THE SORPTION, PENETRATION, AND UPTAKE OF SELECTED BIOLOGICALLY ACTIVE COMPOUNDS THROUGH PLANT CUTICLES AS RELATED TO MOLECULAR STRUCTURE

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ABSTRACT

THE SORPTION, PENETRATION, AND UPTAKE
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A series of studies was begun in 1967 to evaluate the sorption, penetration, and uptake of selected ^{14}C-labeled organic compounds as they relate to molecular structure. Sorption and penetration studies were conducted using enzymatically isolated tomato fruit cuticles, and uptake experiments were conducted with green bean leaf disks.

The sorption, penetration, and uptake of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid increased with time. In general, the descending order of sorption, penetration, and uptake was diphenylacetic acid, N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide.

As the hydrogen ion concentration of the donor solution decreased, the ionized species of diphenylacetic acid increased, resulting in decreased sorption, penetration, and uptake. Undissociated molecules are more lipoidal and more likely to partition into phases such as wax or other lipophilic components of the cuticle. Donor pH

of the three diphenylacetamide molecules did not significantly influence sorption, penetration, nor uptake. Polarity had an influence on the processes studied.

Addition of a nonionic surfactant resulted in an increased penetration and uptake of compounds of different molecular structure. Sorption into isolated tomato fruit cuticles was reduced with the addition of a surfactant. Non-polar (lipophilic) compounds as those used in these studies may be solubilized in the center of micelles which consist of the hydrophilic portions of nonionic surfactants. Thus, apolar compounds may be permitted to take a polar route through the cuticle.

Sorption and penetration of the four different compounds were not influenced by metabolic activity, since respiration of isolated cuticular membranes did not occur. Metabolic activity in the green bean leaf disk may help explain the increased uptake of the four compounds used in these studies.

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Price Hansel Parham

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INTRODUCTION

One of the major points regarding chemicals used for the control of insects, diseases and weed pests of economically important crops is the ability of these pesticides to reach the target site systemically. This is also true for those compounds used to regulate growth response or to correct a nutrient deficiency. Investigations on the absorption of inorganic and organic molecules into living cells have been conducted for many years and are continuing. In both plants and animals a non-living protective or barrier membrane must be traversed before chemicals can penetrate living cells. This membrane is the first vital biological interface in terms of cellular activity and it is the plant's contact with its external environment. As reported by Bukovac and Norris (11,72), Jyung (56), van Overbeek (98), Scott (85), and Crafts and Foy (19), it is the cuticle that is considered the first barrier to penetration into the plant, but there is no question that bioactive chemicals and other substances penetrate it. Skoss (92) reports this is also true for excretion.

The most convenient way to make application of most pesticides is by aqueous spray to aboveground parts. This requires a) that the material become attached to the cuticular surfaces of the plant, b) the material actually transgress the cuticular membrane, and c) that

it be delivered to some reaction site. Without this sequence of events, it is very difficult to get a uniform type response from a plant system.

Since wax may be either superimposed on or interspersed in the plant cuticle, it may form a barrier to penetration of hydrophilic substances.

Most pesticides are studied to observe a biological response by the plant or pest and to determine the metabolic fate of the chemical within the plant. Very little is known about the absorption and translocation of a biologically active compound as related to molecular structure. Because little information is available regarding penetration of exogenously applied organic compounds as they relate to molecular structure, a study was developed to determine: a) the sorption of carbonyl-^{14}C labeled diphenylacetic acid and its dimethyl-, methyl-, and diphenylacetamide analogs into cuticles enzymatically isolated from ripe tomato fruit; b) the penetration of these molecules through isolated tomato fruit cuticles; c) uptake of these ^{14}C-labeled molecules by excised bean leaf disks; and d) the effect of donor pH and surface active agent concentrations upon sorption, penetration, and uptake of these different molecules.

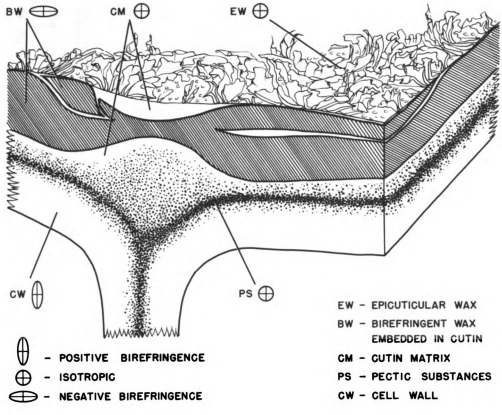
REVIEW OF LITERATURE

Nature of the Cuticular Surface.

In both plant and animal a non-living protective or barrier membrane must be traversed before exogenously applied compounds can penetrate the living cells. External membranes of leaves which are considered the first barriers to penetration are called cuticles, (van Overbeek, 98). The generalized structure of the plant cuticle has been reported by Eglinton and Hamilton (29), Franke (37), and Norris and Bukovac (72). The cuticle is built up of several alternating layers of cellulose, pectin, cutin, and wax (Figure 1). The cutin itself is believed to be composed of polymerized long-chain fatty acids and alcohols. This is bound by epicuticular waxes toward the outside and pectin substances toward the cellulose cell wall.

According to Eglinton and Hamilton (29), surface waxes are complex mixtures of long-chain alkanes, alcohols, ketones, aldehydes, acetals, esters, and acids. The picture is complicated by the positioning and number of functional groups, the degree of chain branching and unsaturation, and the increasing number of other types of constituent. However, the wax composition of a species may differ for different parts of the same plant and may vary with season, age of the plant, locale, humidity, temperature and other internal and external factors.

Figure 1. Diagrammatic representation of the structure of an upper leaf cuticle, not necessarily drawn to scale. (From Norris and Bukovac, 1968).


BW
CM
EW
CW
PS
EW - EPICUTICULAR WAX
BW - BIREFRINGENT WAX EMBEDDED IN CUTIN
CM - CUTIN MATRIX
PS - PECTIC SUBSTANCES
CW - CELL WALL
- POSITIVE BIREFRINGENCE
- ISOTROPIC
- NEGATIVE BIREFRINGENCE

Minute wax protuberances, termed wax rodlets, are observed on the cuticle and according to Mitchell, et al. (66) these often occur in various forms as granular, platelike, crystalline-granular, and rod-shaped. These protuberances can act as a barrier to penetration of exogenously applied aqueous solutions without a surface active agent. Rich and Horsfall (80) observed similar results with toxicants used for the control of fungi. The region directly beneath the epicuticular wax contains wax platelets embedded in the cutin which form the framework and general body of the cuticle.

Pathways of Cuticular Penetration.

Several excellent reviews have been written within the past few years concerning the penetration of exogenously applied compounds into plant cells. Reviews by van Overbeek (98), Crafts and Foy (19), Mitchell (66), Franke (37), Currier and Dybing (23), Wittwer (104), and Sargent (83) have contributed greatly to the understanding of cuticular penetration.

Stomata and internal cuticle.

For several years it was thought that hydrophilic molecules entered the leaves mainly through stomatal pores. This hypothesis was substantiated by many investigators such as Boynton (7), Foy (35), Sargent and Blackman (83), Wittwer and Teubner (105), Wittwer et al. (104), Jyung et al. (56), and Teubner et al. (97) who found that upper surfaces without stomates had less solute uptake than lower surfaces. Most workers agree with Norman et al. (71) and van Overbeek (98) that aqueous solutions do not penetrate through stomatal pores unless the surface tension

is very low. The size of the stomatal pore (3-5μ when fully open) is such that the surface tension of the leaf will prevent the water droplet from gaining entrance into it. Stomatal entry of aqueous solutions must depend, therefore, upon an adequate surface active agent to reduce surface tension. Investigations conducted by Wittwer et al. (104,105), Currier and Dybing (23), Currier et al. (24), and Norris and Bukovac (72) concluded that once the molecule enters the sub-stomatal cavity it must still transgress a lipid membrane called the internal cuticle. The molecule can be considered in the leaf but not in the cell. The cuticular membrane of the stomatal cavity is thinner, more hydrated, and easier to penetrate than the epicuticular surface of the leaf.

Stomatal frequency per unit of leaf area was highly correlated with the rate of rubidium absorption in bean and tomato leaves, (Jyung and Wittwer, 55). The entrance into a stomatal cavity does not preclude the necessity for cuticular penetration. Several investigators (Bukovac and Norris, 11,72; Jyung et al., 56; Wittwer et al., 104; and Yamada et al., 108) have demonstrated that substances applied in aqueous solutions can penetrate intact and isolated astomatous cuticles.

Canals and intercuticular penetration.

Scott et al. (86,87) were of the opinion that canals may exist in the cuticle, but efforts to show that these canals actually exist have not been successful. However, Hall and Donaldson (43) claimed that cuticular pores, approximately 6-7 mμ in diameter, were revealed in epidermal cells of clover (*Trifolium repens*) and cauliflower (*Brassica*

oleraceae) from which wax is extruded. Using microscopy, Bukovac and Norris (11) and Norris and Bukovac (72) showed the pear cuticle to be a uniformly continuous and poreless membrane. If canals were present, they might facilitate the movement of non-polar substances through the cuticle to the hypodermal cells. Scott et al. (86) and Kamimura (57) reported that breaks, fissures, or punctures made by insects or mechanical means are sometimes found in the cuticular membrane. The passage of solutes through these imperfections has been termed intercuticular penetration by Wittwer and Teubner (105).

Leaf pubescence.

Ennis et al. (32) and Mitchell et al. (66) reported that pubescence on the leaves increased cuticular penetration of exogenous aqueous solutions. Since most plant hairs are covered with a cuticle, this possible mode of entry does not eliminate the cuticle as the first barrier to penetration. Harley et al. (47) did not find pubescence to be a factor in NAA penetration of apple leaves. While working with ^{32}P-Systox, Tietz (96) found that hairy leaves of primrose (*Primula obconica*) absorbed three times as much as did the smooth leaves of cyclamen (*Cyclamen persicum*).

Cuticular Structure.

The behavior of the cuticular framework composed of cutin which exhibits moderate hydrophilic properties is significant. Weintraub (102) and van Overbeek (98) reported that after absorbing water the cuticle swells and spreads apart the embedded wax platelets (which exhibit hydrophilic properties). This increases permeability of the

cuticle to water and to certain organic substances that tend to move with water. Conversely, low moisture content would move the wax platelets closer together and therefore reduce water and solute movement through the cuticle. If wax platelets alternate with cutin lamellas, they should prevent the penetration of aqueous solutions because of the apolar lipophilic properties of wax. Investigations conducted by Orgell (73) indicated that the plant cuticle was characterized by an imbricate arrangement of lipoid platelets cemented together by hydrophilic pectinaceous materials. Roberts et al. (81) concluded that "intercuticular penetration" of aqueous solutions should be possible because the pectin layers beneath the epicuticle extend to the middle lamellas of the tissues and should provide a hydrophilic pathway to the vascular bundles close to the epidermal tissue of the leaves. Direct evidence of this has not been clearly demonstrated.

Environmental Factors Affecting Cuticular Penetration.

Cuticular penetration is influenced by several factors other than those discussed above. Such factors as temperature, moisture, light, surface active agents, and chemical formulation may have an influence on cuticular penetration of aqueous solutions.

Temperature.

It is believed that warm temperatures, (10° to 40°C) will promote penetration of solutes through the cuticle. Currier and Dybing (23) and Sargent (84) concluded that temperatures of 10° to 37°C indirectly influenced the penetration rate of aqueous solutions by influencing cytoplasmic viscosity, accumulation, binding, metabolic conversion, and

translocation of the penetrant, i.e., by regulating processes which influence the concentration gradient across the surface layers. Warm temperatures directly influenced the rate of diffusion of lipophilic substances through lipoid-containing membranes. Barrier and Loomis (2), Rice (79), and Sargent and Blackman (84) demonstrated that increased temperatures increased the penetration of 2,4-dichlorophenoxyacetic acid (2,4-D) into plant parts. Luckwill and Jones (63) and Harley (47) showed a direct relationship of increased napthaleneacetic acid absorption to increased temperature. Luckwill and Jones (63) showed that over the range of 5° to 25°C there was a linear relationship using Bramley apple leaves (18% to 45% absorption). Jyung et al. (55) reported that rubidium absorption by green tobacco leaf cells was temperature dependent. It has also been demonstrated that temperature influences the rate of absorption of systemic insecticides. While working with *Vicia faba* (broad bean), Bennett and Thomas (4) found that over a 7-hour period ^{32}P-schradan was absorbed in greater quantities at 26° than at 15°C (64.4% vs. 100%). After 72 hours the absorption was essentially the same.

Moisture.

It has been reported by Palmquist (77) that when a scraped leaf of a water-stressed plant was immersed in a fluorescein solution, the dye moved rapidly through the leaf. Went and Carter (103) reported that sucrose uptake by tomato leaves was independent of the humidity level. While Pallas (76), working with 2,4-dichlorophenoxyacetic acid (2,4-D), found that high humidity increased foliar absorption. Similarly, Volk

and McAuliffe (101) reported increased penetration of urea under high humidity conditions. Clor et al. (12,13) demonstrated that the rate and extent of translocation of 2,4-D and amitrol in cotton leaves was enhanced when the plants were placed in polyethylene bags to produce a high humidity atmosphere. Crafts and Foy (19) suggested that under low atmospheric humidity the leaf was under tension and air blocks prevented union of the spray with the water continuum. Under this condition, the aqueous route of penetration was unavailable but penetration may still have occurred by the lipoidal route. Prasad et al. (78) demonstrated that more dalapon was absorbed and translocated under conditions of high (88 ± 5%) than at low (28 ± 3%) post-treatment relative humidity. Luckwill and Jones (63) found that NAA uptake in apple leaves was 50% and 90% respectively at 37% and 100% relative humidity.

Light.

The effect light has upon penetration of foliar applied solutes is not clear. According to Sargent (82) light may promote absorption by causing an increase in the export of carbohydrates with which growth regulators appeared to be associated during translocation from the leaf. Several investigators have reported an increase in absorption with increased light intensity. Jyung and Wittwer (53) reported a rapid increase in absorption of phosphate and rubidium as the light intensity was increased up to 320 ft-c. While working with green and albino leaves, Juniper (52) reported an increase in the thickness of the cuticle with a rise in light intensity. Sargent and Blackman (83,84) reported a greater increase in the rate of 2,4-dichlorophenoxyacetic

acid penetration in *Phaseolus vulgaris* and *Ligustrum ovalifolium* in the light than in the dark. However, Bennett and Thomas (4) found that light was apparently not very important in the absorption of ^{32}P-schradan over a 72 hour period. Total absorption at 27°C was 79.8% in the dark, and 88.7% under fluorescent light. With *Coleus*, broad bean, and runner beans, darkness post-application also reduced the rate of absorption.

Surfactants.

Surface active agents (surfactants, wetters, detergents, etc.) are materials which are used at very low concentrations to lower the surface tension of water. They are included extensively in the formulation of pesticides to increase wettability which in turn may influence penetration or distribution of aqueous solutions. An effective surface active agent is composed of molecules containing an alkane-type group which is oil soluble and one or more polar groups which are water soluble. These agents are composed of molecules whose structures provide a hydrophilic part called the "head", and a hydrophobic part called the "tail".

In 1890 Gillette (40) found that part of the leaf surface of plum was destroyed when sprayed with a four-ounce-per-gallon concentration of whale oil. An inherent phytotoxicity of several species of plants to sulfonated alcohols in insecticides was reported by Cory and Langford (14). Blackman et al. (2) reported that lowering the surface tension reduced the volume of spray retained by species not readily wetted. These data agreed with those reported by Moore (36) who concluded that some surface active agents increased spreading on waxy or non-waxy surfaces, but not necessarily both. Numerous investigators such as Buchanan (10),

Crafts (18), Currier (22), Ennis (31), Daines et al. (25), Ilnicki et al. (50), Laning and Aldrich (60), Leonard and Crafts (61), Luepschen and Rohrbach (62), McWhorter (64), Mitchell et al. (66), and Prasad et al. (78) have reported the enhancement of pesticide action by the addition of surface active agents.

Other investigators, (Behrens, 1; Jansen et al., 51; Hauser, 48; Sargent and Blackman, 83; Staniforth and Loomis, 94; Skoss, 92; Weintraub et al., 102) reported that 2,4-dichlorophenoxyacetic acid penetration was increased by the inclusion of a surface active agent. While using a fluorescent dye in studying penetration in *Zebrina pendula*, Dybing and Currier (28) found that surface active agents enhanced foliar penetration. In all cases where the surfactant was present, penetration of the lower surface was more rapid than for the upper surface. It was concluded that solutions containing surface active agents penetrated mainly through open stomata. Enhanced stomatal penetration by surfactants has already been discussed on Page 6.

