissue inhibited paraquat translocation. Surfactants were reported to increase the movement of aqueous solutions in intercellular spaces (Currier and Dybing, 1959).

3. PLANT CUTICLES

As mentioned before, the cuticle is the major barrier for pesticide foliar
penetration. To understand the penetration process and how surfactants affect the process, it is necessary to consider the cuticle in detail.

3.1. The structure of the cuticles

The plant cuticle is a thin, continuous layer of predominately lipid material covering the outer wall of epidermal cells and serves as a protective barrier between the plant and its external environment. This extracellular layer is also called cuticular membrane and can be defined on the basis of both its anatomical position and chemical nature. Chemically, it is always characterized by two specific groups of substances: insoluble polymeric cutins that constitute the framework of the membrane and soluble waxes deposited on the surface as epicuticular wax and embedded within the cutin matrix as cuticular wax (Martin and Juniper, 1970; Holloway, 1982a).

In most plants the cuticular membrane is not structurally or chemically homogeneous but is considered to be composed of several layers. The basic structure for some plant species has been investigated intensively. The first or outermost layer of the cuticle in all terrestrial plants is epicuticular wax (Baker, 1982). Beneath the layer of epicuticular wax is the cuticle proper, which can be completely dissolved by treatment with alkali. Below the cuticle proper one or more cuticular layers may be present; and these form the bulk of the cuticular membrane when it is fully developed. The outer region of cuticular layers has no cellulose component and has a similar constitution to the cuticle proper, but
the inner region contains cellulose (Holloway, 1982a). The region of the junction of the cuticular membrane with cell wall of epidermal cells is comprised of a pectin layer (Martin and Juniper, 1970).

The morphological distinction between cuticle proper and cuticular layer is clear for some plant species but not for others. There is no universally occurring plant cuticular membrane structure, and each species must be considered individually (Holloway, 1982a). Cuticle development is also affected by environmental conditions such as light intensity, photoperiod, temperature, humidity, and soil moisture content (Hull et al., 1975; Hunt and Baker, 1982).

3.2. Morphology and chemical constitution of waxes

The crystalline structure of epicuticular waxes could be in the form of plates, tubes, ribbons, rods, filaments, or dendrites (Baker, 1982). Wax morphology is determined mainly by the composition of wax exudates (Jeffree et al., 1975; Chambers et al., 1976). The constituent classes of epicuticular waxes include saturated or unsaturated and branched or unbranched long-chain (C_{12}-C_{n}) aliphatic constituents (acids, aldehydes, alkyl esters, hydrocarbons, primary and secondary alcohols, ketones, and esters), cyclic constituents (terpenoid and sterols), and phenolic constituents (flavonoids and flavonoid glycosides) (Martin and Juniper, 1970; Baker, 1980, 1982).

Fernandes et al. (1963, 1964) reported that waxes washed from mature Bramley apple leaves with chloroform consisted of 32-40% esters, 30-37%
ursolic acid, 16% paraffins, 8% alcohols and ketones, and 3-6% free acids. A similar finding was reported by Leece (1976) with waxes of McIntosh apple leaves.

Leece (1976) also observed that McIntosh apple leaf adaxial cuticle contained 31 μg/cm² surface wax and 65 μg/cm² embedded wax. Haas and Rentschler (1984) reported that epicuticular wax of adaxial leaf cuticles of blackberries amounted to approximate 90% of the total wax, and only 10% present as cuticular wax.

Wax quantity and quality are closely related to pesticide penetration. Bukovac and Norris (1967) reported that successive removal of the surface waxes of pear leaf cuticles resulted in increased NAA penetration, with slight additional increases following removal of the embedded waxes. Wax plated onto dewaxed cuticle reduced the penetration of NAA. They suggested that chemical composition, in addition to overall quantity of waxes, may have a significant impact on uptake of foliar applied chemicals.

3.3. Chemical constitution of cutins

Cutins are insoluble high molecular weight lipid polyesters composed of various characteristic long-chain substituted aliphatic acids. Hydroxyl groups are the most common substituent, but aldehyde, ketone, epoxide and unsaturated groups may also be present (Martin and Juniper, 1970). Monomeric composition varies not only according to plant species but also with position on
the plant. Holloway (1982b) classified cutins into three categories based on the chain lengths of the predominant monomers: \(C_{16}\), \(C_{18}\), and mixed \(C_{16}\) and \(C_{18}\) types. The cutins of apple (*Malus pumila* M.) leaves belong to the mixed \(C_{16}\) and \(C_{18}\) type (Baker et al., 1964). Richmond and Martin (1959) reported that the cutin on the leaves of Merton Worcester and Cox's Orange Pippin apples was present in quantities of about 100 \(\mu\text{g/cm}^2\) of surface.

Very little is known about the intramolecular structure of native cutin biopolymers. Holloway (1982b) observed that only aliphatic compounds were involved in the construction. Similarity in the patterns of esterification in the dihydroxyhexadecanoic types of cutin suggests some form of common structural arrangement of these polymers rather than random inter-esterification. Cutin, like waxes, is also a barrier for foliar penetration of pesticides (Riederer and Schonherr, 1985).

### 3.4. Isolation of plant cuticles

Several methods have been employed to isolate plant cuticles: (1) mechanically (Chamel, 1986), (2) through anaerobic bacterial fermentation (Orgell, 1955), (3) chemically, using zinc chloride-HCl (Schonherr and Huber, 1977) and ammonium oxalate-oxalic acid reflux (Baker et al., 1964), and (4) enzymatically with pectinase and cellulase (Orgell, 1955).

Hoch (1975) compared apple leaf cuticles isolated enzymatically and chemically and observed that cuticles isolated with either pectinase or cellulase
or both appeared most similar to non-isolated cuticles with cutin-embedded microfibrils and the lamellar structure of cuticles preserved. Cuticles isolated by the zinc-chloride-HCl method retained the lamellar structure, but most cutin-embedded microfibrils were dissolved away with this treatment. The ammonium oxalate-oxalic acid reflux procedure for cuticle isolation appeared to yield the most unnatural cuticles. Neither the lamellae nor the cutin-embedded microfibrils were well preserved.

4. SORPTION OF SURFACTANTS BY LEAF CUTICLES

To determine the roles of surfactants in cuticular penetration of pesticides, it is necessary to understand the interactions between surfactants and cuticles in term of cuticular sorption and desorption of surfactants. Unfortunately, little information is available in this area because of the lack of a good method to detect or trace the surfactants. The most common method of determining the amount of surfactant present is by using radioactively-labeled surfactants; however, the fact that only few radioactively-labeled surfactants are available has greatly limited studies on surfactant cuticular sorption (Norris, 1982). Furthermore, many surfactants are not a single chemical compound; they are mixture of several compounds. Labeling different compounds in a surfactant mixture will lead to quite different experimental results (Smith and Foy, 1966).

Sorption is usually expressed by partition coefficient, a ratio of equilibrium concentrations of a surfactant in cuticles and water. In a study on partition
characteristics of surfactants between cuticles isolated from mature tomato or green pepper fruits and water, Shafer et al. (1989) found that the partition coefficient (K) of surfactants (polyethoxy derivatives of octylphenol condensed with 3-40 mol ethylene oxide or EO) was inversely related to EO content for both cuticular membrane (CM) and dewaxed cuticular membrane (DCM). The sorption equilibrium was achieved in 24 hours for surfactants with high EO number but in 432 hours for those with relatively low EO content. Bland and Brian (1972) studied partition of two surfactants, guerbet alcohol and n-dodecanol, between cabbage waxes and water and reported that more surfactants partitioned in cabbage waxes for surfactants with very low or high EO numbers than that with an intermediate EO content.

Shafer and Bukovac (1987) found that the time required for reaching equilibrium of surfactant OP+9.5EO sorption by tomato fruit CMs was longer for surfactant solution above the critical micelle concentration than that below the critical micelle concentration. Sorption of DCMs was significantly greater than that of CMs, and greater differences in sorption between CMs and DCMs occurred at surfactant concentrations below the critical micelle concentration. The partition coefficient (K) of the surfactants between CMs or DCMs and water decreased dramatically as the critical micelle concentration was approached and exceeded. At surfactant concentration above the critical micelle concentration sorption of surfactants by CMs and DCMs was concentration-dependent, suggesting that micelles may participate in surfactant sorption. Sorption of the surfactant OP+9.5EO per unit of CM decreased as
CM amount increased per unit volume of surfactant solution. The sorption was unaffected by pH.

5. EFFECTS OF SURFACTANTS ON PESTICIDE PENETRATION OF LEAF CUTICLES

Surfactants Span 20 and Span 80 were reported to increase 2,4-D penetration through pear leaf cuticles 10-15 fold, but penetration was unaffected by Tween 20 (Norris, 1973). Erickson (1980) observed that increasing MON-0818 surfactant concentrations resulted in increased glyphosate penetration through isolated quackgrass cuticle. Surfactants are believed to affect pesticide penetration by influencing both sorption and diffusion of the pesticide in cuticles (Schonherr and Riederer, 1989).