Brown (8) reported that surface active agents increased the action of defoliants. Increased fungicide penetration with surface active agents has been reported by Daines et al. (25) and Luepschen and Rohrbach (62). Several reports where surface active agents enhanced the penetration of insecticides were reviewed by Mitchell, Smale, and Metcalf (66). Bukovac and Norris (11) reported that the water uptake pattern in immersed isolated tomato fruit cuticle was not altered by the addition of polyoxyethylene-20-sorbitan monolaurate (Tween 20). Sargent and Blackman (83) reported that Tween 20 increased the rate of

penetration of 2,4-D in the dark into the abaxial and the adaxial surfaces of *Phaseolus vulgaris*. Other investigators as well as Teubner et al. (97) found that Tween 20 was ineffective in enhancing the penetration of labeled phosphorus (^{32}P) into bean leaves. However, Neely and Phinney (70) reported that Tween 20 did effectively promote penetration of foliar applied gibberellic acid in maize. Freed and Montgomery (38) concluded that although reduction of surface tension was important, the relationship of molecular interaction between surfactant and the herbicide was of equal or greater importance. They suggested a highly specific requirement for surfactant formulation to fit the herbicide in order to achieve maximum effectiveness. Buchanan (10) reported that it was not possible to classify surfactants as toxic or non-toxic on the basis of ionogenic grouping as there were various levels of toxicity within the ionogenic groups.

Nature of Chemical and Cuticular Penetration.

According to Crafts and Foy (19), there are at least two routes for penetration of exogenously applied materials into plant leaves. These are a polar or aqueous route, and a non-polar or lipoidal route. There is a gradient of polarity from the interior of the cuticular layers and of the cellulose wall which exhibits high polarity. Crafts (15) reported that apolar compounds usually penetrated the cuticular membrane much more rapidly than polar compounds. According to van Overbeek (99) and Veldstra (100) penetration of growth regulators may be improved when the molecule exhibits the proper polar-apolar balance. Middleton (65) reported that many non-polar organic solutes

were absorbed by plant foliage more rapidly than the highly polar inorganic salts. In studies conducted by Yamada et al. (111), it was consistently demonstrated that the dialyzing membranes were more permeable than cuticular membranes to both organic compounds and inorganic ions. These investigators found that permeability of urea through tomato fruit cuticles was twice that of N,N-dimethylaminosuccinamic acid and six times that for maleic hydrazide. Urea also penetrated the tomato fruit cuticle 10 to 20 times more than inorganic ions as Rb^+, Ca^{++}, Cl^-, and SO_4^{--}. Urea was believed to have penetrated by a process of facilitated diffusion. Franke (37) concluded that lipophilic substances may penetrate by a process of solution and the rate determined by the solubility, partition coefficient, and molecular size.

Effects of pH on penetration.

There have been many conflicting results regarding the part played by the pH of donor solutions on the penetration of weak acids into plant parts. In mid-1947, pH was shown by Hamner et al. (45) to influence the regulating compounds of weak acids. Simon et al. (90) reported that the penetration of 3,5-dinitro-O-cresol into leaves was not influenced by the pH of the external solution. Albert (1), Crafts (16,20), and Swanson and Whitney (95) reported that many weak acids penetrated plant cells more readily in the undissociated form, i.e., at pH values below their pK. Crafts (18), Orgell and Weintraub (75), and Sargent and Blackman (83,84) reported that 2,4-dichlorophenoxyacetic acid (2,4-D) penetration was greater in acid solutions than

in neutral solutions. Over the lower range of pH, it was shown that penetration of 2,4-D decreased as the hydrogen-ion concentration of the donor solution decreased. It has been shown by Kuiper (58) that the effect of decenylsuccinic acid and a few of its mono-amides on growth retardation of beans depended on pH level.

Jyung and Wittwer (53) reported a depression in absorption of phosphate with tris-phthalate and the sodium-acetate systems into bean leaves buffered at pH 4.7. They reported that "specific absorption" was greatest at a pH of 3.7.

Shindo and co-workers (88) found that the penetration rate of S-benzoyl-thiamine into red blood cells diminished markedly with decreasing pH of the medium.

While working with enzymatically isolated pear cuticle, Bukovac and Norris (11) found that greater retention of napthaleneacetic acid (NAA) occurred at pH levels below than above its pK. There was no significant change in retention of napthaleneacetamide (NAAm) over a wide range of pH levels. Their data indicated that NAA retention by the pear leaf cuticle was pH dependent whereas NAAm was not.
Van Overbeek (98) suggested that the pH effect from penetrating chemicals was on plant protein rather than on the donor substance applied. Experiments by Volk and McAuliffe (101) suggested that sodium hydroxide increased the permeability of the cuticle. Orgell (74) reported that the cuticle does not have a charge at a pH lower than 5.0, while at a pH above 7.0 the cuticle is negatively charged. This contrasted to the work by Yamada (106) who reported that cuticles from green onion

leaves have a pK value of 2.8, and from ripe tomato fruits, a pK value of 3.2. Bukovac (personal communication) found that the pK value of pear cuticle was 2.8-3.0. Therefore, it seems that pK regulated electrostatic repulsion and attraction phenomena affect cuticular sorption. and, consequently, the rate of foliar uptake of ions. However, Bukovac and Norris (11) found that NAA binding took place in cuticle of pear leaves at pH values greater than its pK of 4.2. They implied that mechanisms other than electrostatic binding were involved in chemical binding to cuticular surfaces.

Size of molecule and penetration.

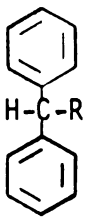
Franke (37) reported the cuticle was negatively charged and these charges were first neutralized by cations. Goodman (41) reported that application of cations as Ca++ and Mn++ brought about dehydration and this increased the entry of larger cationic molecules. Cations such as Ca++ and Mn++ penetrated rapidly because of their small ionic radius. Franke (37) reported that the rapid penetration of small ions facilitated the passage of larger cationic molecules. Craig (21) suggested that molecular size and structure of solutes could be correlated with diffusion rates through calibrated dialyzing membranes. Harley et al. (46) reported that certain 2,4-D formulations gave increased effectiveness in the following order: sodium salt, ammonium salt, amine, and then the ester formulation. Dorschner and Buchholtz (27), Holly (49), Muir and Hansch (68), and Veldstra (100) studied the structure-activity relationship of N-substituted alpha-chloroacetamides.

The influence of slight structural changes on activity was often striking. Penetration may have been a controlling factor in these changes, but there is little direct evidence that this was true.

MATERIALS AND METHODS

Because little information is available regarding sorption, penetration, and uptake of exogenously applied compounds as they relate to molecular structure, this study was conducted to evaluate this effect using isolated tomato fruit cuticles and excised bean leaf disks.

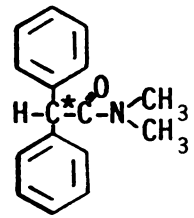
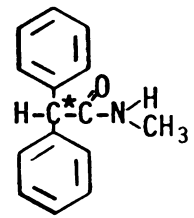
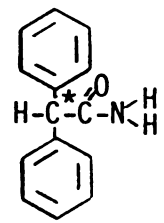
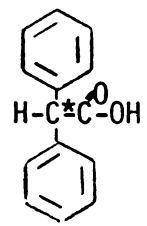
Four radiolabeled (carbonyl-^{14}C) compounds having the general chemical structure



were donated by The Upjohn Company, Kalamazoo, Michigan. These compounds, along with the relevant chemical properties, are shown in Table 1. The compound where R = $-\overset{O}{\overset{\|}{C}}-N\begin{matrix}CH_3\\CH_3\end{matrix}$ is a well-known herbicide named diphenamid (N,N-dimethyl-2,2-diphenylacetamide). The other 3 are derivatives of diphenamid and not commercially available as herbicide products.

Tomato (*Lycopersicon esculentum L.* cv. Michigan-Ohio Hybrid) plants were fruited in the greenhouse at Michigan State University. The astomatous cuticular membranes were enzymatically separated from ripe tomato fruits using the method described by Norris and Bukovac (72).

Table 1. Radiolabeled compounds used in this investigation and the relevant properties of each.

Molecular Structure	Molecular Weight	Chemical Name	Solubility in Water @ 24°C
	239.31	N,N-dimethyl-2,2-diphenyl-acetamide	0.250 mg/ml
	225.28	N-methyl-2,2-diphenylacetamide	0.092 mg/ml
	211.25	Diphenylacetamide	0.110 mg/ml
	212.24	Diphenylacetic acid	0.133 mg/ml

A liquid scintillation spectrometer, Packard Tri-Carb Model 314EX[1] was used to detect radioactivity. Thin layer chromatography procedures were used to identify N,N-dimethyl-2,2-diphenylacetamide which penetrated isolated tomato fruit cuticle. Developing solvent was benzene-chloroform-acetic acid mixture 85:10:5 (v/v); the absorbant was silica gel GF.

Definitions.

In this study sorption refers to the compound that is bound to the cuticular components, either by absorption or adsorption, and is not removed by washing with distilled water. Penetration refers to the process of a compound traversing the cuticle. Uptake refers to the compound that penetrates the cuticle and enters the metabolically active cells and may also involve some movement from cell to cell.

Relation of Molecular Structure to Sorption.

Before cuticular penetration of a compound can take place, it must first pass through the various wax and cutin components of the cuticle. It is reasonable to assume that some of the compound applied to the cuticular surface would be retained by the cuticle. A series of experiments was therefore designed to determine the influence of molecular structure on cuticular sorption.

Cuticular disks (10 mm in diameter) were cut from isolated ripe tomato fruit and placed in glass vials. One milliliter donor solution was added to each vial. All disks were completely submerged in the

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[1]Packard Instrument Company, Inc., LaGrange, Illinois.

donor solution. Each treatment was replicated six times and held at 25°C. After a predetermined length of time, each cuticle was removed from the donor solution, blotted and rinsed three different times in distilled water. After the third rinse, each cuticular disk was dried at ambient temperature and placed in a 20 ml Wheaton glass scintillation vial. Fifteen milliliters solvent mixture (Table 2) was added. These vials were then placed in a liquid scintillation spectrometer for a counting time of 30-90 minutes. An internal standard consisting of 50 μl ^{14}C-Toluene was added to the sample and recounted to determine counting efficiency and disintegrations per minute (DPM). Counting efficiency obtained this way was approximately 68 percent.

Table 2. Scintillation solvent mixture used in the isolated tomato fruit cuticle penetration experiments.

Solvent	Amount	Source
Napthalene (mp 80-81°C)	73 gm	Matheson, Coleman & Bell Cincinnati, Ohio
Liquifluor (25x)	50 ml	Pilot Chemicals, Inc. Watertown, Mass.
1,4-Dioxane	350 ml	Fisher Scientific Company Fair Lawn, New Jersey
Toluene	350 ml	Allied Chemical, Industrial Division Morristown, New Jersey
Methyl Alcohol (anhydrous)	210 ml	Mallinckrodt Chemical Works New York, N. Y.

Influence of time on sorption.

A time course study was conducted to determine the amount of compound retained by the cuticle as influenced by time. Concentration of the donor solution for each of the four compounds was 1.05×10^{-5} M/l. Each donor solution was buffered to pH 3.0, 4.0, or 5.0 (Table 3). After the predetermined length of time had elapsed (3-24 hours), each cuticle was removed from the donor solution and the DPM was determined as above for each sample.

Table 3. Citric acid-phosphate buffer solution used to maintain stated pH level.

pH Level	Citric Acid (0.1 M)	NA_2HPO_4 (0.2 M)
3.0	39.8 ml	10.2 ml
4.0	30.7 ml	19.3 ml
5.0	24.3 ml	25.7 ml
6.0	17.9 ml	32.1 ml
7.0	6.5 ml	43.6 ml

Influence of pH on sorption.

A series of experiments was conducted to determine the influence of donor solution pH on sorption into isolated tomato fruit cuticles. Disks from tomato fruit cuticle were placed in vials containing 1 ml donor solution at 1.05×10^{-5} M/l concentration. The cuticular disks remained submerged in the donor solution for 24 hours. Retention of each compound by the cuticle was first determined without a surfactant

Table 4. Trade names and a summary of the relevant physical and chemical properties of the surface active agents used in this study.

	Surface Active Agent		
	Tergitol 15-S-9	Tween 20	Surfactant DF16
Source	Union Carbide Corp. New York, N.Y.	Atlas Chemical Industries	Rohm & Haas Co. Philadelphia, Pa.
Ionogenic Class	nonionic	nonionic	nonionic
Appearance	clear liquid	yellow liquid	clear liquid
Viscosity (cps. @ 25°C)	68.8 c.p.s.	363 c.p.s.	35 c.p.s.
Specific Gravity @ 25°C	1.000	1.10	0.987
Density	999.29 g/l	1098.74 g/l	987.31 g/l
Chemical Classification	Ethoxylated Alcohol	Ethoxylated fatty acid ester	Ethoxylated & Propylated Alcohol (linear alcohol)

and then with Tergitol 15-S-9 added at 0.3% (v/v). After 24 hours, each cuticular disk was removed from the donor and radioactivity determined.

Influence of surfactant concentration on sorption.

Tomato fruit cuticle disks (10 mm diameter) were placed in donor solutions containing 0.1, 0.2, 0.3, or 0.4 percent Tergitol 15-S-9 surfactant (Table 4). Sorption into these was compared with disks from standard donors which did not contain a surfactant. Donor solution concentration was 1.05×10^{-5} M/l and was buffered to pH 4.0. After 24 hours, each disk was removed from the donor solution, blotted and rinsed three times, and allowed to dry at ambient temperature before radioactivity was determined.

Relation of Molecular Structure to Penetration.

A series of experiments was conducted to evaluate the relationship of molecular structure to penetration. Disks, 15 mm in diameter, were obtained from astomatous ripe tomato fruit cuticle which had been removed enzymatically.

The apparatus used (Figures 2 and 3) was constructed using two L-shaped components from glass tubing of 5 mm inside diameter. The overall length of each component was approximately 12 mm. A flange was made on one of the tubes to which a 1 oz. polyethylene bottle cap was affixed by inserting the tube through a hole punched in the cap. This tube served as the donor tube and will hereafter be referred to as the donor tube. The neck (male part) of a 1 oz. polyethylene bottle was fused onto the second L-shaped component by heating the glass and then

Figure 2. Apparatus used to study the relation of molecular structure to cuticular penetration.

A = Receiver side
B = Donor side
C = Tomato fruit cuticle
D = Teflon washers

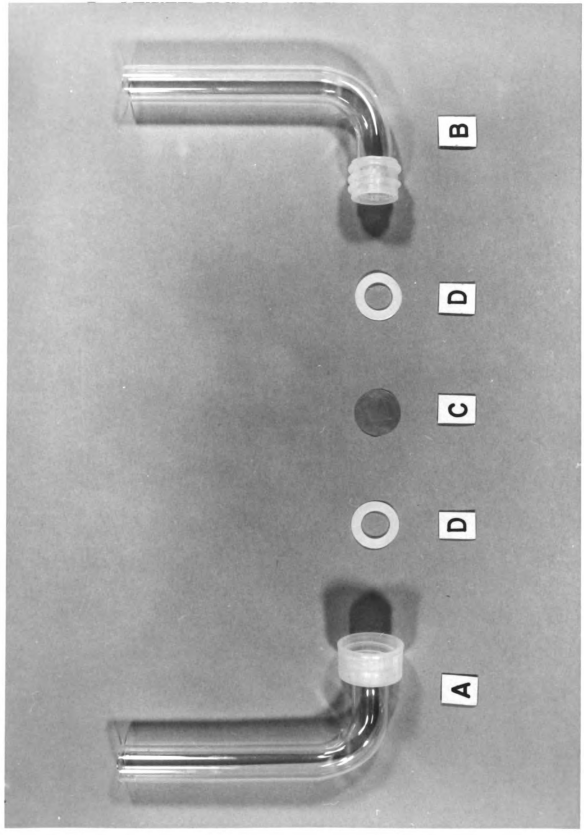
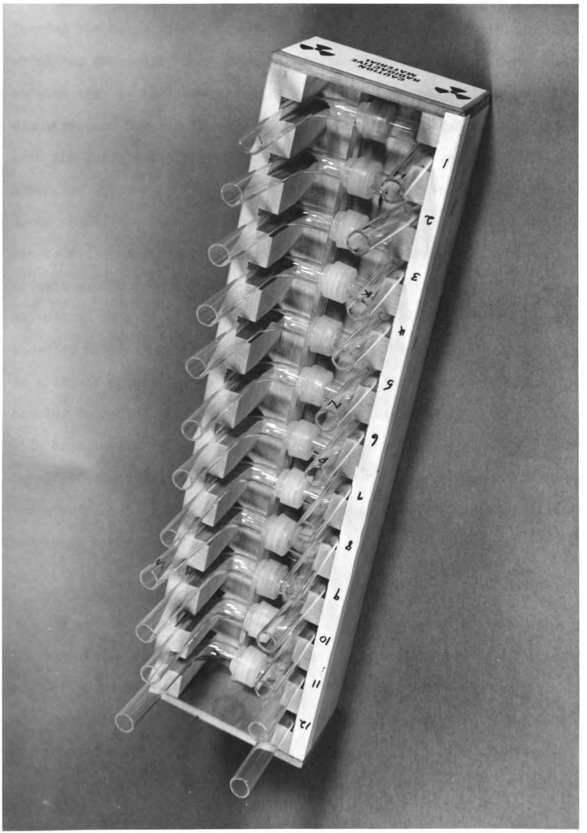

A
D
C
D
B

Figure 3. Set of assembled apparatus for the cuticular penetration studies.