5.1. Effects of surfactants on pesticide sorption by cuticles

Because pesticide flow rate across a membrane is proportional to the concentrations of the pesticide in the membrane rather than to the concentrations of the bulk solution in the source, pesticide sorption by cuticles will affect the chemical concentration gradient and the flow rate (Schonherr and Riederer, 1989). Since pH has great influence on cuticular sorption which is represented by partition coefficient (K), $K_{ph}$ is commonly used and defined as the apparent partition coefficient at a specific pH.
A few studies have focused on the effects of surfactants on cuticular sorption of growth substances. Shafer et al. (1988) found that surfactant Triton X-100 decreased sorption of benzyladenine by tomato fruit cuticles in the pH 4 to 6 range. They (1989) also reported that below the CMC, Triton X-100 had little effect on NAA sorption by tomato fruit cuticles. However, at higher concentrations, NAA sorption was inversely related to Triton X-100 concentrations. Sorption of NAA by dewaxed tomato fruit cuticles was also decreased by Triton X-100.

Sodium dodecyl sulfate and Tween 20 were reported to decrease 2,4-D binding to pear leaf cuticles, but the surfactants did not change permeability of the cuticles to 2,4-D (Norris, 1971; King, 1982), showing that surfactants may change 2,4-D cuticular sorption without altering cuticular penetration.

Schonherr and Bauer (1989) pointed out that surfactants at concentrations above the CMC in aqueous donor solutions reduce the concentration of a lipophilic solute in the cuticle, and this effect invariably decreases the rate of penetration of the solute. However, surfactants may increase the mobility of solutes in the cuticle, leading to an increased rate of penetration.

5.2. Impacts of surfactants on cuticle permeability to pesticides

Several possible independent or overlapping effects of surfactants on cuticles in relation to pesticide penetration have been proposed or substantiated.

Solubilization of cuticular components by surfactants. Kuzych (1984) found
that X-77 could solubilize the outer surface of cabbage head leaves, taking epicuticular components up into solution. Subsequently, as the solution dried or evaporated, the epicuticular materials were randomly redeposited on the plant surface. He observed that should a herbicide also be present with the adjuvant in solution, its penetration into the plant would be greatly facilitated. Takeno and Foy (1974) reported that leaf waxes of cotton were severely eroded 72 hours after treatment with 1% (w/w) polysorbate surfactant solution and the surfactant appeared to solubilize leaf wax. Furmidge (1959) believed that surfactant micelles could dissolve oils and waxes and the solution may remove large areas of the surface leaf wax.

Florence (1981) explained in detail the steps of solubilization of components of biological membranes by non-ionic surfactants and believed that the ratio of surfactant to lipid and the nature of the biological membrane are important in determining the exact nature of the interaction of surfactant and membranes.

Ultrastructure change caused by surfactants. Noga et al. (1988) and Wolter et al. (1988) reported that aqueous surfactant solutions (Triton X series) caused a rapid deterioration of the wax fine-structure filaments of cabbage and sugar beets. Foy (1964) suggested that surfactants may "solubilize" into the cuticle, displacing lipoid molecules and increasing permeability. Some surfactants probably orient polarly and become solubilized in the cuticle, thus causing a loosening or swelling of the cuticular architecture and thereby enhancing penetration. Van Overbeek and Blondeau (1954) explained the increased permeability of plasma membranes by surfactants on the basis that surfactants
solubilize into the membranes, displacing fatty acid molecules and so ‘opening up’ the membrane.

**Hydrophilic channels induced in cuticles by surfactants.** Jansen (1964), in his review on surfactant enhancement of herbicide entry, stated that cuticle swelling caused by imbibition of water by the cellulose matrix and the hydrophilic portion of the cutin results in separation of the wax platelets and increases the area of the submicroscopic channels. If surfactants penetrate the cuticle, the hydrophilic-lipophilic nature of the bulky surfactant molecules would promote the existence of an even greater swelling and an increase in area of the hydrophilic channels and perhaps of the lipophilic portion as well. A proportionality should exist between the number of surfactant molecules in the spray droplet and the number that can be adsorbed along the transport channels. Surfactant adsorption might therefore explain the excess surfactant concentration requirement. Differences in cuticle thicknesses and number of adsorption sites could explain variations in the requirements of different species. In addition, adsorbed surfactant should cause the swelled condition to continue after water evaporates since the large surfactant molecules would tend to prevent complete contraction of the cuticle.

Smith et al. (1966) proposed that surfactant molecules may diffuse from the liquid spray droplet into imperfect areas of cuticles (i.e. cracks, insect punctures and possibly hydrophilic and/or lipophilic areas) along the surfaces of the lipophilic cuticle waxes and cutin. The molecules are perhaps aligning themselves in monolayers. If true, this would result in the lipophilic end being
in or on the cuticle waxes, thus creating a hydrophilic layer or layers in these imperfections. Water molecules would then be attracted to these hydrophilic regions and channels would be formed, conceivably bringing about a slight swelling of the cuticle. They called these areas 'hydrophilic channels'. Water-soluble herbicides would be free to diffuse through these 'channels'. They suggested surfactant molecules with EO content too high or too low were not effective for the channel formation. Foy and Smith (1969) believed that high surfactant concentration increased the diffusion rate of surfactant into the cuticles, thus satisfying all the binding or adsorption sites within the cuticle.

**Phase change of cuticle components.** Noga et al. (1987) suggested that the ultrastructural alterations of cuticles caused by surfactants may be accompanied by a phase transition of the soluble cuticular lipids and subsequent changes both in orientation and location of this lipid fraction within the cutin matrix. These surfactant-induced structural and physical modifications may affect penetration of aqueous pesticide solutions.

Despite it is lack of direct evidence of phase change of cuticles by surfactants, studies of other biological membranes have revealed valuable information on surfactant-induced phase changes in biological membranes. Gurtubay et al. (1980) studied Triton X-100 solubilization of mitochondrial membranes and found the surfactant seemed to act by inducing a phase transition from membrane lamellae to mixed surfactant-protein-lipid micelles. Similar results were also reported by Alosnso et al. (1987). Valpuesta et al. (1986) observed that Triton X-100 withdrawal induces a profound change in
phospholipid architecture from a micellar to a lamellar-like phase.

**Copenetration of pesticides and surfactants through cuticles.** Stevens and Bukovac (1985) observed that the uptake of atrazine and DDT was related to the penetration of Triton X series surfactants in maize leaves. Silcox and Holloway (1989) also reported the intimate association between surfactants and pesticides in foliar penetration.

Copenetration of surfactants with chemicals was also reported in other biological membranes. Levy and Mroszczak (1968) studied the effect of surfactant-drug complex formation on drug penetration of an artificial lipoid membrane and found the surfactant-drug complexes that were more lipoid-soluble than the uncomplexed drug penetrated the lipoid barrier more rapidly than the uncomplexed drug; complexes of lower lipoid solubility than the free drug penetrated the lipoid barrier less rapidly than the drug itself.
CHAPTER 3
RELATIONSHIP OF CHEMICAL CLASSIFICATION AND HYDROPHILIC-
LIPOPHILIC BALANCE OF SURFACTANTS TO UPPER LEAF-
SURFACE PENETRATION OF GROWTH REGULATORS IN APPLES

ABSTRACT

The effect of surfactants on the movement of $^{14}$C-labeled plant growth regulators through the stomatal, adaxial surface of apple (Malus pumila M. 'Golden Delicious') leaf discs was studied by pre-soaking the discs in 10 ml liter$^{-1}$ solutions of nonionic surfactants. Penetration was measured by detecting changes in radioactivity of the solution in glass cylinders sealed to the upper surface of the discs. Penetration of 2,4-D [(2,4-dichlorophenoxy)acetic acid] and β-NAA (2-naphthaleneacetic acid) was significantly increased by Pegosperse 200-ML; no significant effect was observed from Ethox HCO-16, Hyonic NP 40, Pegeste SML, and Span 20 which had the same hydrophilic-lipophilic balance (HLB=8.6±0.5) as Pegosperse 200-ML. Penetration of 2,4-D and β-NAA decreased linearly as surfactant HLB increased from 3.5 (Pegosperse 100-O) to 14.6 (Pegosperse 600-ML). Penetration of maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) was not affected by any surfactant tested.
1. INTRODUCTION

Surfactants reduce surface tension of a pesticide solution and can improve foliar coverage of the solution.\textsuperscript{1-3} However, this is not the only role of surfactants in foliar absorption. In their studies on apples, Westwood and Batjer\textsuperscript{4} observed successive increase in NAA absorption by increasing the concentration of surfactant far beyond that required for minimum surface tension. Lownds et. al.\textsuperscript{5,6} also found a similar phenomena in cowpea and reported the effects depended on the type of surfactant used. They attributed this to the changes in interface area, drying time, and stomatal penetration caused by surfactants and considered this also could be due to interaction between surfactant, NAA, and plant surface. Kirkwood et. al.\textsuperscript{7} reported that the penetration of MCPA and MCPB through isolated abaxial cuticles of broad beans was associated with surfactant HLB. Since the mechanism by which surfactants facilitate foliar entry and movement of pesticides has received little attention,\textsuperscript{2} the specific interactions between plant, solute, and surfactant are still not explained.\textsuperscript{6,8} The objective of this experiment was to investigate the relationship of chemical classification and HLB of surfactants to their effects on the foliar absorption of plant growth regulators. The responses to be studied were not to include effects from changing surface tension, drying time, and stomatal entry of the spray solution.
2. MATERIALS AND METHODS

Fully-expanded apple leaves were sampled in October 1988 from 5-year-old Golden Delicious trees on MM106 rootstocks. Leaf discs were prepared with a 17.5 mm cork borer, avoiding midribs and large veins. The discs were washed with distilled water and put in beakers containing 10 ml liter\(^{-1}\) solutions of different nonionic surfactants. Each surfactant treatment had 5 discs in a beaker. The surfactants with their chemical classification and HLB are shown in Table 3.1. Additional information on the characteristics of these products can be found in McCutcheon's Emulsifiers & Detergents.\(^9\) The discs were soaked in the surfactant solutions for 48 hours at 25 °C in dark and then washed with tap and distilled water.

Glass cylinders with a height of 15 mm and an internal diameter of 12.3 mm were touched to Permabond 910 adhesive (Permabond International Division, Englewood, NJ) and then positioned on the upper surface of the leaf discs. The glass-leaf chamber units were placed in 9 X 2 cm petri dishes lined with 2 pieces of moistened filter papers (Fig. 3.1). After about 45 minutes, when the adhesive had dried, 0.5 ml \(^{14}\)C-labeled plant growth regulator solution, buffered by 40 mM citric phosphate (pH 3.0) and containing 0.1 mM sodium azide, was pipetted into each glass-leaf chamber. Concentrations of 2,4-D, \(\beta\)-NAA, and MH were 0.78, 0.54, and 8.92 mM, respectively. The specific activities of 2-\(^{14}\)C labeled 2,4-D, carboxy-\(^{14}\)C labeled \(\beta\)-NAA, and 4,5-\(^{14}\)C labeled MH were 28.0, 6.6, and 10.2 mCi/mmmole, respectively. The petri dishes were covered and
placed in the dark in a 25 °C water bath. The atmosphere and filter papers inside the petri dishes were kept saturated with distilled water.

After 24 hours, the solution of 14C-labeled growth regulators in each glass-leaf chamber was collected into a liquid scintillation counting vial. Each glass-leaf chamber was washed with 7 ml AquaMix cocktail (ICN Biomedicals, Inc., Costa Mesa, CA) and the washing solution was added to the vial. Radioactivity of the collected solutions was measured with a liquid scintillation counter (Beckman, Model LS 7000). Samples containing 0.5 ml solutions of the initial 14C-labeled and normal, non-radioactive growth regulators were also prepared to determine the original radioactivity present in the solution and background, respectively. The amount of 14C-labeled growth regulator penetrating the leaf surface was determined by subtracting the quantity of 14C-label in the collected solution from the amount originally present.

Trials were conducted with 2,4-D, β-NAA, and MH under the same conditions using completely randomized designs with four replications. Data were subjected to variance analysis. Means of surfactant chemical classification were separated by Newman-Keuls tests and effect of HLB on the penetration of the growth regulators was analyzed with regression.

3. RESULTS AND DISCUSSION

Penetration of 2,4-D and β-NAA through the stomatal, adaxial surface of apple leaf discs was significantly increased by previously soaking the leaf discs in
10 ml liter\(^{-1}\) Pegosperse 200-ML solution (Table 3.2). This treatment doubled and tripled the penetration of 2,4-D and \(\beta\)-NAA, respectively; as compared with the water control. Surfactants from other chemical groups, but which had the same HLB as Pegosperse 200-ML, slightly changed the amount of 2,4-D and \(\beta\)-NAA penetration but the effects were not significant. Chemical classification of the surfactants appears to be an important factor affecting penetration of the plant growth regulators. MH penetration was not affected by any surfactant tested under the conditions of this experiment. The finding that Pegosperse 200-ML increased the penetration of relatively water-insoluble 2,4-D and \(\beta\)-NAA, but did not affect the penetration of water-soluble MH indicates that the effect of surfactants on penetration is also related to type of growth regulators.

Three Pegosperse (polyethylene glycol or PEG) surfactants varying in HLB were used in investigating the relationship between this factor and growth regulator penetration. The three surfactants, Pegosperse 100-O, 200-ML, and 600-ML, had HLBs of 3.5, 8.6, and 14.6, respectively. Penetration of 2,4-D and \(\beta\)-NAA decreased linearly as the HLB of Pegosperse surfactants increased from 3.5 to 14.6 (Figs. 3.2 and 3.3). Although the three Pegosperse materials belong in the same chemical group, Pegosperse 600-ML did not significantly increase the penetration of \(\beta\)-NAA when compared with the water control. It appears that both HLB and chemical classification of surfactants affect the penetration of 2,4-D and \(\beta\)-NAA. As had also been found for the chemical classes, penetration of MH was not influenced by the HLB of Pegosperse surfactants.

In this experiment, since the growth regulator solution had the same
interface area for all surfactant treatments and the upper surface of apple leaves had no stomata, possible effects of surfactants on wetting and stomatal entry of the solution could be excluded. Possible chemical reaction of surfactant and growth regulators before the penetration could be excluded also since surfactants were not added to the growth regulator solutions. The results show that nonionic surfactants could have effects on foliar penetration of plant growth regulators in addition to changing wetting, drying time, and stomatal entry of the spray solution. The effects are related to the chemical classification and HLB of the surfactants and depend on the type of growth regulators.
Fig. 3.1. A system used to study penetration of 2,4-D into apple leaves.
Fig. 3.2. Relationship between the HLB of Pegosperse (PEG) surfactants and penetration of 2,4-D through the stomatal upper surface of apple leaf discs.
Fig. 3.3. Relationship between the HLB of Pegosperse (PEG) surfactants and penetration of β-NAA through the stomatal upper surface of apple leaf discs.
<table>
<thead>
<tr>
<th>Surfactant trade name</th>
<th>Chemical classification</th>
<th>HLB</th>
<th>Manufacturer Name</th>
<th>Address</th>
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</thead>
<tbody>
<tr>
<td>Ethox HCO-16</td>
<td>Ethoxylated triglycerides</td>
<td>8.6</td>
<td>Ethox Chemicals, Inc.</td>
<td>P.O. Box 5094 Sta. B Greenville, SC 29606</td>
</tr>
<tr>
<td>Hyonic NP 40</td>
<td>Alkyl phenoxy polyoxyethylene ethanol</td>
<td>8.6</td>
<td>Henkel Corp.</td>
<td>350 Mt. Kemble Ave. Morristown, NJ 07960</td>
</tr>
<tr>
<td>Pegeste SML</td>
<td>Sorbitan esters</td>
<td>8.6</td>
<td>GAF Chemicals Corp.</td>
<td>1361 alps Rd. Wayne, NJ 07470</td>
</tr>
<tr>
<td>Pegosperse 100-O</td>
<td>Diethylene glycol oleate</td>
<td>3.5</td>
<td>Lonza Inc.</td>
<td>17-17 Route 208 Fair Lawn, NJ 07410</td>
</tr>
<tr>
<td>Pegosperse 200-ML</td>
<td>Polyethylene glycol 200 monolaurate</td>
<td>8.6</td>
<td>Lonza Inc.</td>
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<tr>
<td>Pegosperse 600-ML</td>
<td>Polyethylene glycol 600 monolaurate</td>
<td>14.6</td>
<td>Lonza Inc.</td>
<td></td>
</tr>
<tr>
<td>Span 20</td>
<td>Sorbitan monolaurate</td>
<td>8.6</td>
<td>ICI Americas Inc.</td>
<td>Concord Pike &amp; New Murphy Rd. Wilmington, DE 19897</td>
</tr>
</tbody>
</table>
Table 3.2. Relationship of chemical classification of nonionic surfactants to penetration of $^{14}$C-labeled 2,4-D, β-NAA, and MH through the stomatal upper surface of apple leaf discs.

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>Absorbed by leaf discs (cpm)</th>
<th>Absorption percentage (%)</th>
<th>Absorbed by leaf discs (cpm)</th>
<th>Absorption percentage (%)</th>
<th>Absorbed by leaf discs (cpm)</th>
<th>Absorption percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water control</td>
<td>1800 a</td>
<td>33</td>
<td>780 a</td>
<td>9</td>
<td>73 a</td>
<td>1</td>
</tr>
<tr>
<td>Ethox HCO-16</td>
<td>2500 a</td>
<td>45</td>
<td>620 a</td>
<td>7</td>
<td>79 a</td>
<td>1</td>
</tr>
<tr>
<td>Hyonic NP 40</td>
<td>2800 a</td>
<td>51</td>
<td>1600 ab</td>
<td>18</td>
<td>92 a</td>
<td>2</td>
</tr>
<tr>
<td>Pegeste SML</td>
<td>2700 a</td>
<td>49</td>
<td>860 a</td>
<td>10</td>
<td>111 a</td>
<td>2</td>
</tr>
<tr>
<td>Pegosperse 200-ML</td>
<td>4100 b</td>
<td>74</td>
<td>2340 b</td>
<td>26</td>
<td>73 a</td>
<td>1</td>
</tr>
<tr>
<td>Span 20</td>
<td>3000 a</td>
<td>55</td>
<td>540 a</td>
<td>6</td>
<td>96 a</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$ Counts per minute (cpm) absorbed by leaf discs = (cpm placed in the glass cylinder originally) - (cpm left in the cylinder).

$^b$ Absorption percentage = (cpm absorbed by leaf discs)/(cpm placed in the cylinder originally) X 100.