1
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12

forcing the polyethylene neck onto it. Two teflon washers (Figure 2), which had previously been dipped in melted petroleum jelly, were placed on each side of the tomato disk. The teflon washers had 14 mm outside diameters and 9 mm inside diameters. The 2 L-shaped tubes were joined with the cuticle fastened between the donor and receiver parts of the apparatus. Outer surface of the cuticle was always in contact with the donor solution. Each treatment was replicated six times and held in an air-conditioned laboratory at 25 $\pm$ 1°C. After a predetermined time, an aliquot was removed from the receiver side of the tube. Each aliquot was placed in a 20 ml Wheaton glass scintillation vial. To this was added 15 ml of a scintillation solvent mixture (Table 2). Counting time in a liquid scintillation spectrometer was 30-90 minutes for each sample, depending upon the level of radioactivity found; those samples with high levels were counted for the shorter period of time. An internal standard of ^{14}C-Toluene (5.10×10^{-5} dpm/g $\pm$ 3%) was added to the counted sample and recounted to determine the efficiency of counting. For these experiments an efficiency of 60 $\pm$ 1 percent was obtained.

Influence of time on penetration.

A series of experiments was conducted to compare the rate of penetration of diphenylacetic acid and its 3 diphenylacetamide derivatives as influenced by time. The concentration of the donor solution of the compounds was 1.05×10^{-5} M/l. Each solution was buffered to pH 4.0, the pK of diphenylacetic acid being 3.94. A surfactant (0.3% Tergitol 15-S-9) was added to each donor solution. Four milliliters (ml) of the

concentration was used as the donor solution and 4 ml distilled water served as the receiver solution. After the predetermined time (3-24 hours), 0.5 ml aliquots were removed from the receiver and donor and the radioactivity determined.

Influence of donor pH on penetration.

Several experiments were conducted to determine the influence of donor pH on the penetration of different molecular structures through tomato fruit cuticles. Donor solutions were used at a concentration of 1.05×10^{-5} M/l and buffered to pH 3.0, 4.0, or 5.0 with a citric acid-phosphate buffer as listed in Table 3. The influence of donor pH on cuticular penetration was compared first without a surfactant, and then with Tergitol 15-S-9 surfactant at 0.3% (v/v). After 24 hours, a 0.5 ml aliquot sample was taken from the receiver solution and the amount of radioactivity determined as above.

Influence of surfactants on penetration.

The influence of surfactants on the penetration of the 4 compounds through isolated tomato fruit cuticle was determined using several different nonionic surfactants (Table 4). Technical data were obtained either from brochures provided by the respective companies, or from personal communication with formulation chemists. In all experiments, the concentration of surfactant added to the donor solution ranged from 0.05% to 0.4% (percentages were based on a volume to volume ratio). All donor solutions of the compounds were used at a concentration of 1.05×10^{-5} M/l in distilled water or buffered to

pH 4.0. After a predetermined period of time, usually 24 hours, a 0.5 ml aliquot sample was taken from the receiver solution and radioactivity determined.

Influence of surface wax on penetration.

Surface waxes were removed from tomato fruit cuticles to determine whether these influence penetration of the compounds under investigation. Cuticular disks of 15 mm diameter were allowed to remain submerged in chloroform for 24 hours. They were then rinsed in distilled water, blotted with a Kimwipe and allowed to dry at ambient temperature in the laboratory. The apparatus and experimental procedures used were the same as described earlier (Page 25). Penetration was permitted to proceed for 24 hours and the level of radioactivity was determined and compared to the donor solution.

Relation of Molecular Structure to Uptake.

In order to compare a metabolically active system with isolated tomato fruit cuticles, bean plants (*Phaseolus lunatus* cultivar Henderson Bush) were grown in vermiculite in the greenhouse. Disks 15 mm in diameter were cut from smooth, fully expanded primary leaves immediately upon removal of a leaf from the plant. Care was exercised to avoid removing disks containing part of the midrib or large veins. Immediately after cutting, 6 leaf disks were placed on Whatman No. 4 filter paper in a 9 centimeter petri dish. One milliliter of distilled water was used to moisten the filter paper and maintain cell turgidity. At no time were the leaf disks permitted to become flaccid. Glass

cylinders 13 mm high and of 9 mm inside diameter were affixed to the bean leaf disks by placing a small bead of petroleum jelly on the cylinder end in contact with the disk. A donor solution (0.1 ml) was added to each cylinder. All treatments were replicated six times and held at approximately 25°C. After a predetermined length of time (1 to 24 hours), the cylinders were removed and the excess donor solution washed off with distilled water. Each leaf disk was cleansed with a cotton ball saturated with xylene (mixed isomers) to remove epicuticular waxes and any ^{14}C-labeled donor material binding to the surface waxes. Remaining chemical would be that in the cuticle and which had been taken up by the living cells of the bean leaf disk. After cleansing with xylene, the disks were allowed to dry at ambient temperature for 10-15 minutes. The entire leaf disk was then placed in a glass Wheaton scintillation vial containing 15 ml solvent mixture (Table 5). Samples were then placed in a Packard Tri-Carb liquid scintillation spectrometer and counted for a period of 30-90 minutes each. Fifty microliters of ^{14}C-Toluene internal standard (5.10×10^{-5} dpm/g $\pm$ 3%) was added and the sample recounted to obtain counting efficiency and disintegrations per minute. The figure obtained for counting efficiency was approximately 60%.

Influence of time on uptake.

A time course study was conducted to determine the relationship between time and uptake of the four compounds. The concentration of the donor solution for each compound was 1.05×10^{-5} M/l, buffered to pH 4.0. After the predetermined times of 1, 3, 6, 12, or 24 hours

had elapsed, the amount of radioactivity was determined for each bean leaf disk.

Table 5. Scintillation solvent mixture used for counting samples from the bean leaf uptake experiments.

Solvent	Amount	PPO[1]	POPOP[2]	Source
Liquifluor 25x	63 ml	7.3 gm	93.75 mg	Pilot Chemicals, Inc. Watertown, Mass.
Toluene	937 ml	-	-	Allied Chemical, Industrial Division Morristown, New Jersey

[1]2,5-Diphenyloxazole

[2]1,4-Bis-2-[5-phenyloxazolyl]benzene

Influence of pH on uptake.

A series of experiments was conducted to determine the influence of donor pH on uptake of compounds by green bean leaf disks. The donor concentration for each compound was 1.05×10^{-5} M/l, buffered to pH 3.0, pH 4.0, or pH 5.0. Tergitol 15-S-9 nonionic surfactant (0.3% v/v) was added to each donor solution. The influence of donor pH was compared 6 and 24 hours later by determining the radioactivity in the leaf disks.

Influence of surfactant on uptake.

Disks of 15 mm diameter were cut from fully expanded green primary leaves of bean plants. These were then placed on moist filter paper in a 9 cm petri dish. Donor solutions of 1.05×10^{-5} M/l

concentration were applied to six green bean leaf disks for each treatment. A nonionic surfactant (Tergitol 15-S-9) was added to each compound at a concentration of 0.1, 0.2, 0.3, or 0.4 percent (v/v). After a predetermined period of time (usually 6 or 12 hours), each leaf disk was placed in a scintillation solvent mixture and the disintegrations per minute determined as described above.

Relation of Molecular Structure to Partitioning.

The plant cuticle is primarily a layer of cutin composed of cross-linked hydroxy fatty acid bound by a layer of wax. According to Eglinton and Hamilton (29) the surface waxes are complex mixtures of long-chain alkanes, alcohols, ketones, aldehydes, acetals, esters, and acids. The solubility of externally applied compounds in these cuticular substances is involved in sorption. This experiment was conducted to evaluate the degree of partitioning of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid from an aqueous solution into two organic solvents. Chloroform and oleic acid were used as the organic solvents. Donor aqueous solutions were buffered to pH 4.0 or pH 7.0 with citric acid-phosphate buffer solution (Table 3).

Equal portions (1.5 ml) of the donor and solvent were placed in a 2 ml Kimax volumetric flask and then sealed with a ground glass stopper. Each treatment was replicated 5-6 times. At the beginning of the experiment, each flask was agitated for 5 seconds using a S8223 Vortex Genie Mixer set at speed No. 9. Another agitation, identical to the first, was given 22 hours later. A 0.5 ml aliquot was taken from both

the water and organic phases 24 hours after the beginning of the experiment. The upper phase was always sampled first. An aliquot from the lower phase was obtained by carefully inserting a 0.5 ml Kimax volumetric pipette through the upper phase while forcing air through the pipette to minimize contamination. Each pipette was wiped with a Kimwipe prior to placing the aliquot in a 20 ml Wheaton glass scintillation vial. Fifteen milliliters scintillation solvent (Table 2) was added to each vial. Samples were then placed into a Packard Tri-Carb liquid scintillation spectrometer and counted for 30-90 minutes. Background counts were obtained using a scintillation solvent sample containing 0.5 ml aliquot chloroform, oleic acid and water, depending upon the phase being counted. An internal sample of 50 μl ^{14}C-Toluene (5.10×10^{-5} dpm/g $\pm$ 3%) was added to each sample before recounting to determine efficiency.

RESULTS

Relation of Molecular Structure to Sorption.

Influence of time on sorption.

The quantity of diphenylacetic acid sorbed into enzymatically isolated tomato fruit cuticles is shown in Figure 4-IV. There was a rapid increase in sorption for the first 6 hours, followed by a leveling-off or decline with increasing time. The latter characteristic was more evident at pH values of 4.0 or pH 5.0 than when diphenylacetic acid was buffered to pH 3.0.

A summary of the sorption of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide into tomato fruit cuticles is shown in Figures 4-I, 4-II, and 4-III. In general, sorption of the three amide molecules increased with time. The sorption was rapid during the first 3 to 6 hours and increased 3-fold by the end of the 24-hour treatment. The descending order of sorption of the amide molecules was N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide respectively.

Influence of pH on sorption.

The influence of donor pH on the sorption of the 4 different compounds into tomato fruit cuticles is shown in Figure 5. The amount of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide,

Figure 4. Comparative rates of cuticular sorption of different compounds through isolated tomato fruit cuticles as influenced by time at different pH levels.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid

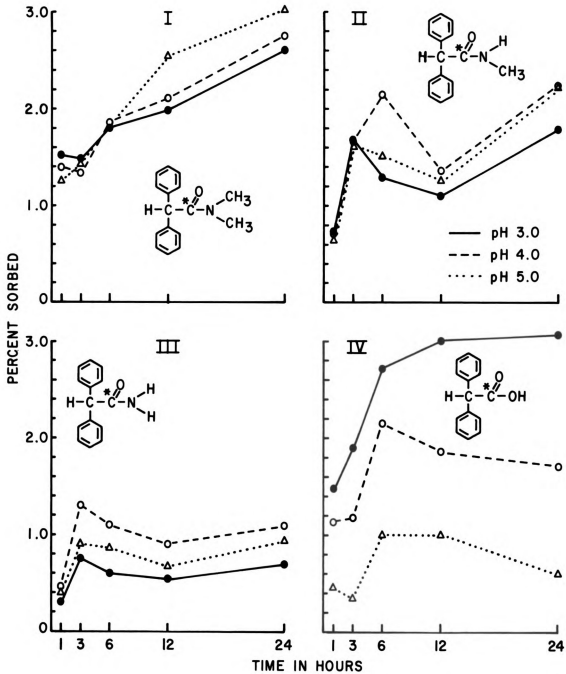

I
II
III
IV
PERCENT SORBED
TIME IN HOURS
pH 3.0
pH 4.0
pH 5.0
0
1.0
2.0
3.0
1
3
6
12
24

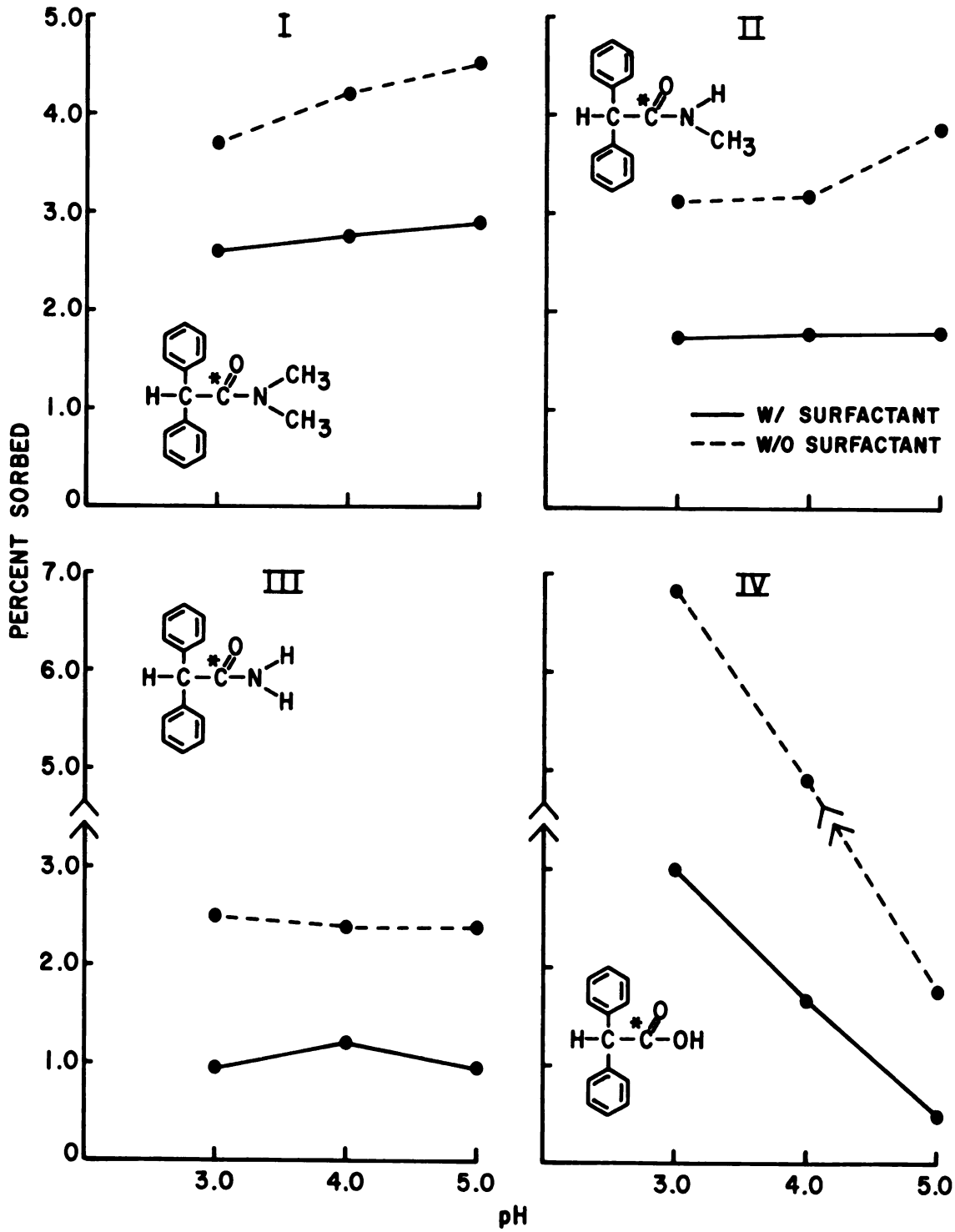
Figure 5. Influence of donor pH on the sorption of different compounds into isolated tomato fruit cuticles, with and without a surfactant, 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


I
II
III
IV
PERCENT SORBED
pH
W/ SURFACTANT
W/O SURFACTANT
5.0
4.0
3.0
2.0
1.0
0
7.0
6.0
3.0
4.0
5.0
H-C-C-N
CH3
H
OH
O

or diphenylacetamide sorbed into the cuticle was not affected by the different pH levels in these experiments (Figures 5-I, 5-II, and 5-III). However, there was a highly significant difference (Appendix A) in the amount of diphenylacetic acid sorbed into the tomato fruit cuticles from donors buffered at different pH levels (Figure 5-IV). More diphenylacetic acid was sorbed into the cuticular membrane at pH 3.0 and 4.0 than at pH 5.0. The amount of diphenylacetic acid sorbed was inversely proportional to the pH levels used; e.g., 6.8% at pH 3.0, 4.9% at pH 4.0, and 1.8% at pH 5.0 (Appendix B). Similar sorption curves were obtained at the different pH levels for each molecular structure regardless of whether or not a surfactant was used.