$^c$ Mean separation in columns by Newman-Keuls tests at 5% level.
REFERENCES


CHAPTER 4
EFFECTS OF NONIONIC SURFACTANTS ON CUTICULAR SORPTION AND PENETRATION OF (2,4-DICHLOROPHENOXY)ACETIC ACID

ABSTRACT

Surfactants increase uptake of some foliar applied chemicals to a greater extent than expected from effects on surface tension and spray coverage. This study of uptake of 2,4-D [(2,4-dichlorophenoxy)acetic acid] evaluated surfactant effect on penetration and sorption in isolated cuticles of apple leaves. $^{14}$C-2,4-D was placed in glass cylinders affixed to enzymatically isolated adaxial apple leaf cuticles after the cuticle segments had been treated with Pegosperse (PEG) surfactants. The same surfactant pretreatments were included in sorption studies in which cuticle segments were immersed in $^{14}$C-2,4-D for 96 hours. Quantities of 2,4-D passing through or sorbed by the cuticle were determined. Similar experiments were conducted with unaltered cuticles and cuticles dewaxed with chloroform. The hydrophilic-lipophilic balance (HLB) of polyethylene glycol based surfactants was inversely related to sorption of those surfactants by the cuticles and penetration of 2,4-D. Sorption of 2,4-D by apple leaf cuticles was unaffected by surfactant pretreatment. Dewaxed cuticle membranes showed a similar response of 2,4-D penetration and sorption following the surfactant pretreatment.
1. INTRODUCTION

Surfactants increase foliar absorption of pesticides to a greater extent than what can be explained by decreasing surface tension and increasing coverage of the pesticide solution.\(^1\)\(^2\) A possible effect of the surfactants in the foliar absorption is their direct interaction with leaf cuticles.\(^1\) Norris reported that pretreatment with 10 ml liter\(^{-1}\) solutions of surfactants Span 20 and Span 80 increased the permeability of isolated pear leaf cuticles to 2,4-D by 7- and 14-fold, respectively.\(^3\) Span 20 and Span 80 belong to the same surfactant series and have a hydrophilic-lipophilic balance (HLB) of 8.6 and 4.3, respectively. This inverse relationship of HLB and enhanced penetration of Span surfactants suggests that surfactant HLB is an important determinant in cuticular penetration.

The change in permeance (P) of cuticle to an applied compound can be attributed to changes in the diffusion coefficient (D) and the partition coefficient (K) for certain thickness of cuticles (l) with \(P = \frac{DK}{l}\). The ratio K denotes the equilibrium concentrations of a pesticide in the cuticle and water, and usually represents sorption by cuticles. K is important because the flow rate across the cuticular membrane is proportional to the concentration of the solute in the cuticle rather than to the concentrations in the bulk solution.\(^4\)

A few studies have focused on the effects of surfactants on sorption of growth substances. Shafer et al. found that the surfactant Triton X-100 decreased sorption of benzyladenine by tomato fruit cuticles in the pH 4 to 6
They also reported that below the critical micelle concentration (CMC), Triton X-100 had little effect on naphthaleneacetic acid (NAA) sorption by tomato fruit cuticles. However, at higher concentrations, NAA sorption was inversely related to Triton X-100 concentration. Sorption of NAA by dewaxed tomato fruit cuticles was also decreased by Triton X-100. These researchers focused on sorption because they believed that cuticular sorption is important to cuticular penetration. To understand the effects of surfactants on different phases of cuticular penetration, it would be necessary to study the relationship between cuticular sorption and penetration as influenced by surfactant.

The objectives of this research were to investigate the effects of polyethylene glycol (PEG) surfactant HLB on 2,4-D penetration through and sorption by apple leaf cuticles and to study the relationship between surfactant altered penetration and sorption.

2. MATERIALS AND METHODS

2.1. Cuticle isolation

Fully-expanded apple leaves were sampled from 6-year-old Golden Delicious (Malus pumila M.) trees on MM 106 rootstocks. Leaf discs were cut with a 19.6 mm cork borer, avoiding midribs and large veins. The abaxial surface of the leaf discs was abraded with sand paper (Grit size 220) to disrupt the abaxial cuticles and enhance the infiltration of enzyme solution into the leaf
tissues. The discs were washed with tap and distilled water and placed in 20 g liter\(^{-1}\) pectinase (U.S. Biochemical Co., Cleveland, Ohio) and 20 g liter\(^{-1}\) cellulase (U.S. Biochemical Co.) solution buffered by 8 mM citric phosphate (pH 4.0) and containing 0.1 mM sodium azide. The tissue was vacuum infiltrated and then kept at room temperature for 2-5 weeks. The leaf discs were gently washed with tap water to separate adaxial cuticles from other tissues. The cuticles were then washed with distilled water, air dried, and stored at room temperature for future use.

2.2. Cuticular penetration of 2,4-D

Cuticular membrane (CM) discs weighing 1.5±0.1 mg were checked with an optical microscope for defects. The CMs were soaked in distilled water and 10 ml liter\(^{-1}\) PEG 100-O (diethylene glycol oleate), PEG 200-ML (polyethylene glycol monolaurate) or PEG 600-ML (polyethylene glycol monolaurate) for 24 hours at 20 °C. The surfactants all contain \((\text{CH}_2\text{-CH}_2\text{-O})_n\) chain and the average chain length (n) for PEG 100-O, PEG 200-ML, and PEG 600-ML are 2, 4, and 13, respectively. The treated CMs were washed with running tap water for 20 minutes, rinsed with distilled water, and then air dried. Glass cylinders with a 12.3 mm internal diameter and 15 mm length were touched to Wonder Bond Adhesive (Borden Inc. Columbus, Ohio) and then positioned on the external surface of the CMs. When the adhesive had dried, the glass-cuticle chamber units were placed on a wet filter paper and were filled with distilled water to
detect defects of the cuticle and seal. After drying, the CMs were placed on an aluminum screen 10 mm above the bottom of a pan in a 25 °C water bath (Fig. 4.1). A half milliliter of 0.78 mM 2-14C-labeled 2,4-D, buffered by 40 mM citric phosphate (pH 3.0) and containing 0.1 mM sodium azide, was pipetted into each glass-cuticle chamber. The 14C-labeled 2,4-D (Amersham Corp., Arlington Heights, Illinois) had a specific activity of 28.0 mCi mmol⁻¹ and a purity of 98%. The distilled water (pH 3, adjusted with HCl) level in the pan, adjusted to the same height as the 2,4-D solution in the glass-cuticle chamber, was changed at a rate of 2 liters hr⁻¹ with uniform flow across the pan. After 6 hours, the 2,4-D solution in each glass-cuticle chamber was collected in a liquid scintillation counting vial. The glass cylinder was separated from the CM which was placed in another counting vial. The glass cylinder was washed with 0.5 ml distilled water and the washing solution was added to the first vial. Radioactivities of the collected solution and the CM were counted in AquaMix cocktail (ICN Biomedicals, Inc., Costa Mesa, California) with a liquid scintillation counter (Beckman Model LS 7000). The amount of 14C-labeled 2,4-D passing through the CM was determined by subtracting the quantity of 14C-label in the collected solution and CM from the amount originally present. Each experimental treatment had five replications.

2.3. Cuticular sorption of 2,4-D

Cuticular membranes, three for each replicate of a treatment, were weighed
after being oven dried at 30 °C for eight hours, and then soaked in distilled water and 10 ml liter\(^{-1}\) PEG 100-O, PEG 200-ML or PEG 600-ML at 20 °C for 24 hours. Each treatment was replicated five times. The treated CMs were washed with distilled water after tap water for 20 minutes, and then oven dried. The dried CMs were placed in liquid scintillation counting vials. One milliliter of \(^{14}\)C-labeled 2,4-D solution, as described earlier, was added to the vials and they were placed in a 25 °C water bath. After 96 hours, the CMs were taken out and washed with 0.5 ml distilled water for 1 second; the washing solution was added to the vial. Radioactivity of the collected solution was measured by liquid scintillation counting. The amount of \(^{14}\)C-labeled 2,4-D in the CMs was determined by subtracting the quantity of \(^{14}\)C-label in the collected solution from the amount originally present. Sorption is represented by the apparent partition coefficient, \(K_{\text{pH}}\), which is calculated as followings:

\[
K_{\text{pH}} = \frac{\text{Radioactivity in CM/CM weight}}{\text{Radioactivity in buffer/buffer weight}}
\]

or

\[
2,4\text{-D concentration in CM}
\]

\[
2,4\text{-D concentration in buffer}
\]

2.4. Cuticular sorption of the PEG surfactants

CM discs, three for each replicate of a treatment, were weighed after being oven dried at 35 °C for eight hours and were soaked in 1.5 ml 10 ml liter\(^{-1}\) PEG 100-O, PEG 200-ML, and 600-ML solutions or distilled water, respectively.
Each treatment was replicated three times. The vial containing PEG surfactants was previously filled with the same surfactant solutions for 24 hours to eliminate possible sorption by the glass vials, and the solutions were discarded. The vials were then placed in a 20 °C water bath for 72 hours. The CM discs were removed from the vials and placed between blotting papers to absorb any extra surfactant solution left on the cuticle surface. The CMs were dried at 35 °C for eight hours in an oven and were weighed immediately after drying. The amount of PEG surfactant sorbed by the CMs was calculated based on the weight change of the CMs before and after the soaking.

2.5. Penetration and sorption of the dewaxed cuticles

CMs were soaked in chloroform solution for one hour and moved to fresh chloroform for another hour at 20 °C to remove wax from the CMs. The process reduced CM weight by 24 percent, the maximum reduction achievable with chloroform soaking. The dewaxed cuticular membranes (DCMs) were used to repeat the penetration and sorption experiments as described for the CMs except treatment with PEG was limited to the 100-O product.

Since quenching was constant for all treatments within each penetration or sorption experiment, the radioactivity of each treatment was represented by counts per minute. Experiments were conducted using completely randomized designs. Data were subjected to variance analysis. Mean difference between water check and surfactants was evaluated with orthogonal contrast tests, and
effect of surfactant HLB was analyzed with regression.