Influence of surfactant on sorption.

Significantly less radiolabeled compound sorbed into the tomato fruit cuticles when a surfactant was used (Figure 6 and Appendix B). All concentrations (0.1% to 0.4% v/v) of the nonionic surfactant Tergitol 15-S-9 decreased retention of the 4 different compounds compared to the quantity sorbed without the addition of a surfactant. The amount sorbed by the cuticles 24 hours after treatment with a surfactant was one and one-half times less for N,N-dimethyl-2,2-diphenylacetamide, and approximately two and one-half times less for N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid than from donors without a surfactant.

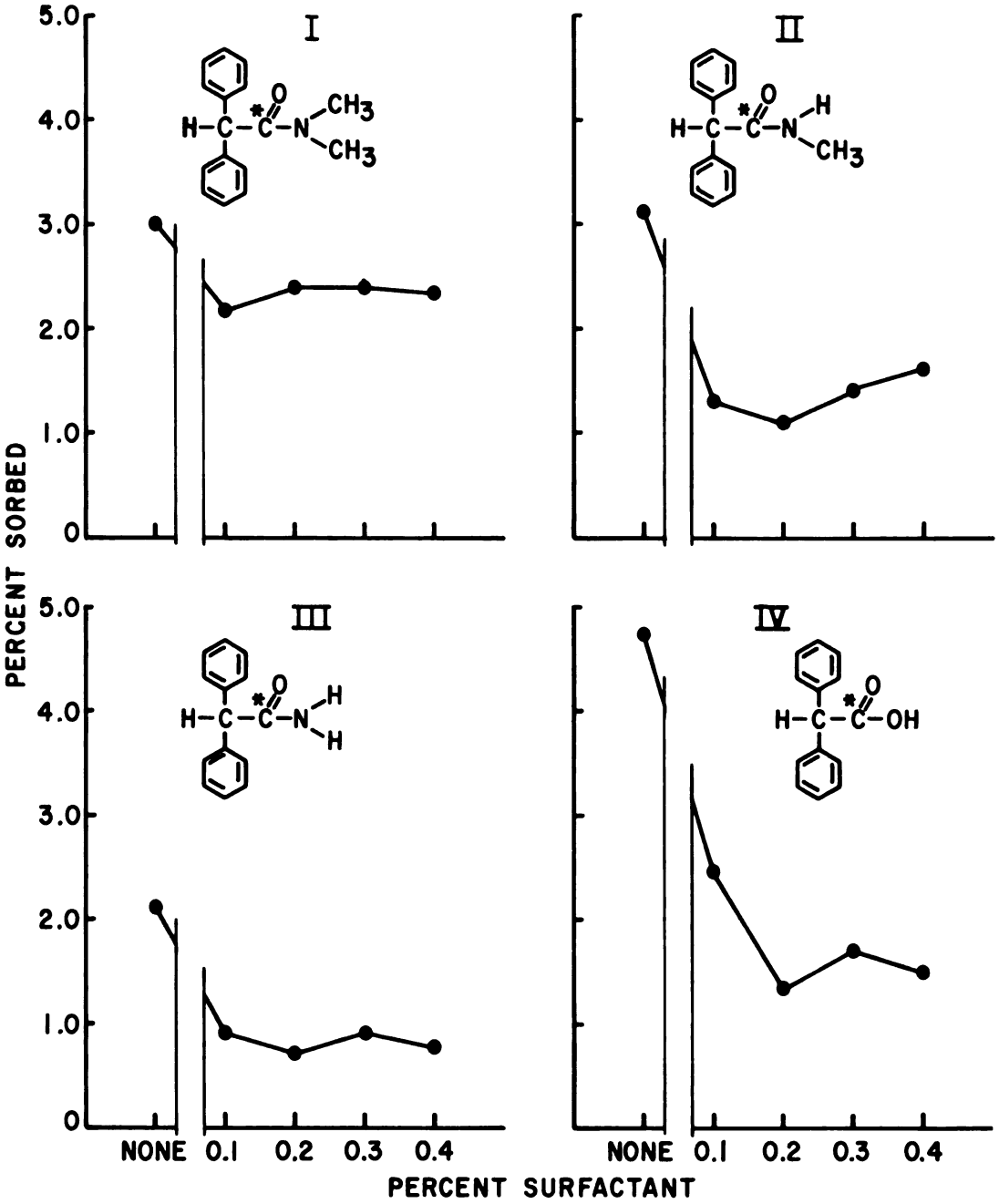
Figure 6. The concentration influence of the nonionic surfactant Tergitol 15-S-9 on the sorption of different compounds into tomato fruit cuticles, 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


I
II
III
IV
PERCENT SORBED
5.0
4.0
3.0
2.0
1.0
0
NONE
0.1
0.2
0.3
0.4
PERCENT SURFACTANT

Relation of Molecular Structure to Penetration.

Influence of time on penetration.

The penetration of N,N-dimethyl-2,2-diphenylacetamide, N-methyl 2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid through enzymatically isolated tomato fruit cuticles is illustrated in Figure 7. Penetration of all compounds increased with time. At first there was a gradual increase in penetration rate for all compounds, and then a more rapid linear increase from 6 to 24 hours. In all instances, significant quantities (Appendix C) of diphenylacetic acid molecule penetrated at a more rapid rate than diphenylacetamide, N-methyl-2,2-diphenylacetamide, or N,N-dimethyl-2,2-diphenylacetamide. The descending order of penetration for these compounds was diphenylacetic acid, N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide respectively.

Influence of pH on penetration.

There was a highly significant difference (Appendix D) in the amount of diphenylacetic acid which penetrated isolated tomato fruit cuticles from donor solutions buffered to different pH levels (Figure 8). Greater amounts of diphenylacetic acid penetrated the cuticular membrane at pH values below than above the pK value (3.94).

There was a 2- to 4-fold increase in penetration when a surfactant was added to the donor solution (Figure 8). Still, the influence of pH was evident regardless of whether or not a surfactant was used. However, the pH level used in these experiments appeared to

Figure 7. Penetration of different compounds through cuticles enzymatically isolated from tomato fruit, as influenced by time.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid

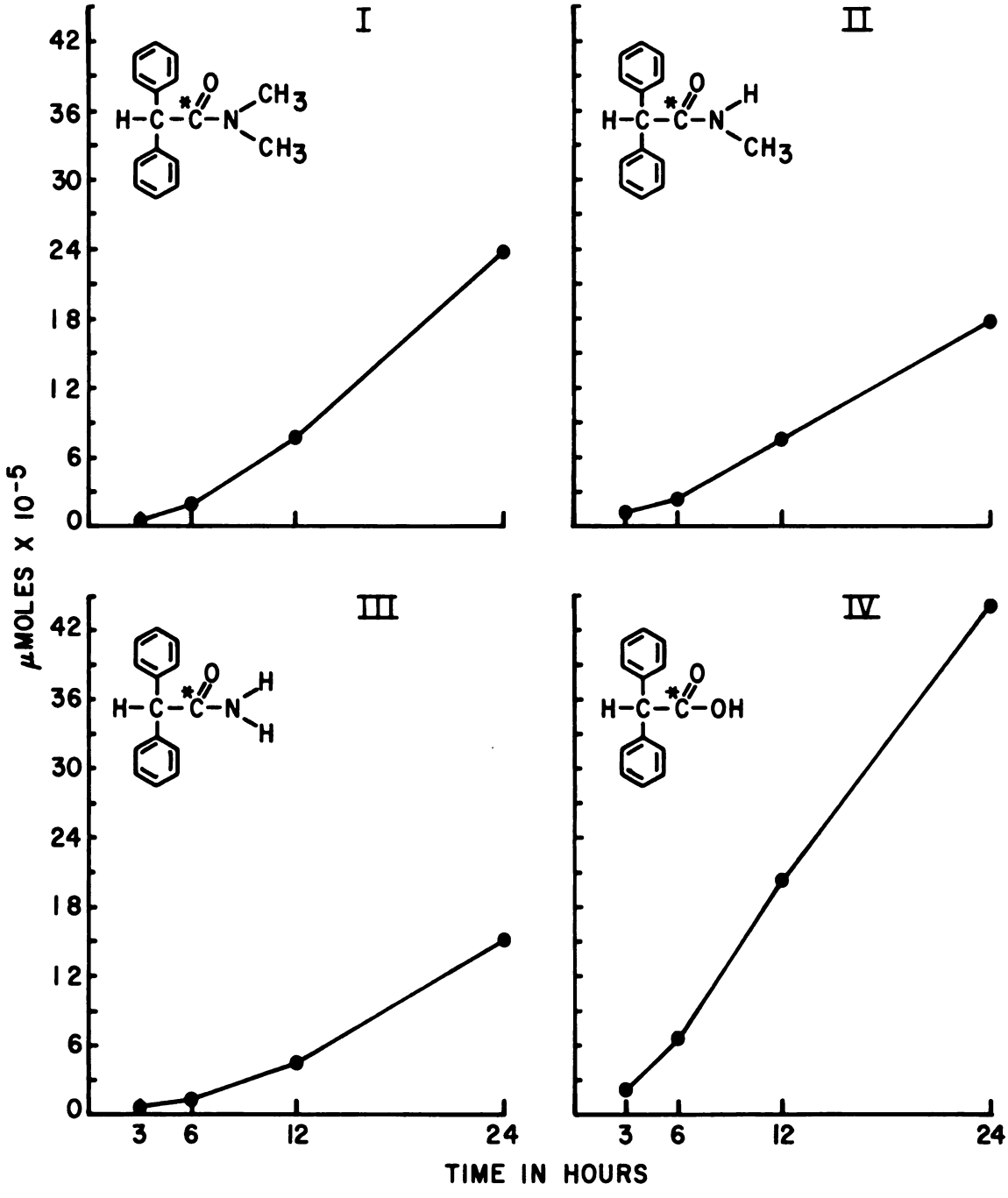

I
II
III
IV
42
36
30
24
18
12
6
0
μMOLES X 10⁻⁵
3
6
12
24
TIME IN HOURS

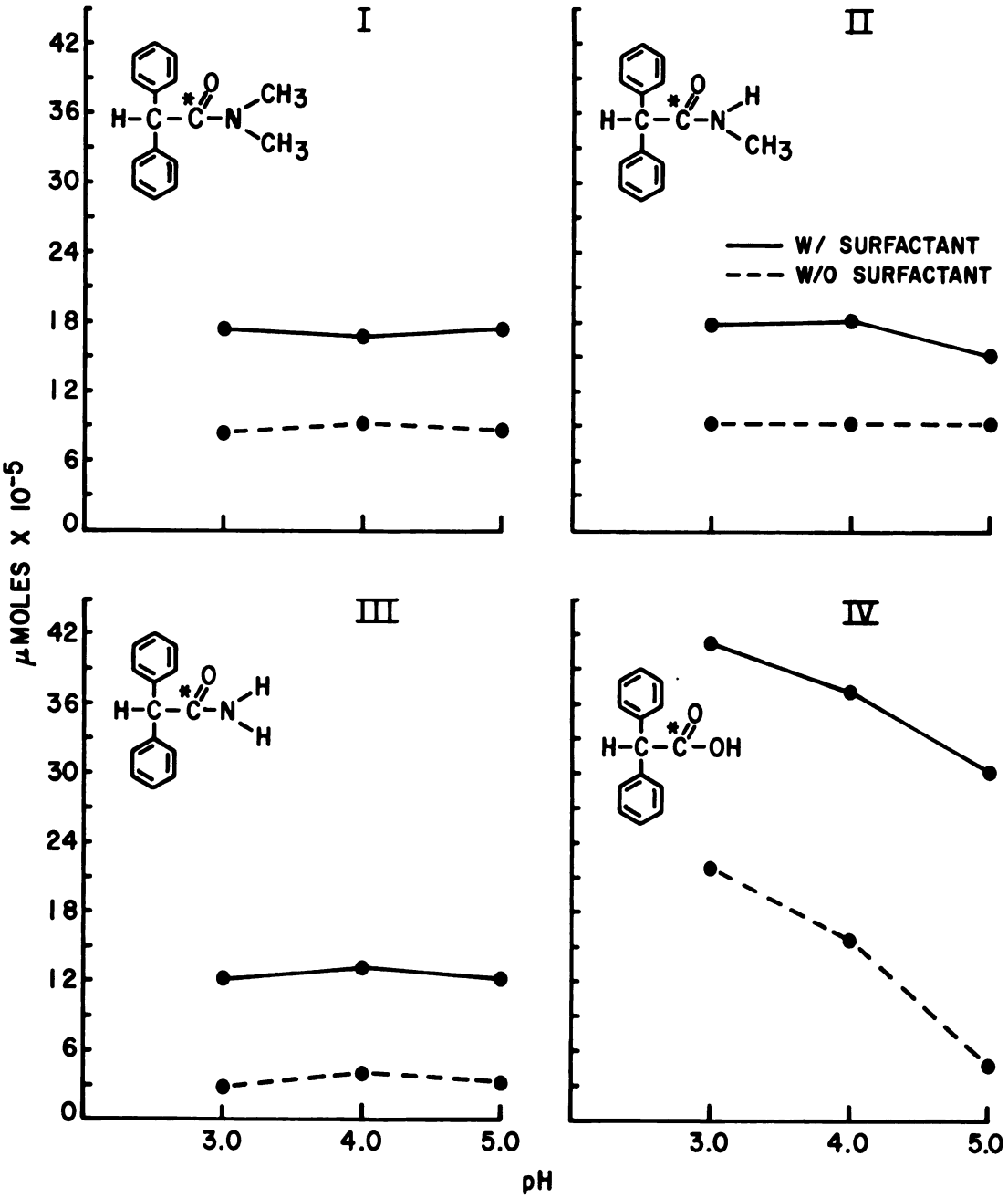
Figure 8. The influence of donor pH on penetration of different compounds, with and without a surfactant, through enzymatically isolated tomato fruit cuticles, 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


I
II
III
IV
W/ SURFACTANT
W/O SURFACTANT
μMOLES X 10⁻⁵
42
36
30
24
18
12
6
0
3.0
4.0
5.0
pH

have little to no effect on penetration of the N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide. At a pH level of 4.0, the amount of diphenylacetic acid which penetrated was twice that for N,N-dimethyl-2,2-diphenylacetamide and N-methyl-2,2-diphenylacetamide, and three times that for diphenylacetamide.

Influence of surfactants on penetration.

The addition of the nonionic surfactant Tergitol 15-S-9 to the donor solution promoted penetration of all four compounds through isolated tomato fruit cuticles. This was independent of the pH level of the donor solution. Note that the penetration pattern of each compound was the same for all pH levels tested with the variation being amount, as influenced by the addition of the surfactant.

Several nonionic surfactants were evaluated to determine their influence on penetration of N,N-dimethyl-2,2 diphenylacetamide through tomato fruit cuticles. The results are illustrated in Figure 9. These data show that after 24 hours all surfactants increased penetration. However, Tergitol 15-S-9 gave the greatest increase in penetration; Tween 20, the least; while surfactant DF-16 was intermediate. This order of penetration was similar for both the 6 and 24-hour treatments. Tergitol 15-S-9 surfactant (0.1% v/v) resulted in penetration of N,N-dimethyl-2,2-diphenylacetamide after 6 hours being equal to 24 hours without a surfactant (Figure 9).

The influence of concentration of the surfactants Tween 20 and Tergitol 15-S-9 is shown in Figures 10 and 11. These data again show

Figure 9. Influence of different nonionic surfactants (0.1% v/v) on penetration of N,N-dimethyl-2,2-diphenylacetamide through isolated tomato fruit cuticles.

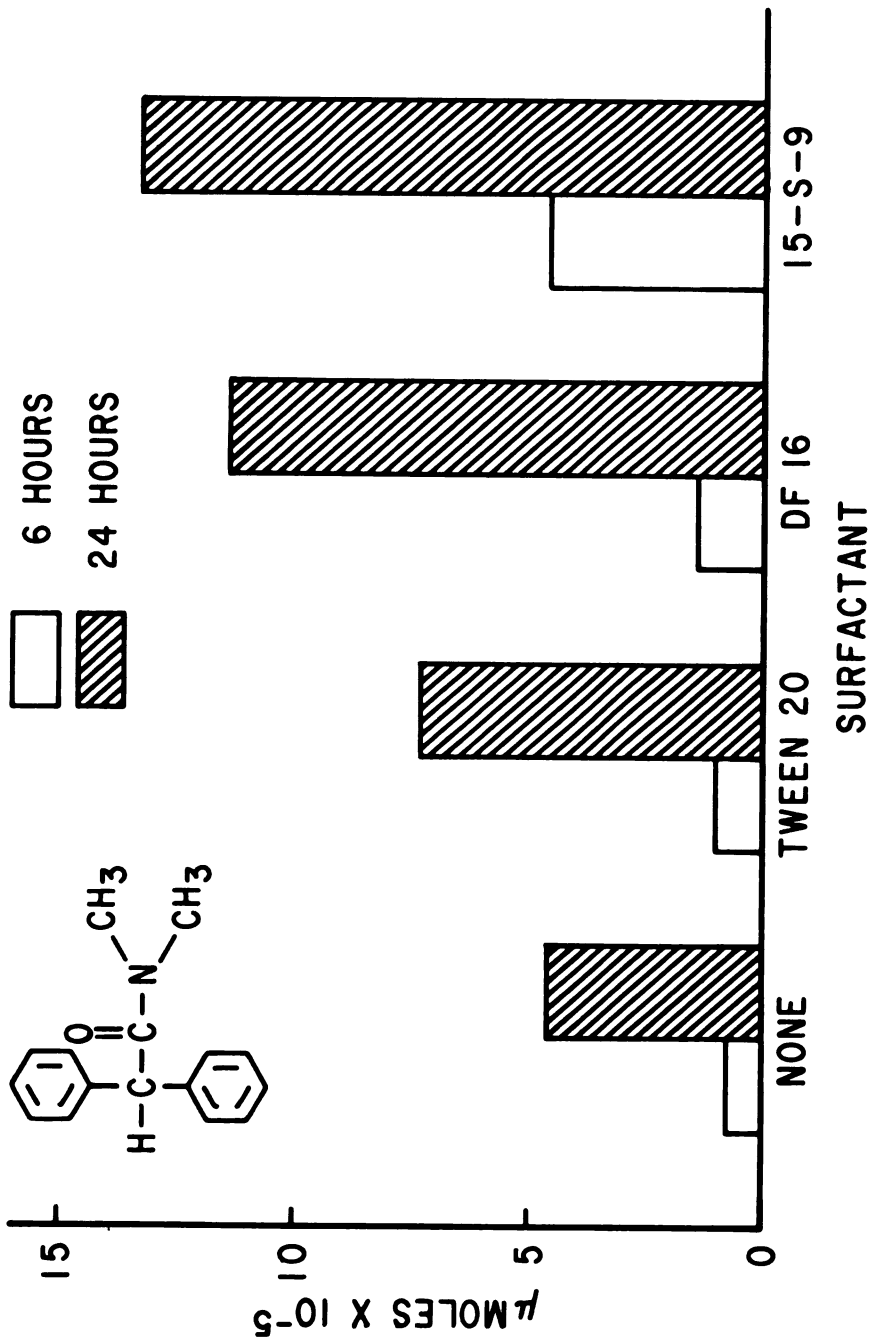

6 HOURS
24 HOURS
μMOLES X 10⁻⁵
0
5
10
15
NONE
TWEEN 20
DF 16
15-S-9
SURFACTANT
CH3
CH3
N
C
O
H

Figure 10. Influence of concentration of the nonionic surfactant Tween 20 on penetration of N,N-dimethyl-2,2-diphenyl-acetamide through isolated tomato fruit cuticles, 18 hours after treatment.

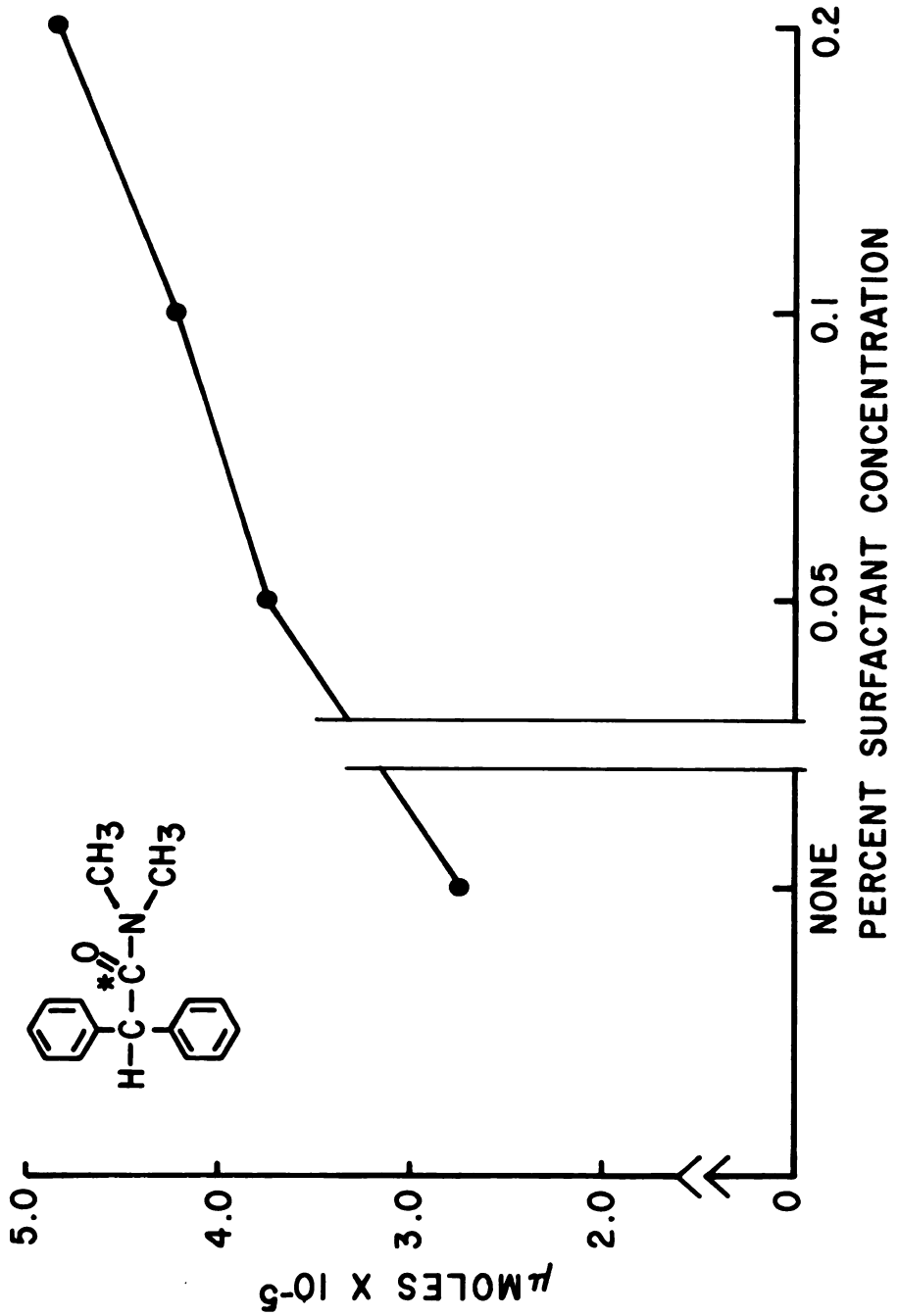
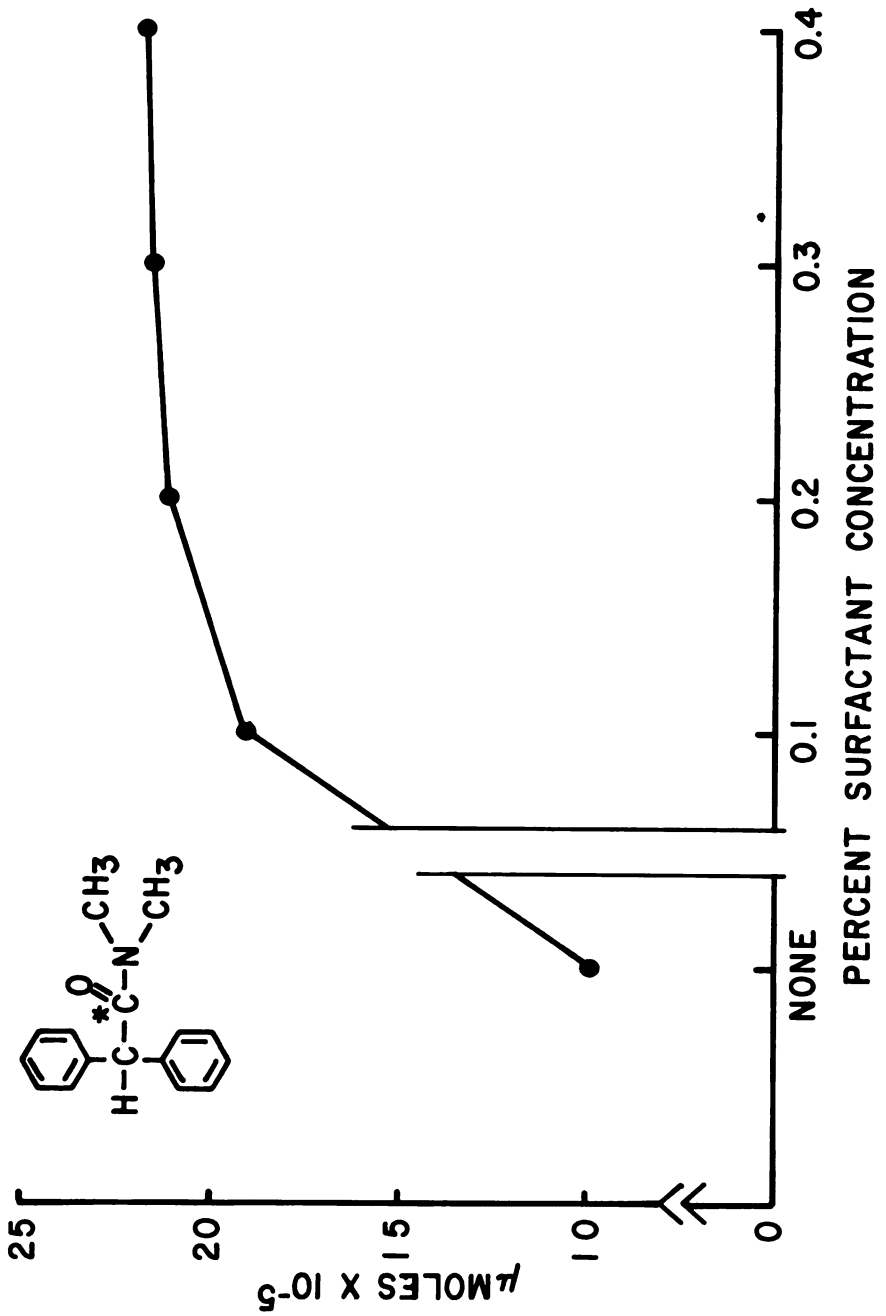

5.0
4.0
3.0
2.0
0
μMOLES X 10-5
NONE
0.05
0.1
0.2
PERCENT SURFACTANT CONCENTRATION
H
C
C
O
N
CH3
CH3

Figure 11. Influence of concentration of the nonionic surfactant Tergitol 15-S-9 on penetration of N,N-dimethyl-2,2-diphenylacetamide through isolated tomato fruit cuticles, 24 hours after treatment.


μMOLES X 10⁻⁵
25
20
15
10
0
NONE
0.1
0.2
0.3
0.4
PERCENT SURFACTANT CONCENTRATION
H–C–C–N
CH3
CH3
O
*

an increase in penetration of N,N-dimethyl-2,2-diphenylacetamide through tomato fruit cuticles when a surfactant was added to the donor solution. This increased quantity of penetrant was influenced by the concentration of surfactant. In general, as the concentration of surfactant was increased from no surfactant to 0.2%, the amount of compound which penetrated the isolated tomato cuticle also increased. There was almost a two-fold decrease in penetration of N,N-dimethyl-2,2-diphenylacetamide without a surfactant than when either Tween 20 or Tergitol 15-S-9 was added at a 0.2% (v/v) concentration.

The influence of the nonionic surfactant Tergitol 15-S-9 (0.3% v/v) on penetration of different compounds through tomato fruit cuticles is illustrated in Figure 12. After 24 hours, there was a marked increase in penetration of compound with the addition of the surfactant Tergitol 15-S-9 when compared to the amount of the same compound which penetrated the cuticle without addition of a surfactant.

Influence of surface wax on penetration.

A comparison of the influence of surface wax on the penetration of different molecular structures through tomato fruit cuticles is summarized in Figure 13. In all instances, there was a greater increase in penetration after removal of the epicuticular waxes. In the case of diphenylacetic acid, removal of surface waxes resulted in a five-fold increase in penetration compared to the quantity which penetrated when waxes were left intact on tomato fruit cuticles. The removal of waxes permitted a 2- to 3-fold increase in penetration of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide.

Figure 12. Influence of the nonionic surfactant Tergitol 15-S-9 (0.3% v/v) on penetration of different compounds through isolated tomato fruit cuticles, 24 hours after treatment.

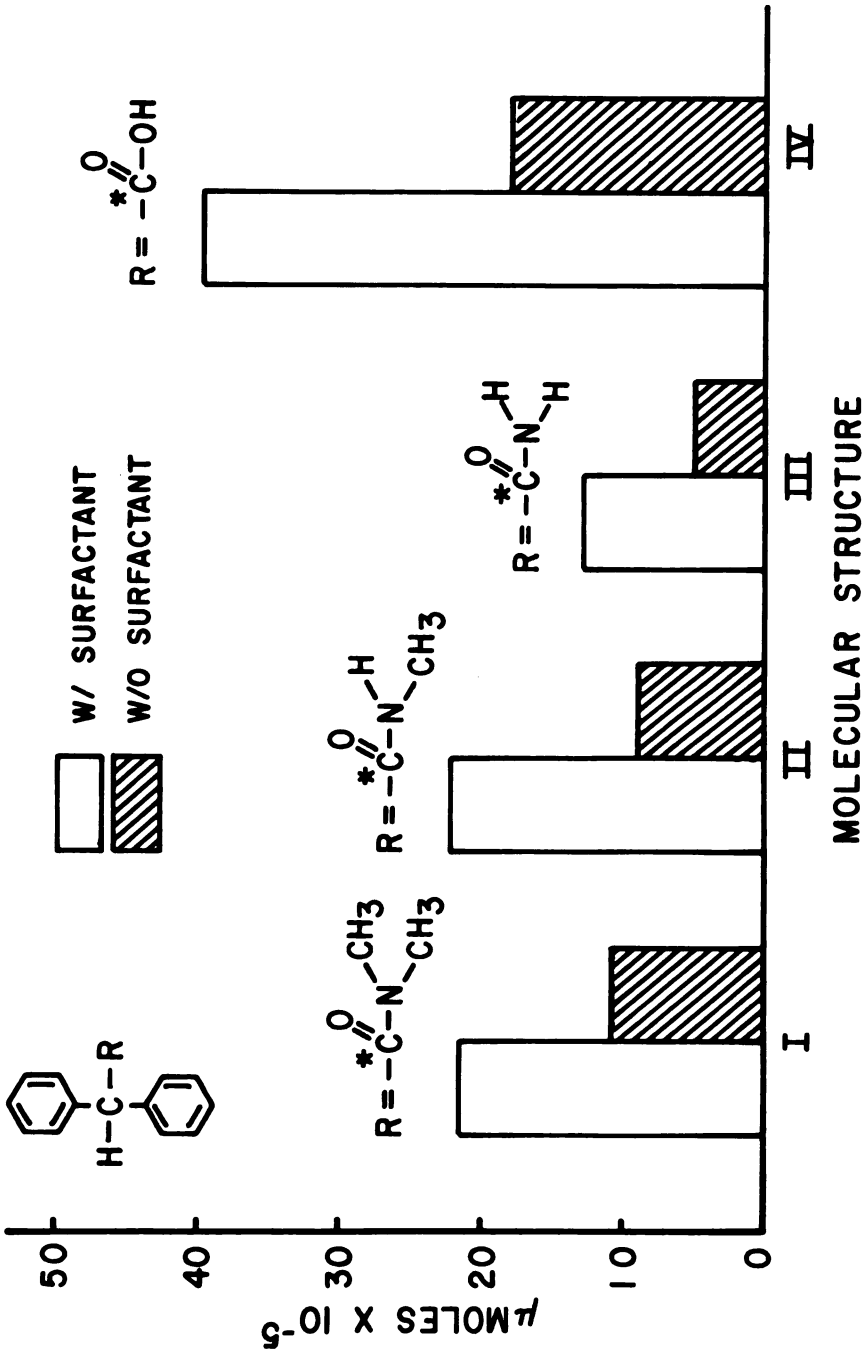

μMOLES X 10⁻⁵
0
10
20
30
40
50
W/ SURFACTANT
W/O SURFACTANT
H–C–R
R = –C(=O)–N(CH3)–CH3
R = –C(=O)–N(H)–CH3
R = –C(=O)–N(H)–H
R = –C(=O)–OH
I
II
III
IV
MOLECULAR STRUCTURE