3. RESULTS AND DISCUSSION

The surfactant treatments significantly increased the penetration of 2,4-D through the CM, and the penetration appears to decrease as surfactant HLB increased (Table 4.1). At HLB 14.6, the penetration of PEG 600-ML treatment was not significantly different from that of the water check. Surfactant HLB appears to be an important factor affecting modification of 2,4-D penetration. Norris also reported that penetration of 2,4-D through pear leaf cuticles could be increased by Span 20 and Span 80.\(^3\) The increase seems to be inversely related to the HLB of the Span surfactants. He also found that Triton N-101 (HLB 13.4) and Tween 20 (HLB 16.7), both with relatively high HLBs, had no effect on the permeability of pear leaf cuticles. The differences between these two surfactants and Span surfactants in affecting the permeability to 2,4-D could be due to either differences in chemical groups, differences in HLBs, or both.

Stevens and Bukovac, in their study of octylphenoxy surfactant (Triton X series) effects on atrazine and DDT uptake by maize leaves, observed that uptake of the two pesticides was inversely related to the surfactant HLB.\(^7\) However, Kirkwood et al. reported that penetration of MCPA and MCPB through broad bean abaxial leaf cuticles was increased as HLB of the surfactant mixture (Span series and Tween series) increased.\(^8\) For the latter two experiments, the surfactants could affect both stomatal and astomatal penetration.
Penetration of 2,4-D through the DCM was also significantly increased by PEG 100-O (Table 4.1). The doubled 2,4-D penetration through both CM and DCM led by PEG 100-O suggests that the surfactants enhance 2,4-D penetration by interacting with both cutin and cuticular waxes. Irrespective of surfactant effects, penetration of 2,4-D through the DCM was about 2.5 times of that through the CM, indicating that waxes of the cuticle were an important part of the barrier for 2,4-D penetration under conditions of this study.

Sorption of PEG surfactants by the cuticles was inversely related to HLB of the surfactants. The DCM sorbed more PEG 100-O than the CM did (Table 4.1). The latter result is similar to the finding of Shafer and Bukovac.9 PEG surfactants did not change the apparent partition coefficient, Ksp, of 2,4-D between the CM or DCM and a buffer solution (Table 4.1). This result is different from those of several other studies on pears and tomatoes.5,6,10 Those researchers reported that Triton X-100 decreased 2,4-D binding to pear leaf cuticles and sorption of NAA and benzyladenine by tomato fruit cuticles. Orgell reported that surfactant Tween 81 decreased slightly the sorption of 2,4-D by apricot cuticles at pH 3 and had very little effect at pH 9.11

Sodium dodecyl sulfate and Tween 20 were also reported to decrease 2,4-D binding to pear leaf cuticles, but the surfactants did not change permeability of the cuticles to 2,4-D.3,10 These studies show surfactants may change 2,4-D cuticular sorption without altering cuticular penetration. Data from our experiments suggest that there was no correlation between surfactant effects on 2,4-D sorption and penetration of cuticles, and surfactants may change 2,4-D
penetration through cuticles without changing 2,4-D sorption by the cuticles. The change in 2,4-D penetration resulting from pretreatment with surfactants may be attributed to change in surfactant mediated 2,4-D diffusion rather than sorption. Schonherr and Bauer pointed out that surfactants at concentrations above the CMC in aqueous donor solutions reduce the concentration of a lipophilic solute in the cuticle, and this effect invariably decreases the rate of penetration of the solute. However, surfactants may increase the mobility of solutes in the cuticle, leading to an increased rate of penetration. How surfactants change the mobility of solutes in the cuticles needs further study.

4. CONCLUSIONS

Penetration of the CM by 2,4-D was increased by PEG surfactant pretreatment, and the increase was inversely related to the HLB of the surfactant. The cuticular sorption of the surfactants and the increased 2,4-D penetration after CMs and DCMs were treated with surfactants indicate that surfactants may interact with both cutin and waxes of cuticles. Modification of 2,4-D cuticular sorption by PEG surfactants was not significant. The increased 2,4-D penetration through the CMs and DCMs pretreated with PEG surfactants was not attributed to changes in 2,4-D sorption by CMs and DCMs, implying that PEG surfactants might have changed 2,4-D mobility through the cuticles.
Fig. 4.1. A system used for studying penetration of 2,4-D through apple leaf cuticles.
Table 4.1. 2,4-D penetration through and sorption by apple leaf cuticles as influenced by pre-soaking the cuticles in 10 ml liter⁻¹ PEG surfactant solutions and sorption of the surfactants by the cuticles.

<table>
<thead>
<tr>
<th>Surfactant Name</th>
<th>Penetrated 2,4-D (cpm)ᵃ</th>
<th>2,4-D partition coefficient (Kᵢ)ᵇ</th>
<th>Wt. of sorbed surfactant (mg) per mg cuticle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuticular membrane (CM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water check</td>
<td>700</td>
<td>434</td>
<td>0</td>
</tr>
<tr>
<td>PEG 100-O</td>
<td>1500</td>
<td>457</td>
<td>0.5</td>
</tr>
<tr>
<td>PEG 200-ML</td>
<td>1470</td>
<td>471</td>
<td>0.1</td>
</tr>
<tr>
<td>PEG 600-ML</td>
<td>870</td>
<td>439</td>
<td>0</td>
</tr>
<tr>
<td>Orthogonal contrast test of ck vs. treatment</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Regression for surfactant HLB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>*</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Quadratic</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

Dewaxed cuticular membrane (DCM)

| Water check               | 1900                    | 495                              | 0                                           |
| PEG 100-O                 | 3800                    | 492                              | 0.7                                         |
| T-test                    | **                      | NS                              | **                                          |

ᵃPenetrated counts per minute = (cpm originally placed in the glass-cuticle chamber) - (cpm left in the chamber and the cuticle).
ᵇPenetrated % = (penetrated cpm/cpm originally placed in the chamber) x 100.
ᶜKᵢ = 2,4-D concentration in cuticle/2,4-D concentration in buffer solution.
ᵈNS, *, ** Nonsignificant, significant at the 5% and 1% levels, respectively.
REFERENCES


CHAPTER 5
CUTICULAR SORPTION AND DESORPTION OF NONIONIC SURFACTANT PEGOSPERSE 100-O

ABSTRACT

Sorption and desorption of Pegosperse 100-O (diethylene glycol oleate) surfactant by unaltered (CM) and dewaxed (DCM) adaxial cuticle membranes isolated from apple (*Malus pumila* M.) leaves were studied. The aim of this study was to understand interactions between surfactants and cuticles. Enzymatically-isolated cuticles were soaked in buffer or Pegosperse 100-O (PEG 100-O) solution (pH 7.0), and the weight change of cuticles or the surface tension change of the solutions was measured to determine the amount of surfactant sorbed or desorbed by the cuticles. PEG 100-O sorption by both the CMs and the DCMs occurred mainly in the first three hours and was concentration-dependent. The DCMs always sorbed more surfactant than the CMs did. Desorption of PEG 100-O from both CMs and DCMs was rapid in the first few hours and then at relatively low rates until the surfactant was totally desorbed from the cuticles after about two months. The sorption and complete desorption of the surfactant by both CMs and DCMs show that PEG 100-O interacts with both cutins and waxes of the cuticles and the interactions are reversible.
1. INTRODUCTION

Surfactants are commonly used for formulation and as spray adjuvants of pesticides. Surfactants can improve the characteristics of the spray solution by acting as a utility-modifier, such as a compatibility agent, and a spray-modifier, such as a spreader.\(^1\) Surfactants also enhance pesticide activity through increasing pesticide foliar penetration, serving as an activator.\(^1\) However, information on the mechanism of surfactant-enhanced foliar penetration is limited.\(^4\) The enhancement may be the result of a complex interaction between pesticides, surfactants, plant surfaces, and environmental conditions. Since plant cuticle is the primary barrier for pesticide penetration,\(^5\) the cuticle is logically the layer of the leaf surface to be considered. Interactions between plant, solute, and surfactant are still not clear and more studies are needed to understand how surfactants modify cuticles.\(^6\) With the inherent difficulties in studying and understanding a complex, four-factor interaction, a simpler approach was used here to focus on understanding the interaction between surfactants and cuticles by examining the cuticular sorption and desorption of a nonionic surfactant.

Little attention has been given, and limited information is available for the interaction between surfactants and cuticles.\(^7\) Shafer and Bukovac found that removing soluble lipids from isolated cuticles of tomato fruits and increasing surfactant concentrations significantly increased sorption of the surfactants Triton X-45 and Triton X-100 by the cuticles.\(^4\) The time course of surfactant
sorption was characterized by a rapid initial sorption phase followed by a plateau.

The objectives of this study were to investigate (1) time courses of the cuticular sorption and desorption of PEG 100-O surfactant and the influence of cuticle waxes on the sorption and desorption, and (2) to refine a simple method to measure surfactants in low concentration based on surface activity (reduction of surface tension) of the surfactants.