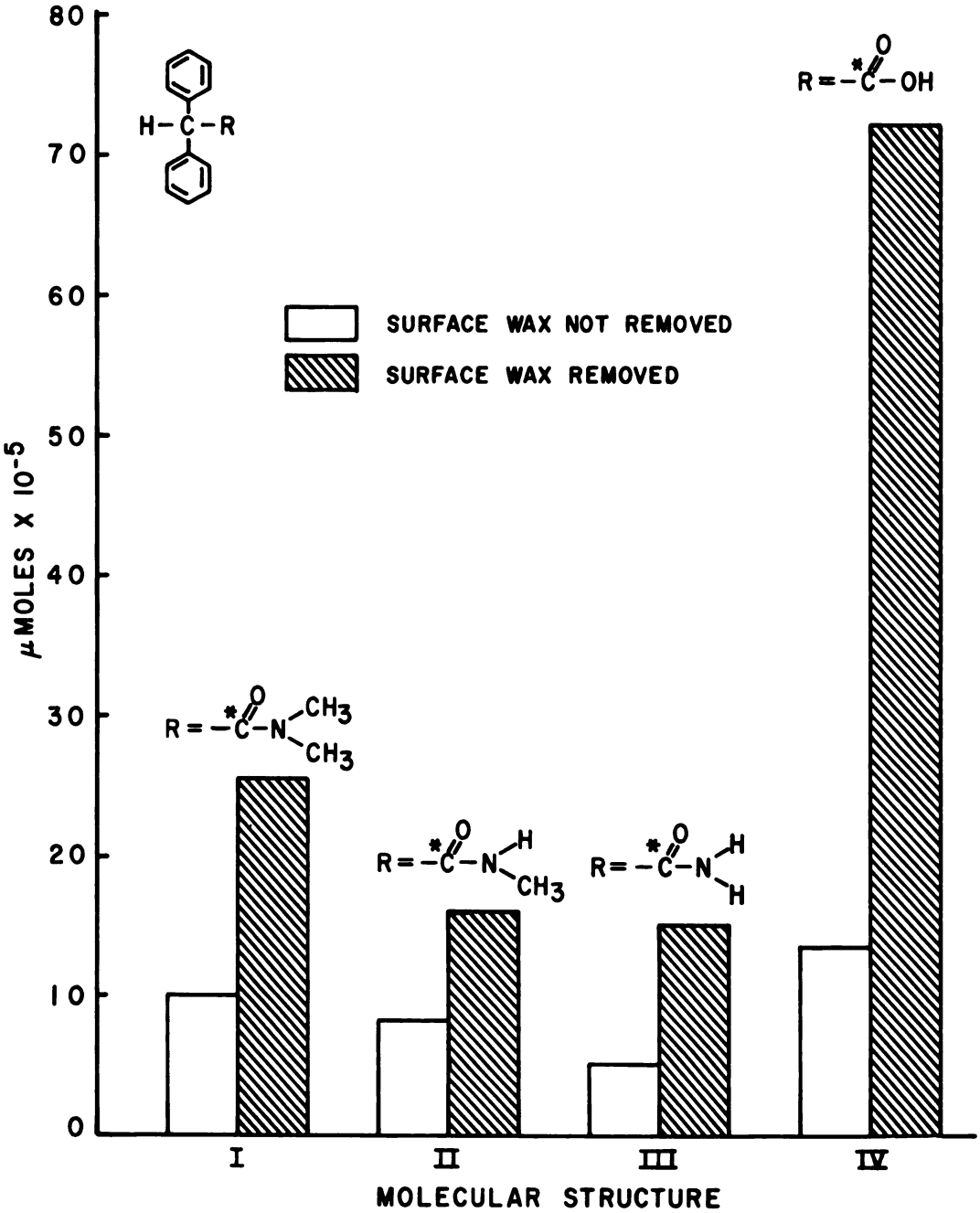
Figure 13. Influence of surface wax on the penetration of different compounds through isolated tomato fruit cuticles, 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


80
70
60
50
40
30
20
10
0
μMOLES X 10-5
SURFACE WAX NOT REMOVED
SURFACE WAX REMOVED
H-C-R
R=-C-OH
R=-C-N(CH3)2
R=-C-NH-CH3
R=-C-NH2
I
II
III
IV
MOLECULAR STRUCTURE

Relation of Molecular Structure to Uptake.

Influence of time on uptake.

The comparative amount of uptake of 4 different compounds into green bean leaf disks is illustrated in Figure 14. There was a slight to rapid increase in uptake of all four compounds 1, 3, and 6 hours following application of the donor solution. Twelve hours following application, there was a leveling-off or slight decline in uptake of the three amide molecules; quantity being 5.2%, 5.1%, and 5.5% respectively for N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide. Uptake of diphenylacetic acid (Figure 14-IV) was a steady increase for the 1, 3, 6, 12, and 24-hour periods (2.4, 6.7, 7.0, 10.4, and 20.1 percent).

Twenty-four hours after treating bean leaf disks, the descending order of compound uptake was diphenylacetic acid (20.1%), N,N-dimethyl-2,2-diphenylacetamide (15.0%), diphenylacetamide (13.9%), and N-methyl-2,2-diphenylacetamide (7.4%).

In these experiments, the four donor solutions were buffered to pH 4.0 and contained 0.3 percent Tergitol 15-S-9 surfactant.

Influence of pH on uptake.

The relation of molecular structure to uptake of radioactive compounds into green bean leaf disks as influenced by donor pH is illustrated in Figures 15 and 16. After a treatment period of 24 hours, the percent uptake of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid from

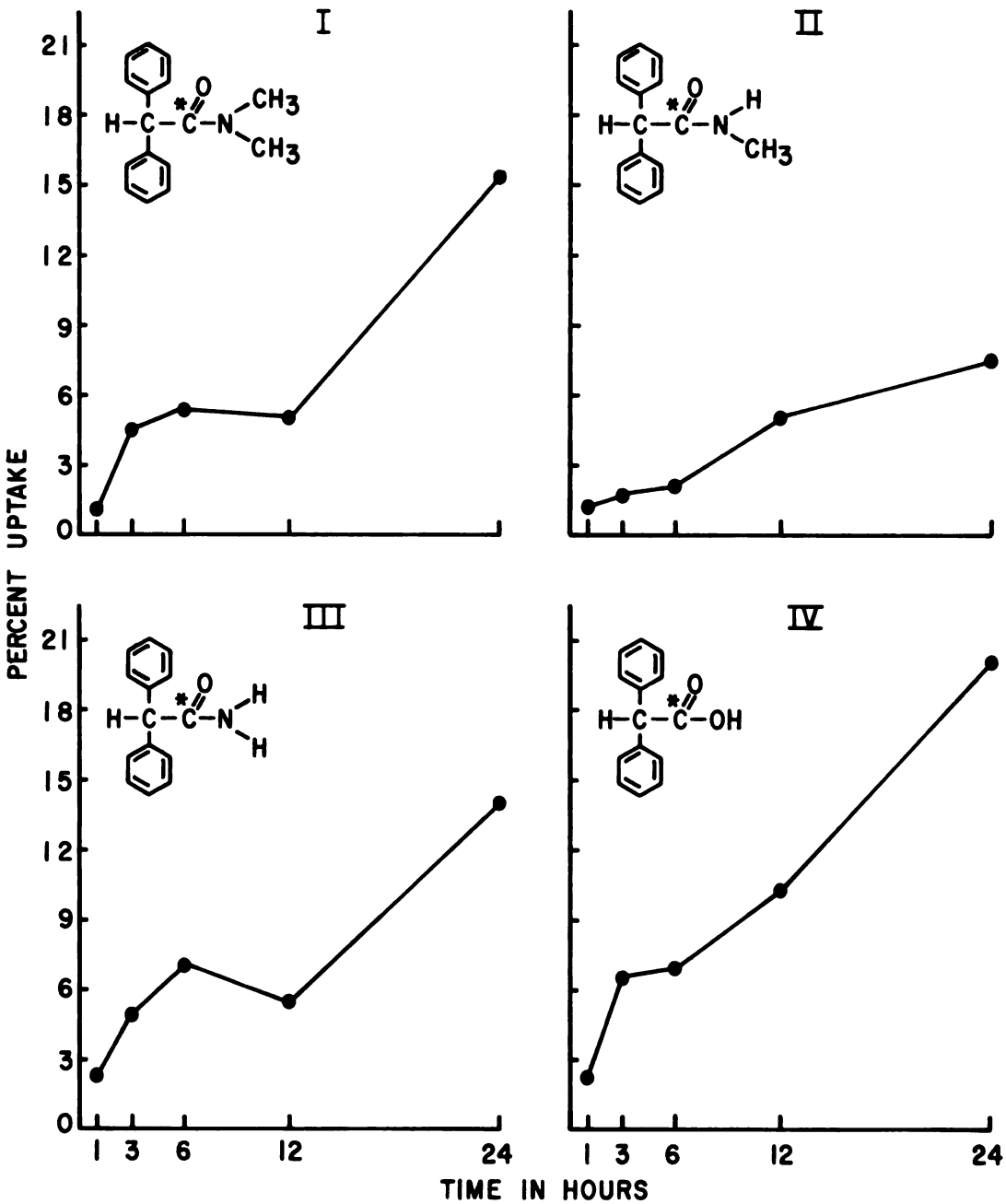
Figure 14. Comparative amounts of uptake of different compounds (at pH 4.0) into green bean leaf disks as influenced by time.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


I
II
III
IV
PERCENT UPTAKE
0
3
6
9
12
15
18
21
1
3
6
12
24
TIME IN HOURS

distilled water ($_p$H 6.6) was 6.2, 5.6, 6.2, and 2.0 percent (Figure 15). These data indicate there was little or no difference in the uptake of the three amide molecules (6.2%, 5.6%, 6.2%). However, the uptake of diphenylacetic acid (Figure 15-IV) was only 2.0% from a distilled water ($_p$H 6.6) donor solution.

As illustrated in Figure 16, the $_p$H level of the donor solution had little effect on the uptake of the 3 amide molecules. Uptake of N,N-dimethyl-2,2-diphenylacetamide was 4.9% at $_p$H 3.0, 5.0% at $_p$H 4.0, and 6.2% at $_p$H 5.0 after 6 hours; and 14.2, 15.0, and 17.4 percent 24 hours following application. Percent uptake of N-methyl-2,2-diphenylacetamide after 6 hours was 3.0, 3.2, and 3.4 at $_p$H 3.0, 4.0, and 5.0 respectively; and 9.5%, 8.7%, and 9.3% after 24 hours. Uptake of diphenylacetamide after 6 and 24 hours at $_p$H 3.0, 4.0, and 5.0 was 4.6, 5.3, 5.9 and 13.2, 13.9, and 15.0 percent respectively. Difference in uptake of the three amide molecules was influenced by time and not by $_p$H of the donor solution.

Lesser amounts of diphenylacetic acid were taken up by green bean leaf disks from donors buffered to $_p$H 5.0 than from donors buffered to $_p$H 4.0 or 3.0 (Figure 16-IV). After 6 hours, uptake was 7.2% at $_p$H 3.0, 6.3% at $_p$H 4.0, and 3.0% at $_p$H 5.0. Twenty-four hours following treatment, uptake was 21.7, 20.0, and 15.4 percent for the three $_p$H levels. The greater uptake of diphenylacetic acid occurred at $_p$H levels below its $_p$K (3.94). This is essentially the same $_p$H level effect observed in the penetration and sorption studies discussed earlier.

Figure 15. Relation of molecular structure of ^{14}C-carbonyl labeled compounds into green bean leaf disks 24 hours after treatment. Donor solution was distilled water without a surfactant.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid

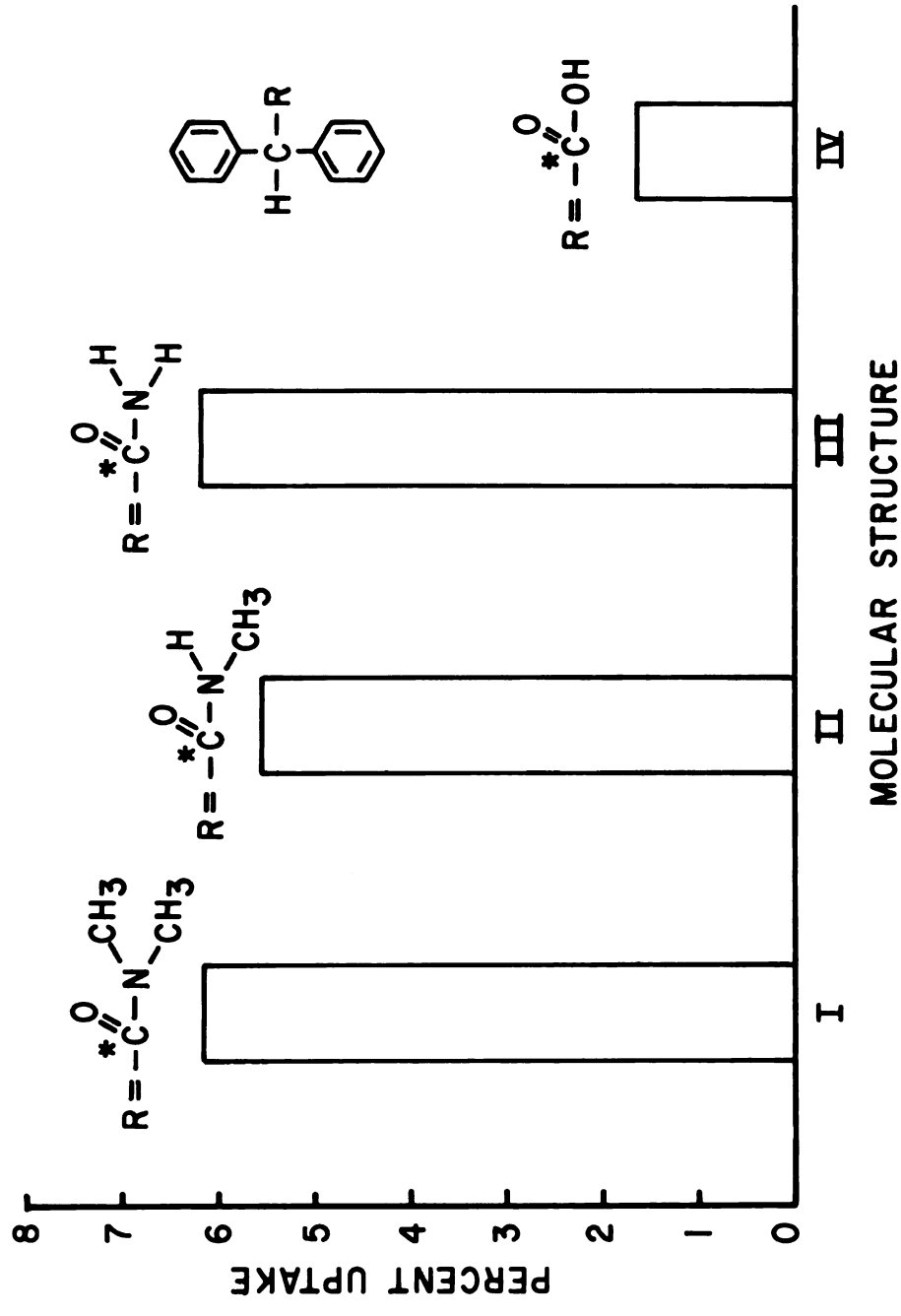

PERCENT UPTAKE
0
1
2
3
4
5
6
7
8
I
II
III
IV
MOLECULAR STRUCTURE
R=-C(=O)*-N(CH3)CH3
R=-C(=O)*-N(H)CH3
R=-C(=O)*-N(H)H
R=-C(=O)*-OH
H-C-R