2. MATERIALS AND METHODS

2.1. Cuticle isolation and removal of cuticle waxes

Fully-expanded apple leaves were sampled from 6-year-old field grown Golden Delicious (Malus pumila M.) trees on MM 106 rootstocks. Leaf discs were cut with a 19.6 mm cork borer, avoiding midribs and large veins. The cuticles of leaf discs were enzymatically-isolated using a method similar to the one reported by Norris and Bukovac. The abaxial surface of the leaf discs was abraded with sand paper (Grit size 220) to disrupt the abaxial cuticles and enhance the infiltration of enzyme solution into the leaf tissues. The discs were washed with tap and distilled water and incubated in 8 mM citric phosphate buffer (pH 4.0) containing 20 g liter\(^{-1}\) pectinase (U.S. Biochemical Co., Cleveland, OH), 20 g liter\(^{-1}\) cellulase (U.S. Biochemical Co.), and 0.1 mM sodium azide. The tissue was vacuum infiltrated and then kept at room
temperature for 2-5 weeks. The leaf discs were gently washed with tap water to separate adaxial-astomatal cuticles from other tissues. The cuticular membranes (CMs) were then washed with distilled water, air dried, and stored at room temperature for future use. Dewaxed cuticular membranes (DCMs) were obtained by soaking the cuticular membrane twice for one hour each time in fresh chloroform at 20 °C.

2.2. Sorption of PEG 100-O by cuticles

CM discs, four for each of the four replicates, were weighed after being oven dried at 35 °C for eight hours and then soaked in 1.5 ml of 10 ml liter⁻¹ PEG 100-O aqueous solution buffered by 40 mM citric phosphate (pH 7.0) or solution with buffer only as control in a 20 ml glass vial. To eliminate the effect of sorption by the glass, vials were pretreated by filling them with the same concentration of PEG 100-O solution and allowing them to stand for 24 hours and discarding the pretreatment solution. Vials containing the CM discs were placed in a 20 °C water bath. At each designated time (1/6, 1, 3, 5, and 7 hours), the CM discs were taken from the PEG 100-O solution and placed between blotting papers to remove any extra surfactant solution on the cuticle surface. The CMs were oven dried and weighed. The amount of PEG 100-O sorbed by the CMs was calculated according to the weight change of the CMs before and after the soaking.

In a study of PEG 100-O concentration effect on the sorption during 24
hours, treatments were replicated three times with three CMs in each replicate. The same procedures as the time course study were employed for concentrations of 5 and 10 ml liter\(^{-1}\). For concentrations from 0.01 to 0.1 ml liter\(^{-1}\), the sorption was determined by measuring surface tension change of PEG 100-O solution in the vials before and after soaking the CMs in 1.5 ml surfactant solution. In order to determine the relationship between surface tensions and PEG 100-O concentrations, the surface tensions of surfactant solutions with concentrations from 0 to 500 ml liter\(^{-1}\) were measured with a Surface Tension Apparatus (Central Scientific Co., Chicago, IL) at 20 °C with three replications. A standard calibration equation of surface tension vs. surfactant concentration was established within a concentration range from 0.005 to 0.1 ml liter\(^{-1}\). Concentrations of the surfactant in the vials with cuticles were calculated based on measured surface tensions according to the standard calibration equation, and consequently the amount of PEG 100-O left in the solution or sorbed by CMs could be determined. All procedures for studying surfactant sorption by DCMs were the same as that for CMs.

2.3. PEG 100-O desorption from cuticles

CM discs, four for each of four replicates, were weighed and soaked in 10 ml liter\(^{-1}\) PEG 100-O solution for 24 hours to get maximum sorption of the surfactant. After extra surfactant solution left on the CM surface was removed by blotting paper, the CMs were dried and weighed in the same manner as
mentioned in the sorption experiment to determine the amount of PEG 100-O sorbed by the CMs. The four CMs with surfactant were placed in 1.5 ml buffer solution (40 mM citric phosphate, pH 7) in a 20 ml glass vial and were removed at intervals of 10-15 minutes before being dried and weighed. The amount of PEG 100-O desorbed or released from the CMs at each time was reflected by the weight change of the CMs. Percent of PEG 100-O desorption was calculated from the amount of surfactant desorbed at each time divided by the surfactant amount sorbed at the beginning. After 90 minute desorption in 1.5 ml buffer solution, the four CMs were transferred into 50 ml fresh buffer solution in a flask. The CMs were removed at intervals from 30 minutes to several days, dried, and weighed. Procedures for studying surfactant desorption from DCMs were the same as that for CMs.

3. RESULTS AND DISCUSSION

3.1. Relationship between PEG 100-O concentration and surface tension

The surface tension decreased slightly as surfactant concentration increased up to 0.001 ml liter$^{-1}$ and then changed greatly within a concentration range between 0.001 and 0.1 ml liter$^{-1}$ (Fig. 5.1). Since surface tension was very sensitive to the change in concentration, this character was used to determine PEG 100-O quantitatively. A function of surface tension vs. log concentration of PEG 100-O was calculated within a concentration range from 0.005 to 0.1 ml
liter⁻¹. The regression equation was found to be \( \text{Surface Tension} = 88 + 80 \times (\log \text{Concentration}) + 31 \times (\log \text{Concentration})^2 \) with \( R^2 \) and \( P \) values of 0.97 and 0.001, respectively. When PEG 100-O concentrations increased from 0.1 ml liter⁻¹ up to 500 ml liter⁻¹, surface tension decreased only slightly. The function curve of surface tension vs. log concentration of the surfactant solution found in this study agrees with typical plots of surface tension against the logarithm of surfactant concentration.¹¹⁻¹²

3.2. Sorption of PEG 100-O by cuticles

PEG 100-O was sorbed rapidly by both CMs and DCMs in the first 3 hours of treatment (Fig. 5.2). After that only slightly additional PEG 100-O was sorbed and no further sorption was detectable after 5-hour soaking of the cuticles in the surfactant solution. DCMs always sorbed more PEG 100-O than CMs did. These sorption trends are similar to the findings by Shafer and Bukovac on sorption of Triton X-100 by tomato fruit cuticles.⁴

Sorption of both CMs and DCMs increased as PEG 100-O concentrations increased (Fig. 5.3). At concentrations below 0.1 ml liter⁻¹, the sorption increase as a function of concentration was nearly linear. When the concentration increased up to 10 ml liter⁻¹, the sorption was still concentration-dependent, but the concentration effect was relatively small compared with the effect at low concentrations. The DCMs sorbed more surfactant than CMs at all concentrations.
3.3. PEG 100-O desorption from the cuticles

Desorption of PEG 100-O from both CMs and DCMs was rapid in the first few hours, and both had similar desorption rates as expressed in percent desorption (Fig. 5.4). Desorption was apparently affected by PEG 100-O present in the buffer solution. Before the CMs or DCMs were transferred into fresh buffer solution, desorption and sorption seemed to reach equilibrium in the 1.5 ml buffer solution. After transfer, the desorption continued with another rapid period and then at relatively low rates until the surfactant was totally desorbed from the cuticles after about two months. Soaking the membranes further in the buffer solution did not lead to additional weight change of the membranes. The final weight of CMs and DCMs was the same as that at the beginning, showing that the sorption-desorption process did not change CM and DCM weight. It is unlikely that any constituent of cuticles was removed as a result of the surfactant sorption and desorption. The complete desorption of the surfactant from both CMs and DCMs shows the sorption of the surfactant by cuticles was reversible, indicating a simple physical interaction between the surfactant and the cuticle.

4. CONCLUSIONS

Tracing surfactant PEG 100-O in aqueous solution by measuring its surface tension proved to be a sensitive method. Using this procedure, we could detect
the surfactant at a concentration as low as 0.001 ml liter⁻¹. A considerable amount of PEG 100-O was sorbed by both the CMs and DCMs, showing the surfactant interacts with both cutins and waxes of the cuticles. Complete desorption of PEG 100-O from the cuticle indicates that binding of surfactant to the cuticles was reversible. It is unlikely that PEG 100-O surfactant removed any constituent of the cuticle.
Fig. 5.1. Relationship between surface tension and concentrations of PEG 100-O at 20 °C.
Fig. 5.2. Time course of PEG 100-O sorption by cuticular membranes (CM) and dewaxed cuticular membranes (DCM) of apple (*Malus pumila*) leaves. Each point is an average of four replicates, and the vertical bars are standard error bars. The initial PEG 100-O concentration was 10 ml liter⁻¹.
Fig. 5.3. Sorption of PEG 100-O by cuticular membranes (CM) and dewaxed cuticular membranes (DCM) of apple (Malus pumila) leaves as affected by PEG 100-O concentrations. Each point is an average of three replicates. The vertical bars are standard error bars and are not shown when the bars are shorter than the plot points.
Fig. 5.4. Time course of PEG 100-O desorption from cuticular membranes (CM) and dewaxed cuticular membranes (DCM) of apple (*Malus pumila*) leaves. Each point is an average of four replicates, and the vertical bars are standard error bars.
REFERENCES


CHAPTER 6

CUTICULAR PENETRATION OF (2,4-DICHLOROPHENOXY)ACETIC ACID AS AFFECTED BY INTERACTION BETWEEN PEGOSPERSE 100-O SURFACTANT AND APPLE LEAF CUTICLES

ABSTRACT

Impacts of pH and sorption-desorption of Pegosperse 100-O (diethyleneglycol oleate) surfactant by apple (Malus pumila M.) leaf cuticles on surfactant-enhanced cuticular penetration of 2,4-D [(2,4-dichlorophenoxy)acetic acid] were studied. Glass cylinders were affixed to enzymatically-isolated adaxial apple leaf cuticles after the cuticle segments had been soaked in 10 ml l\(^{-1}\) Pegosperse 100-O (PEG 100-O) solution and washed for 20 and 120 minutes, respectively. The glass-cuticle chamber units were placed in petri dishes containing buffer solutions. Quantities of \(^{14}\)C-2,4-D in the glass-cuticle chambers passing through the cuticles at pH from 1 to 6.5 were determined. PEG 100-O significantly increased cuticular penetration of dissociated 2,4-D at pH 4.5; the surfactant had no effect on penetration of undissociated 2,4-D at pH 1.0. Surfactant-enhanced penetration of 2,4-D occurred only when the surfactant was in the cuticles, while the process of surfactant sorption-desorption alone had no effect on the penetration. These results support a 'hydrophilic channel' hypothesis, that surfactants may create hydrophilic channels or increase the area of the channels in the cuticle and, consequently, enhance the passing of
polar molecules like dissociated 2,4-D through the cuticle.