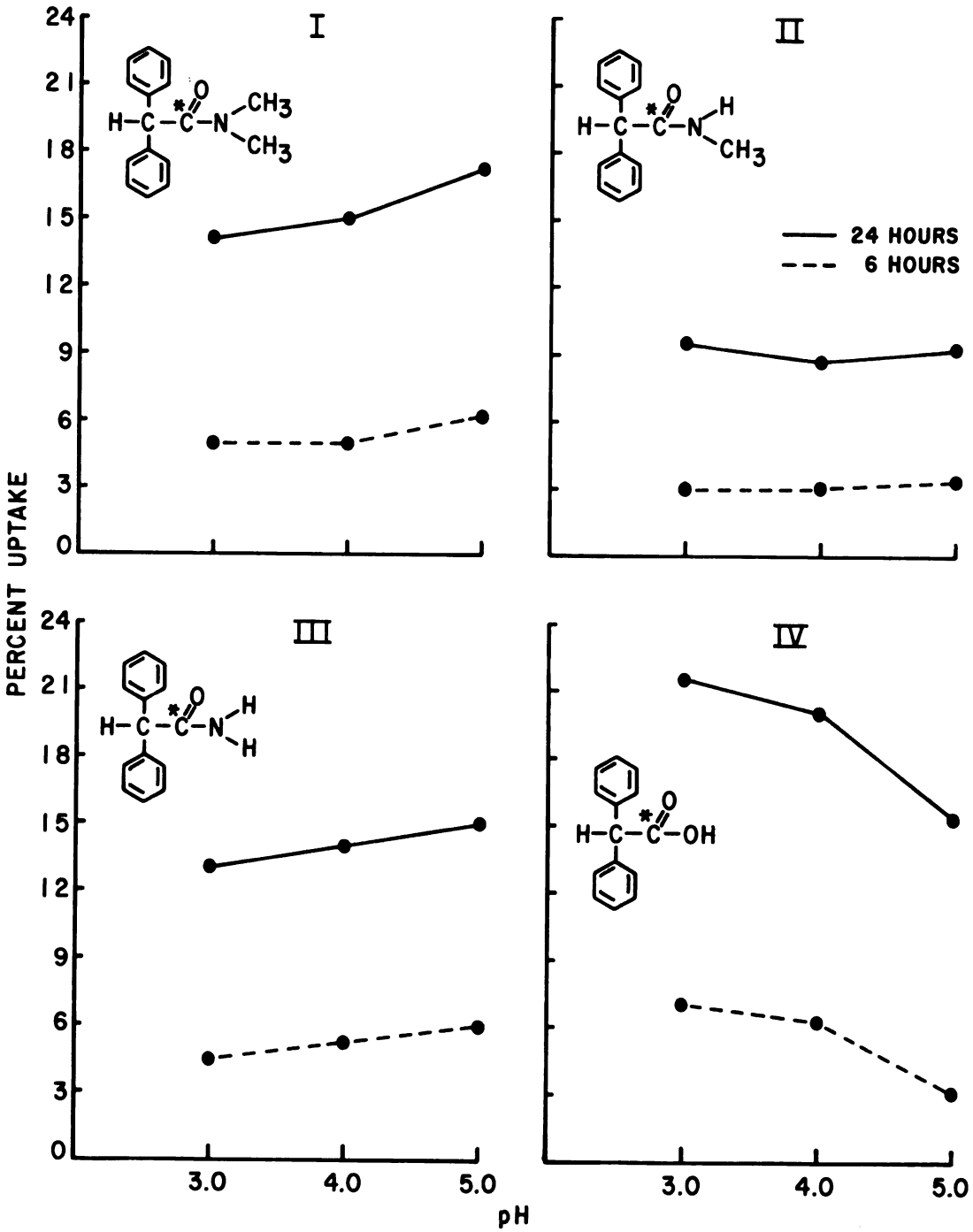
Figure 16. Influence of donor pH on uptake of 4 different compounds into green bean leaf disks, 6 and 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


I
II
III
IV
24 HOURS
6 HOURS
PERCENT UPTAKE
pH
0
3
6
9
12
15
18
21
24
3.0
4.0
5.0
H-C-C-N-CH3
CH3
H-C-C-N-H
CH3
H-C-C-N-H
H
H-C-C-OH

Influence of surfactant on uptake.

The addition of the surfactant (0.3% v/v) Tergitol 15-S-9 to the donor solution resulted in an increased uptake of all 4 compounds into bean leaf disks. As shown in Figure 17, the surfactant resulted in a 2-fold increase in uptake of each compound. The values for this increase in uptake due to a surfactant were 5.13 vs. 10.97 percent for diphenylacetic acid, 2.05 vs. 5.48 percent for diphenylacetamide, 2.89 vs. 5.17 percent for N,N-dimethyl-2,2-diphenylacetamide, and 2.25 vs. 4.87 percent for N-methyl-2,2-diphenylacetamide. At a donor $_{p}H$ of 4.0, the quantity of diphenylacetic acid without a surfactant taken up by the green bean leaf disks was equal to that of the three amides with a surfactant (Figure 17).

The influence of different concentrations of the nonionic surfactant Tergitol 15-S-9 on the uptake of N,N-dimethyl-2,2-diphenylacetamide into bean leaf disks is shown in Figure 18.

The use of the surfactant at all concentrations resulted in an increase in uptake of the compound for both the 6 and 12-hour treatments. The maximum uptake occurred at a 12-hour treatment time when the Tergitol 15-S-9 was 0.2 percent (v/v).

In the 6-hour treatments there appeared to be more than one maxima for uptake of N,N-dimethyl-2,2-diphenylacetamide. However, these differences for the surfactant concentrations are probably the result of experimental error and are not significant (Appendix E).

Figure 17. Influence of nonionic surfactant (0.3% v/v) Tergitol 15-S-9 on uptake of different compounds into green bean leaf disks, 12 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid

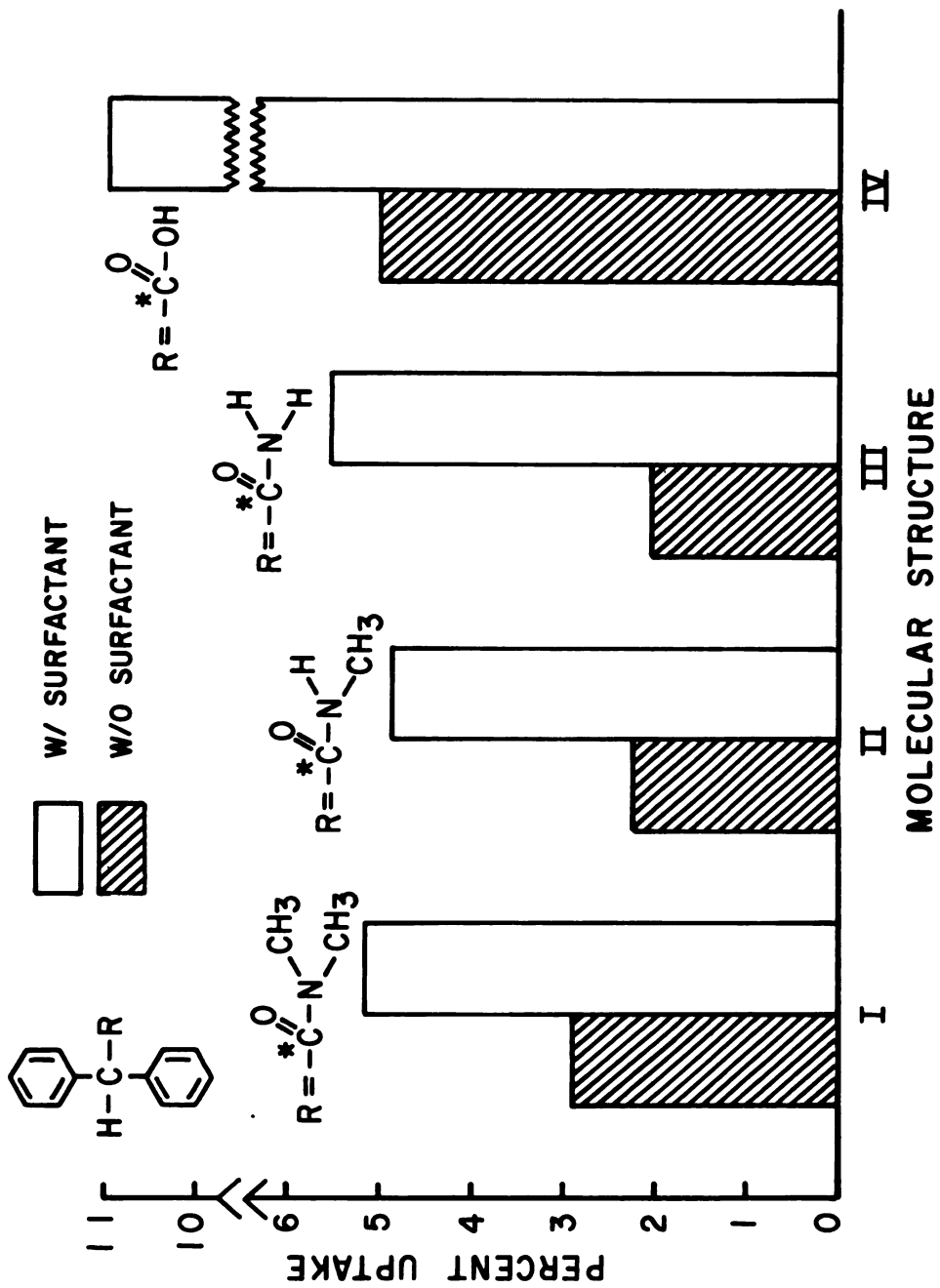
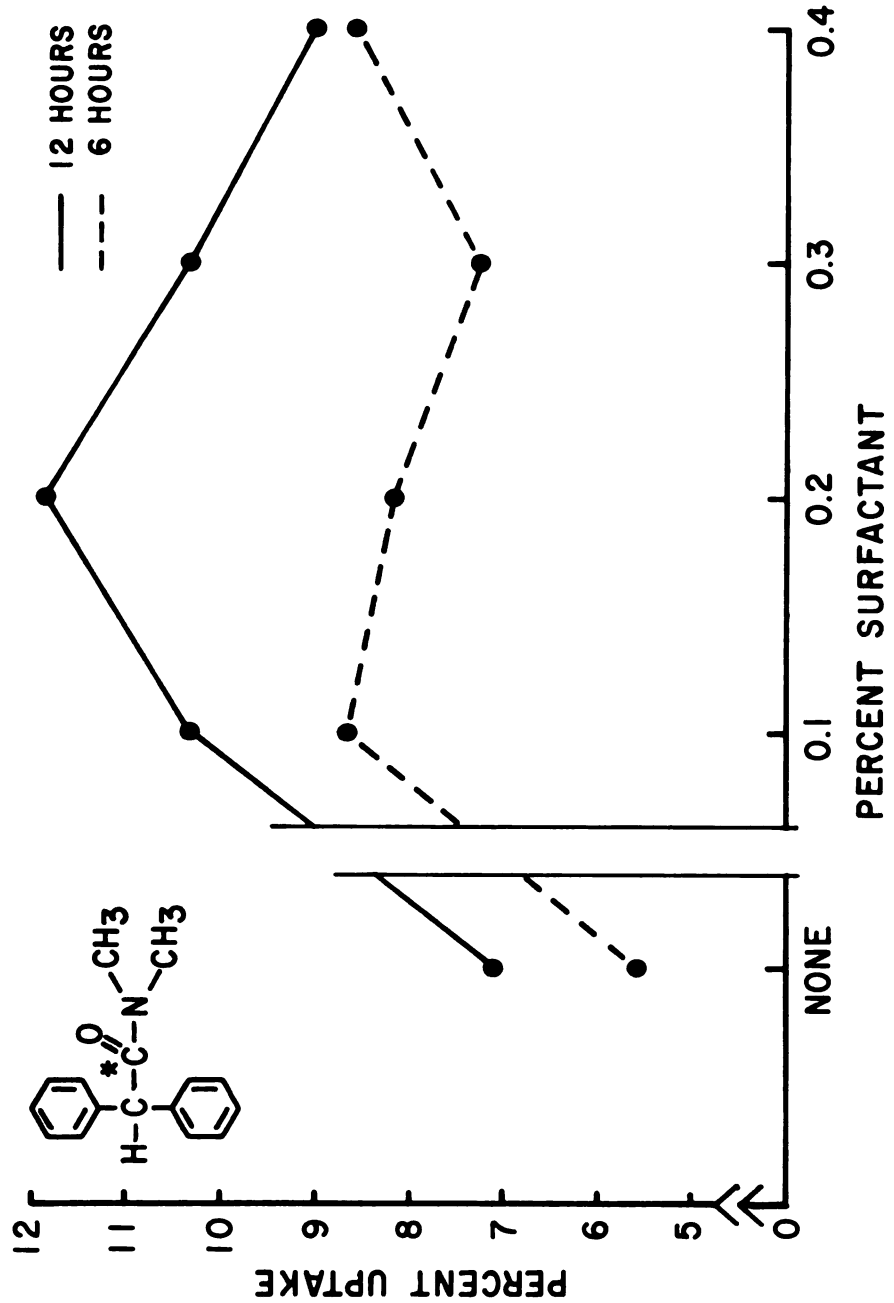

PERCENT UPTAKE
0
1
2
3
4
5
6
10
11
W/ SURFACTANT
W/O SURFACTANT
H–C–R
R=–C(=O)–N(CH3)–CH3
R=–C(=O)–N(H)–CH3
R=–C(=O)–N(H)–H
R=–C(=O)–OH
I
II
III
IV
MOLECULAR STRUCTURE

Figure 18. Influence of concentration of the nonionic surfactant Tergitol 15-S-9 on uptake of N,N-dimethyl-2,2-diphenyl-acetamide into green bean leaf disks, 6 and 12 hours after treatment.


12 HOURS
6 HOURS
PERCENT UPTAKE
12
11
10
9
8
7
6
5
0
NONE
0.1
0.2
0.3
0.4
PERCENT SURFACTANT
H
C
O
N
CH3
CH3

Relation of Molecular Structure to Partitioning.

Influence of organic solvents on partitioning.

There was very little difference in the amount of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, or diphenylacetamide partitioning from water to chloroform or oleic acid solvents (Figures 19-I, 19-II, and 19-III). The percentages of the above amides partitioning into chloroform were 99.09, 96.30, and 96.05 respectively. There was no significant difference (Appendix G) in the amount of diphenylacetic acid (95.34%) which partitioned into the oleic acid solvent (Figure 19-IV) when compared to the three diphenylacetamides.

Influence of pH on partitioning.

As seen in Figure 19, a donor pH of 4.0 or 7.0 did not affect the partitioning of these compounds into oleic acid. When donors were buffered to pH 4.0, the amount of partitioning of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid was 93.54, 94.85, 94.80, and 92.89 percent respectively. From donors buffered to pH 7.0, the partitioning into oleic acid was 98.51, 95.77, 97.85, and 97.79 percent.

The donor pH level had no significant effect on the percent of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, or diphenylacetamide partitioning into chloroform (Figures 19-I, 19-II, 19-III), the amount being 94.98 to 99.16 percent. As seen in Figure 19-IV and Appendix F, a significantly greater amount of diphenylacetic acid partitioned into chloroform from donors at pH 4.0 than from donors at pH 7.0 (94.47% vs. 66.17%).

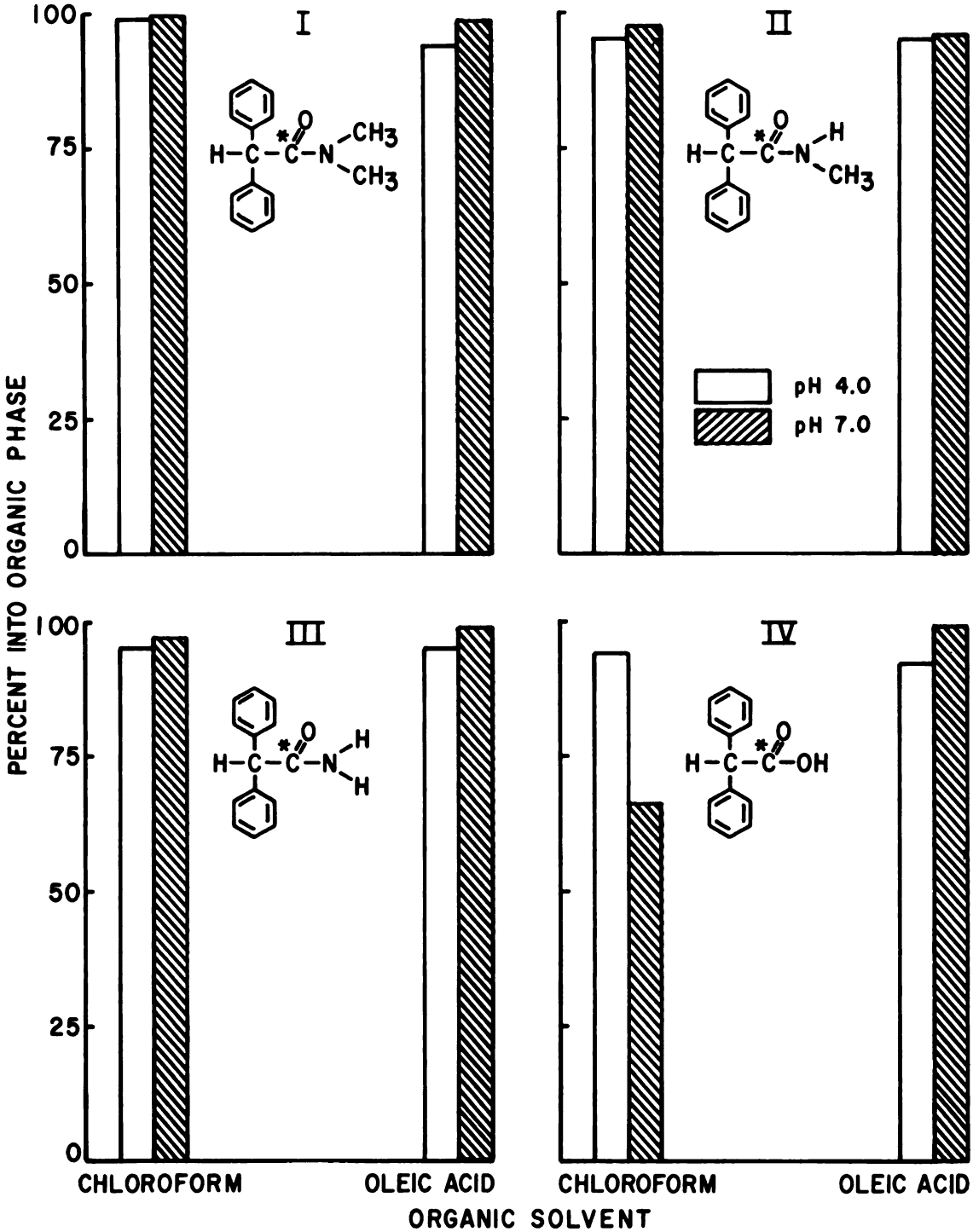
Figure 19. Relation of molecular structure and pH of ^{14}C-carbonyl labeled compounds to partitioning into different organic solvents from aqueous donor solutions, 24 hours after treatment.