1. INTRODUCTION

Inadequate penetration of pesticides into the leaf surface of plants represents a direct financial loss to farmers and a potential contribution to environmental pollution.¹ Surfactants have been found to enhance the penetration and, consequently, reduce costs and residues of the pesticides in the environment.²⁻⁴ However, the mechanism by which surfactants facilitate foliar entry of herbicides has received little attention,⁵ and detailed information on the mechanism of surfactant-enhanced penetration or on the nature of surfactant interaction with the cuticular membranes is limited.⁵ This report summarizes part of our research on roles of surfactants in foliar uptake of pesticides.

It has been suggested that surfactants enhance pesticide activity in many possible ways.³⁻⁷,⁸⁻¹⁰ One important mechanism is through surfactant enhancement of cuticular penetration of pesticides. This has been proposed or found to be a result of several possible effects. Surfactants were reported to decrease cuticular sorption of several growth substances.¹¹⁻¹² This reduced concentration of a solute in a cuticle would lead to a low rate of solute penetration; however, surfactants may increase the mobility of solutes in the cuticle, resulting in an increased rate of penetration.¹³ How surfactants increase the mobility of solutes in cuticles is still open to question. Solubilization of cuticle waxes by surfactants was observed and may facilitate pesticide
Surfactants could change the ultrastructure of cuticles and may modify their permeability. Also there are reports that surfactants copenetrated with pesticides.

Other possible impacts of surfactants on cuticular penetration of pesticides have been suggested. Jansen (1964) and Smith et al. (1966) proposed that surfactants may penetrate cuticles or diffuse into imperfect areas of cuticles and form or increase areas of 'hydrophilic channels' in cuticles. Water-soluble pesticides would be free to diffuse through these channels. Noga et al. (1987) suggested that surfactants may induce a phase transition of the soluble cuticular lipids and subsequently change both the orientation and location of the lipid fraction within the cutin matrix. This modification may affect penetration of aqueous pesticide solutions.

In our previous studies, we found that PEG 100-O surfactant enhanced penetration of 2,4-D through apple leaf cuticles even though 2,4-D sorption by the cuticle was not changed. Apparently, sorption could not explain the increased 2,4-D penetration, and suggested that this enhancement might be related to increased mobility of 2,4-D in the cuticles. The objective of this study was to find possible explanations for enhanced 2,4-D cuticular penetration brought about by PEG 100-O.

2. MATERIALS AND METHODS

Fully-expanded apple leaves were sampled from 6-year-old field grown
Golden Delicious (Malus pumila M.) trees on MM 106 rootstocks. Leaf discs were cut with a 19.6 mm cork borer, avoiding midribs and large veins. The cuticles of leaf discs were enzymatically-isolated using a method similar to the one reported by Norris and Bukovac (1968).\(^\text{23}\) The abaxial surface of the leaf discs was abraded with sand paper (Grit size 220) to disrupt the abaxial cuticles and enhance the infiltration of enzyme solution into the leaf tissues. The discs were washed with tap and distilled water and incubated in 8 mM citric phosphate buffer (pH 4.0) containing 20 g liter\(^{-1}\) pectinase (U.S. Biochemical Co., Cleveland, OH), 20 g liter\(^{-1}\) cellulase (U.S. Biochemical Co.), and 0.1 mM sodium azide. The tissue was vacuum infiltrated and then kept at room temperature for 2-5 weeks. The leaf discs were gently washed with tap water to separate adaxial, stomatal cuticles from other tissues. The cuticular membranes (CMs) were then washed with distilled water, air dried, and stored at room temperature for future use.

2.1. Impact of pH on PEG 100-O enhanced cuticular penetration of 2,4-D

CM discs were soaked in 10 ml l\(^{-1}\) PEG 100-O solution or distilled water at 20 °C for 24 hours. After being washed with running tap water for 20 minutes, the CMs were rinsed with distilled water, dried, and checked with an optical microscope for any defect. A glass cylinder with a 12.3 mm internal diameter and 15 mm length was touched to Wonder Bond Adhesive (Borden Inc. Columbus, OH) and positioned on the external surface of each CM disc. After
the adhesive had dried, the glass-cuticle chamber units were placed in a petri
dish lined with a piece of Whatman 4 filter paper (Fig. 6.1). Six glass-cuticle
chamber units were placed in each dish; three of the units had CMs pre-soaked
in PEG 100-O solution and the rest had CMs pre-soaked in water as a control.
Each of the four pH levels was applied to two dishes, thereby treating 12 glass-
cuticle chamber units with each pH. A half milliliter of 0.78 mM 2-\textsuperscript{14}C-labeled
2,4-D, buffered by 40 mM citric phosphate (pH 3.0-6.5) or HCl-KCl (pH 1.0)
and containing 0.1 mM sodium azide, was pipetted into each glass-cuticle
chamber. Specific activity of the \textsuperscript{14}C-labeled 2,4-D was 28.0 mCi mmole\textsuperscript{-1}.
Buffer solution (25 ml) corresponding to each pH was pipetted into each petri
dish so that the buffer solution level was the same as the 2,4-D solution in the
chamber. The petri dishes were covered and placed in a 25 °C water bath.
After 24 hours, the 2,4-D solution in each glass-cuticle chamber was collected in
a liquid scintillation counting vial. The glass cylinder was separated from the
CM and was washed with 0.5 ml distilled water and 6 ml AquaMix cocktail
(ICN Biomedicals, Inc., Costa Mesa, CA). Both the CMs and washing solution
were also placed in the counting vial. Radioactivities of the solution and the
CM in the vial were counted with a liquid scintillation counter (Beckman Model
LS 7000). The amount of \textsuperscript{14}C-labeled 2,4-D passing through the CM was
determined by subtracting the quantity of \textsuperscript{14}C-label in the collected solution and
CMs from the amount originally present.
2.2. Effects of PEG 100-O cuticular sorption-desorption on 2,4-D penetration

CM discs were soaked in 10 ml l\(^{-1}\) PEG 100-O solution and distilled water, respectively at 20 °C for 24 hours. Twelve CMs soaked in PEG 100-O solution were washed with running tap water for 20 minutes and another twelve for 120 minutes. Experimental treatments then consisted of a water control, a 20-minute or a 120-minute desorption after surfactant sorption. All CMs were rinsed with distilled water, dried, weighed, and checked with an optical microscope for any defect. Glass-cuticle chamber units were prepared following the same procedures as the experiment on pH effects. In this experiment each of two petri dishes had six glass-cuticle chamber units prepared with CMs from the three treatments with two units of each treatment. Procedures for 2,4-D solution preparation, collection, and radioactivity detection were the same as in the pH experiment except that all 2,4-D solutions were prepared at pH 3.0.

3. RESULTS AND DISCUSSION

3.1. Impact of pH on PEG 100-O enhanced cuticular penetration of 2,4-D

Cuticular penetration of 2,4-D decreased as pH increased when PEG 100-O was absent (Fig. 6.2). This trend was related to dissociation of 2,4-D. Ashton and Crafts (1981) reported that dissociated 2,4-D at high pH is negatively charged and does not penetrate the cuticle as easily as the un-ionized form.\(^{24}\)
However, in this study both un-ionized and dissociated 2,4-D penetrated the cuticle to some degree. The pH may also influence 2,4-D penetration by changing the electrical charge of cuticles which act as polyelectrolytes. At a pH higher than the isoelectric point of the cuticle, the cuticle possesses a net negative charge and is permselective to cations but not to negatively charged, dissociated 2,4-D. At low pH the cuticles carry positive charges and are permselective to anions like dissociated 2,4-D. Parr and Norman (1965) found that un-ionized 2,4-D could easily penetrate charged membranes. At high pH the tendency for 2,4-D dissociation and the negative charge of cuticles do not favor penetration, while at low pH both the tendency of 2,4-D to be in the un-ionized form and the positive charge of the cuticles are favorable for penetration.

When PEG 100-O surfactant was introduced into the system, the penetration trend was altered (Fig. 6.2). The highest 2,4-D penetration was achieved at pH 3.0 instead of at 1.0 in the control. The surfactants significantly increased 2,4-D penetration at pH 3.0 and 4.5 but had no effect at pH 1.0 and 6.5. At pH 1.0, more than 98 percent of 2,4-D was un-ionized and PEG 100-O did not increase penetration of un-ionized 2,4-D. At pH 4.5, more than 98 percent 2,4-D was dissociated, and the surfactant significantly increased the dissociated 2,4-D penetration. There was essentially no difference in percent of 2,4-D dissociation between pH 4.5 and 6.5; however, surfactant significantly enhanced 2,4-D penetration at pH 4.5 but had no effect at pH 6.5, showing an interaction between surfactant, cuticle, and pH. Conversely, penetration of
2,4-D in the control treatment was constant over this pH range, indicating that pH and cuticle did not interact with each other in affecting penetration of dissociated 2,4-D.

3.2. Effects of PEG 100-O cuticular sorption-desorption on 2,4-D penetration

PEG 100-O surfactant was sorbed by apple leaf cuticles (Table 6.1). After 120 minute washing, the weight of the washed cuticles was exactly the same as the original weight of the cuticles, indicating the surfactant could be completely desorbed from the cuticle. Washing the treated cuticles for 240 minutes also resulted in exactly the same average cuticle weight, suggesting that cuticle constituents were not dissolved and removed by the surfactant sorption-desorption process. Much of the sorbed surfactant (0.11 mg/cuticle) was left in cuticles if the cuticles were washed for 20 minutes after surfactant treatment. The surfactant-treated cuticles with total and partial desorption of the surfactant provided good materials for studying how the surfactant interacts with the cuticle and effects of the interaction on 2,4-D penetration.

PEG 100-O increased 2,4-D penetration only when the surfactant was still present in the cuticles (Table 6.1). When, after being sorbed, PEG 100-O was completely desorbed from cuticles, the surfactant had no effect on 2,4-D penetration. This indicated that the cuticular sorption-desorption process of the surfactant and any alteration of the cuticle by the process was not important in affecting 2,4-D penetration.
If the surfactant increases 2,4-D penetration by changing cuticular ultrastructure or by causing a cuticular phase transition, these possible effects are operative only when the surfactant is present in the cuticles, and the effect is reversible when the surfactant is removed. Explaining why surfactant-enhanced 2,4-D penetration was pH-dependent (Fig. 6.1) would be difficult with the ultrastructure change or phase transition hypotheses.

Copenetration of the surfactant and 2,4-D was unlikely because the surfactant was in the cuticle and not with the 2,4-D in the uptake chambers. In addition, there was no concentration gradient of the surfactant between the source and the sink.

A considerable amount of surfactant was left in cuticles after sorption and brief desorption (Table 6.1). Surfactants and cuticles both have hydrophilic and lipophilic properties, and hydrophilic-hydrophilic or lipophilic-lipophilic interactions between the surfactant molecule and the cuticle or between the surfactant molecules themselves would be expected. The 'hydrophilic channel' hypothesis might explain the results obtained from this study. Jansen (1964) suggested that the hydrophilic-lipophilic nature of "bulky" surfactants in the cuticle may increase cuticle swelling and the area of the 'hydrophilic channels'. Development of these channels is a result of separation of the wax platelets led by imbibition of water by the cellulose matrix and the hydrophilic portion of the cutin. Smith et al. (1966) proposed that surfactant molecules may diffuse into the leaf through imperfections in the leaf surface. These imperfections may consist of cracks and hydrophilic and/or lipophilic areas (perhaps not pores in
the usual sense) along the interfaces of the lipophilic cuticle waxes and cutin. The surfactant molecules perhaps align themselves in monolayers, thus creating a hydrophilic layer or layers in these imperfections. Water molecules would be attracted to these hydrophilic regions as they are formed, thus creating channels. Water soluble pesticides would be free to diffuse through these 'hydrophilic channels'.

Increasing the areas of 'hydrophilic channels' or creating 'hydrophilic channels' by PEG 100-O may explain why in this study the surfactant must be present in the cuticle to affect 2,4-D penetration and why the surfactant sorbed by the cuticles increased penetration of dissociated 2,4-D at pH 4.5 without influencing penetration of un-ionized 2,4-D at pH 1.0 (Fig. 6.1). The increased areas of 'hydrophilic channels' or created 'hydrophilic channels' by the surfactant in the cuticle may facilitate penetration of polar 2,4-D (dissociated form) without affecting nonpolar 2,4-D (un-ionized form). The increased areas of 'hydrophilic channels' or created 'hydrophilic channels' by the surfactant would be diminished as the surfactant was removed or disappeared from the cuticle.

4. CONCLUSIONS

Increase in 2,4-D cuticular penetration by PEG 100-O surfactant was pH-dependent. The surfactant did not affect penetration of un-ionized 2,4-D at pH 1.0. However, the surfactant enhanced the cuticular penetration of dissociated 2,4-D and the effect was influenced by interaction between surfactant, cuticle,
and pH. This enhancement occurred only when surfactant was present in cuticles. Solubilization of cuticle constituents by the surfactant and copenetration of surfactant and 2,4-D were not satisfactory explanations for the increased 2,4-D penetration. Although enhancement of penetration by reversible ultrastructural changes, phase transition of the cuticle induced by surfactant, and other possible mechanisms could not be excluded, increasing areas of 'hydrophilic channels' and forming 'hydrophilic channels' in the cuticle by the surfactant could reasonably explain the results of this study. The 'hydrophilic channels' formed in this way may facilitate the penetration of cuticles by water soluble or polar molecules like dissociated 2,4-D.
Fig. 6.1. A system used to study cuticular penetration of 2,4-D.
Fig. 6.2. Effects of pH on PEG 100-O enhanced 2,4-D penetration of adaxial cuticles isolated from apple (Malus pumila) leaves and on 2,4-D dissociation. The vertical bars are standard error bars. Percent of 2,4-D dissociation was calculated based on Henderson-Hasselbalch equation,
\[ \text{pH} = \text{pK}' + \log\left(\frac{\text{proton acceptor}}{\text{proton donor}}\right) \]
or
\[ \text{Dissociation} \% = \left[10^{\text{pH}-\text{pk}'}/(1 + 10^{\text{pH}-\text{pk}'})\right] \times 100, \text{ at pk}'_{2,4-D} = 2.76. \]
Table 6.1. PEG 100-O sorption-desorption by cuticular membranes (CMs) isolated from apple (*Malus pumila*) leaves and its impacts on 2,4-D penetration of the CMs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cuticle dry wt. before treatment (mg/cuticle)</th>
<th>Cuticle dry wt. after treatment (mg/cuticle)</th>
<th>PEG 100-O in CMs (mg/cuticle)</th>
<th>24 hr 2,4-D penetration (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water control</td>
<td>1.40&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.40</td>
<td>0 a&lt;sup&gt;x&lt;/sup&gt;</td>
<td>26 a</td>
</tr>
<tr>
<td>120 minute desorption after sorption</td>
<td>1.30</td>
<td>1.30</td>
<td>0 a</td>
<td>28 a</td>
</tr>
<tr>
<td>20 minute desorption after sorption</td>
<td>1.36</td>
<td>1.47</td>
<td>0.11 b</td>
<td>37 b</td>
</tr>
</tbody>
</table>

<sup>a</sup>Penetration percent = (penetrated cpm/cpm originally placed in the chamber) x 100  
<sup>y</sup>Average of five replicates  
<sup>x</sup>Mean separation by Fisher’s LSD test at P=5%.
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APPENDICES
Fig. A.1. Time course of chloroform effect on weight of cuticular membranes isolated from upper surfaces of apple leaves. Three cuticles in 4 ml chloroform for each of five replicates.
Fig. A2. Time course of 2,4-D sorption by adaxial cuticular membranes of apple leaves. The volume of 0.78 mM 2,4-D solution was 2 ml, the weight of the cuticular membranes was 1.9 mg, and pH was 3.0.
Fig. A.3. Penetration of 2,4-D through adaxial cuticular membranes (CMs) of apple leaves (pH 3.0) as affected by soaking the CMs in 10 ml liter\(^{-1}\) PEG 100-O (diethylene glycol oleate) surfactant and its hydrolyzates for 24 hours followed by 20 minute washing with water. Mean separation by Fisher’s LSD test at P = 5%.
Table A.1. Penetration of 2,4-D into upper surfaces of fresh and dead (by freezing) apple leaves and through adaxial cuticular membranes of apple leaves (Ph 6.5) as affected by pre-soaking the leaves and cuticular membranes in 10 ml liter\(^{-1}\) PEG 100-O surfactant for 24 hours.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Penetrated 2,4-D (cpm)</th>
<th>Penetrated 2,4-D (%)(^{x})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh leaves</td>
<td>4888</td>
<td>53</td>
</tr>
<tr>
<td>Dead leaves</td>
<td>4795</td>
<td>52</td>
</tr>
<tr>
<td>Cuticles</td>
<td>3482</td>
<td>38</td>
</tr>
<tr>
<td><strong>Orthogonal contrast tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves/Cuticles</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Fresh/Dead leaves</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Surfactant treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With PEG 100-O</td>
<td>5029</td>
<td>54</td>
</tr>
<tr>
<td>Without surfactant</td>
<td>3747</td>
<td>40</td>
</tr>
<tr>
<td><strong>T-test</strong></td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td><strong>F-test for the interaction of tissue type and surfactant</strong></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^{x}\) Penetrated 2,4-D % = (penetrated cpm/cpm originally placed in the chamber) x 100.
Table A.2. Weights of filter paper (Whatman 541) and waxes collected from apple leaf surfaces as influenced by PEG 100-O surfactant treatment (10 ml liter\(^{-1}\), 24 hours).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight of filter paper (mg)</th>
<th>Amount of waxes deposited (mg)</th>
<th>Weight increase by surfactant (mg)(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With wax deposition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>20.2</td>
<td>3.2</td>
<td>0.4</td>
</tr>
<tr>
<td>PEG 100-O</td>
<td>20.7</td>
<td>3.4</td>
<td>1.7</td>
</tr>
<tr>
<td>F-test</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Without wax deposition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>20.1</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>PEG 100-O</td>
<td>20.4</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>F-test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^*\)Weight increase by surfactant (mg) = weight of filter paper (with or without deposited waxes) after the surfactant treatment - Weigh of the filter paper (with or without deposited waxes) before the treatment.