I. N,N-dimethyl-2,2-diphenylacetamide

II. N-methyl-2,2-diphenylacetamide

III. Diphenylacetamide

IV. Diphenylacetic acid


100
75
50
25
0
I
II
III
IV
pH 4.0
pH 7.0
PERCENT INTO ORGANIC PHASE
CHLOROFORM
OLEIC ACID
CHLOROFORM
OLEIC ACID
ORGANIC SOLVENT

DISCUSSION

Sorption and penetration of externally applied chemicals to plant surfaces are part of a multi-step process. Before becoming accessible to living cells, a chemical must first traverse the cuticle which is considered the first barrier. The cuticle itself is composed mainly of cutin and waxes. Pectin substances separate the cuticle from underlying cell walls. Enzymatically isolated cuticular membranes have been used to study the mechanism of absorption and penetration by several investigators (Weintraub et al. 102; Yamada et al., 107; Jyung et al., 53,54; and Norris and Bukovac, 72). Ripe tomato fruit cuticle was isolated enzymatically without any apparent physical alteration to it. Thus, factors affecting sorption and penetration could be studied without being influenced by a metabolically active plant cell.

Cuticular Sorption and Penetration.

In this study, sorption refers to the compound that is bound to the cuticular components, either by absorption or adsorption, and is not removed by repeated washing with distilled water. Penetration refers to the process by which the compound traverses the cuticle.

The sorption of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid into

tomato fruit cuticles occurred rapidly (Figure 4) for the first 3 to 6 hours, followed by a leveling-off or decline with increasing time. The behavior of the cuticular framework, composed of cutin, which exhibits moderate adhesion was significant to sorption of the compounds. Weintraub (102) and van Overbeek (98) suggested that after absorbing water the cuticle swells and spreads apart the embedded wax platelets (which exhibit low adhesion). This increases the permeability of the cuticle to water and perhaps to the four compounds of different molecular structure used in this study. In the sorption experiments, tomato fruit cuticles were immersed in the donor solution and the rapid sorption for the first 3 to 6 hours could be a result of rapid water uptake through the inner hydrophilic surface. After a certain length of time, a saturation point was reached and rate of sorption was much reduced (the pH influence on diphenylacetic acid will be discussed later). These results are similar to those obtained by Bukovac and Norris (11) who suggested that isolated cuticular membranes from tomato fruit will absorb water much faster through the inner hydrophilic surface than through the outer surface. The decrease in sorption of N-methyl-2,2-diphenylacetamide and diphenylacetamide at 6 and 12 hours is difficult to explain (Figure 4-II and 4-III). It could be that cuticular surface changes were occurring during exposure to the donor solution. Crafts and Foy (19) suggested that more lipophilic groups are accumulated outside rather than inside the cuticle. This results in a gradient of polarity from the exterior which is apolar to the interior which is polar. This degree of polarity could

explain the increase in sorption of the 3 diphenylacetamide molecules at 24 hours. After the cuticle had become saturated during the first 3 to 6 hours, the sorption of chemicals was then much slower.

The amount of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid which penetrated tomato fruit cuticle increased with time (Figure 7). For the first 6 hours, it is possible the organic compound was being sorbed to the components of the cuticle. Then after a certain amount of binding, the compound molecules were permitted to penetrate the cuticle at a more rapid rate. This could explain the increased penetration observed for the 12- and 24-hour treatments (Figure 7). It is assumed that penetration of these organic molecules was by diffusion, which is in agreement with evidence reported by Yamada (106), and Yamada et al. (109) that penetration of organic solutes was by diffusion.

The review by Franke (37) stated that lipid molecules are in constant thermodynamic motion oscillating permanently around a medium portion, dynamically changing ultraporosity of the wax layers allowing the penetration of larger hydrophilic molecules. In this study, the diphenylmethyl part is larger than the carboxamide part of the molecules used. This could explain the descending order of penetration rate through astomatous tomato fruit cuticle after 24 hours of the diphenylacetic acid, N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide (44.2, 23.1, 17.9, and 15.3 μmoles x 10^{-5}). The molecular weights of the above

compounds are 212.14, 239.31, 225.28, and 211.25 respectively. However, it must be realized that molecular weight is not always a true measure of molecular size, and that conformation of the molecule is important in determining the size opening which it can penetrate.

If tomato fruit cuticle is made up of alternating wax layers and cutin lamellas, then the wax quantity should inhibit the penetration of these aqueous solutions because of its apolar properties. This was not the case with the compounds used in this study, since penetration did occur. There was very little difference in the amount of ^{14}C-carbonyl labeled compounds partitioning into chloroform or oleic acid solvents (Figure 19); quantities being 95.0 to 99.4 percent into chloroform, and 93.5 to 94.9 percent into oleic acid. This shows that these organic molecules of different molecular structure can partition into apolar substances. The descending order of polarity for the four compounds used in these tests is diphenylacetic acid, diphenylacetamide, N-methyl-2,2-diphenylacetamide, and N,N-dimethyl-2,2-diphenylacetamide. Excluding the influence of donor $_{p}H$ on diphenylacetic acid, sorption and penetration was inversely proportional to polarity. At a $_{p}H$ greater than the $_{p}K$ of 3.94 of diphenylacetic acid, a proton is lost from the carboxyl group. This molecule then becomes an anion ($R-\overset{O}{\overset{\|}{C}}-O^-$) which should be more polar than the acid ($R-\overset{O}{\overset{\|}{C}}-OH$). These data show that sorption and penetration of diphenylacetic acid was decreased as the $_{p}H$ level of the donor solution was increased (Figures 5 and 8). As the $_{p}H$ level above $_{p}K$ 3.94 increased, the ionized ($R-\overset{O}{\overset{\|}{C}}-O^-$) species (highly polar) also increased and sorption and penetration decreased.

It is possible that water-soluble polar compounds may follow a special aqueous route through the cuticle, while apolar lipid-soluble compounds may use a lipoidal route through the cuticle. This possibility was first suggested by Crafts (18).

Metabolic activity did not influence sorption nor penetration of the compounds used in these experiments, since respiration of isolated cuticular membranes did not occur.

Uptake.

The comparative rates of uptake into bean leaf disks of the ^{14}C-carbonyl labeled compounds used in this study showed a slight progressive increase for the first 6 to 12 hours, and then a more rapid increase at the 24-hour treatment (Figure 14). These data also show a greater quantity of uptake of each compound than was sorbed by or penetrated through the isolated tomato fruit cuticle (Figures 4 and 7). The bean leaf is more pubescent, less waxy, and, therefore, more easily wetted. Jyung and Wittwer (53) suggested the overall process of foliar absorption and translocation of ions is metabolic. Certain protein synthesis inhibitors as chloramphenicol have been reported by Jyung and Wittwer (53) to reduce the amount of rubidium uptake in leaf cells. This means that leaf cell absorption of rubidium is closely parallel with protein synthesis, and that rubidium carriers may be proteinaceous. Chloramphenicol is a specific inhibitor in the transfer of amino acids from soluble ribonucleic acid (RNA) to protein. Thus, the increased uptake of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide,

and diphenylacetic acid may have been influenced by the metabolically active cell. Respiration did not occur in the isolated tomato fruit cuticles. Thus, lack of metabolic activity could explain the reduced amount of compound which was sorbed by or penetrated through the tomato cuticle.

It has been observed by Crafts (17), Foy (36), and Yamada et al. (109) that penetration and absorption of certain chemicals increased over veinal areas or along anticlinal cell walls. This increased uptake of compounds is often explained on the basis of thinner cuticle over veins. Yet Norris and Bukovac (72) reported thicker cuticle in veinal areas of pear leaves. Therefore, increased uptake of the four compounds used in this study cannot be conclusively explained on the basis of cuticular thickness over veins.

Franke (37) suggests that ectodesmata have a distinct role in penetration and uptake of foliar applied chemicals. Ectodesmata are predominantly found along anticlinal cell walls, guard cells, and above, beneath, and on both sides of veins. The occurrence of ectodesmata in the bean leaf disks most likely had a significant influence on the uptake of molecules of different molecular structure used in this study. After the bean leaf cuticle had become saturated with the exogenously applied compound (usually 12 to 24 hours), then movement into and from cell to cell may have involved an active uptake process. It is assumed that thinner cuticle on pubescence also permitted increased uptake of the organic compound applied to the bean leaf.

Influence of pH on Sorption, Penetration, and Uptake.

Sorption, penetration, and uptake of diphenylacetic acid through isolated tomato fruit cuticle and into green bean leaf disks were definitely influenced by the hydrogen ion concentration of the donor solution. As the hydrogen ion concentration increased, the amount of ^{14}C-carboxyl labeled compound detected in the bean leaf disk or tomato fruit cuticle increased. After 24 hours, the amount of diphenylacetic acid recovered from bean leaf disks was 21.67 percent at pH 3.0 and 15.44 percent at pH 5.0. The reduced uptake was even more pronounced when distilled water (pH 6.6) without a surfactant was used for the donor solution (Figure 15). This further substantiates the effect high pH levels have on uptake of diphenylacetic acid into bean leaf disks.

The pK of diphenylacetic acid is 3.94, and greater sorption, penetration, and uptake occurred at pH values below rather than above this pK. Yamada (106) reported that cuticles from green onion leaves have a pK value of 2.8, and those from ripe tomato fruits have a pK value of 3.2. Therefore, it would seem that pH regulated electrostatic repulsion and attraction phenomena affect cuticular sorption, penetration, and uptake of organic compounds. At pH values greater than 3.94 there would be more of the ionized species of diphenylacetic acid ($R\text{-}\overset{O}{\overset{\|}{C}}\text{-}O^-$). The free carboxyl groups of the cuticle would impart a negative charge at the surface interface at pH values greater than pK of the cuticle. Therefore, electrostatic repulsion of the negative charges occur. If the cuticular surface is negatively charged, we would expect hydrogen bonding of the carboximide group of diphenylacetic

acid to the surface. Thus, this would satisfy the negative charge of the cuticular surface and reduce or eliminate the repulsion effect on the molecule. Bukovac and Norris (11) found that napthaleneacetic acid (NAA) binding took place in cuticles of pear leaves at pH values higher than its pK of 4.2. This implies that mechanisms other than electrostatic binding are involved in chemical binding to cuticular surfaces of pear leaves. It is suggested that pH is not influencing the membrane, but has a primary influence upon the diphenylacetic acid molecule.

There was very little change in the amount of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, or diphenylacetamide which penetrated through or into tomato fruit cuticles or was taken up by the leaf disks over the range of pH solutions used in this study. These data agree with those reported by Bukovac and Norris (11) that more napthaleneacetic acid penetrated pear cuticles at pH levels below its pK (4.2) than above it. The same authors reported penetration of napthaleneacetamide was not influenced by the pH levels used (3.2 to 6.2). Albert (1), Crafts (18), and Swanson and Whitney (95) are in agreement with data reported herein which show that weak acids penetrated plant cells more readily in the undissociated form. Undissociated molecules are usually more lipid soluble and are more likely to partition into phases such as wax or other lipid components of the cuticle. The three diphenylacetamide molecules used in this study are considered to be almost totally undissociated at the levels of pH used, thus no change in lipid solubility was noted.

Influence of Surfactant on Sorption, Penetration, and Uptake.

In this investigation, it was found that there was greater penetration and uptake of all four molecules of different molecular structure when a nonionic surfactant was added to the donor solution (Figures 12 and 17). Sorption into isolated tomato fruit cuticles was reduced (Figure 6) with the addition of a surfactant. Tergitol 15-S-9 nonionic surfactant provided as much penetration of N,N-dimethyl-2,2-diphenylacetamide after 6 hours as the treatment without a surfactant provided after 24 hours (Figure 9). The increased penetration obtained with the addition of a surfactant is in agreement with data reported by Buchanan (10), Weintraub et al. (102), Dybing and Currier (28), Jansen et al. (51), Sargent and Blackman (83). Yet, Tuebner et al. (97) found that the nonionic surfactant Tween 20 was ineffective in enhancing the penetration of ^{32}P-labeled phosphorus into bean leaves. Comparing 0.1% concentration of nonionic surfactants in this study, Tergitol 15-S-9 (ethoxylated alcohol) gave the greater penetration increase of N,N-dimethyl-2,2-diphenylacetamide; Tween 20 (ethoxylated fatty acid ester), the least; and surfactant DF16 (ethoxylated + propylated alcohol), intermediate (Figure 9).

It is believed that the addition of a surfactant to aqueous solutions reduces the surface tension of a chemical and, therefore, permits better wetting of the leaf consequently increasing penetration and uptake. Boynton (7), Foy (35), and Wittwer et al. (104) reported that upper leaf surfaces without stomates had less solute uptake than lower leaf surfaces with stomates. Since the diameter of a stomatal

pore is small (3-5μ), it is doubtful that an aqueous solution could enter unless surface tension is reduced. Norman et al. (71) agreed that stomatal entry of aqueous solutions must depend upon a surfactant to reduce surface tension. Still, the entrance of a molecule into the substomatal cavity does not preclude cuticular penetration, as substomatal cavities are lined with a cuticle (Norris and Bukovac, 72).

The lowering of surface tension and stomatal penetration cannot sufficiently explain the results obtained from this investigation. Only the astomatous upper bean leaf surface was used in the uptake studies, and astomatous isolated tomato fruit cuticles were used in the sorption and penetration studies. This means that the influence of surfactant on increased uptake and penetration of the 4 compounds used in this study included something other than merely reducing surface tension. Reports by Freed and Montgomery (38), and Staniforth and Loomis (94) are in agreement with this conclusion. They noted that the major effect on herbicidal activity was found in the surfactant range above the critical micelle range (concentration at which maximum reduction of surface tension occurs). In these studies, there was an increase in penetration (Figure 12) of N,N-dimethyl-2,2-diphenylacetamide at concentrations higher (0.1 to 0.4%) than those reported above (0.01 to 0.1%). Therefore, it is suggested that the higher surfactant concentrations resulted in the formation of micelles with lipoidal properties which had the ability to solubilize cutin and wax. Furmidge (39) agreed that micelles of a surfactant can remove large areas of wax from leaf surfaces. Data (Figure 13) obtained from

this study suggested that cuticular wax definitely influenced penetration of the 4 compounds used. With the removal of epicuticular waxes, there was a 2- to 5-fold increase in penetration of all compounds through isolated tomato fruit cuticular membranes. Therefore, micelles of the nonionic surfactant Tergitol 15-S-9, by solubilization, may have removed some of the wax from bean leaf and tomato fruit cuticles. This permitted increased penetration and uptake of the 4 molecules of different molecular structure. The reduced sorption of the same 4 molecules (Figure 6) into isolated tomato fruit cuticles was, in turn, influenced by the solubilization and removal of wax from the cuticular membrane, and the molecules were allowed to diffuse through and be washed out more easily (less retention by the cuticle).

Non-polar (lipophilic) molecules, as those used in this study, may be solubilized in the micelles which consist of the hydrophilic portions of nonionic surfactants. Smith et al. (93) and Mysels (69) suggested that non-polar compound molecules can be incorporated in the center of the micelle. And, even if micelles are unable to cross membranes themselves, they may be helpful in transporting the non-polar compounds to the membrane and picking it up again on the other side.

In this study, it is assumed that cuticles were hydrated with the donor solution, swelled, and spread apart the embedded wax platelets. The nonionic surfactant could, therefore, be aligned on the surfaces of these embedded wax platelets in the cuticle. These wax platelets, plus the areas that may have been removed by solubilization, provided areas for the nonionic surfactant molecules to align

themselves with the hydrophilic part of the chain oriented away from the surfaces. This allowed for less binding or sorption of the different compounds into hydrated isolated tomato fruit or bean leaf cuticles. Smith et al. (93) suggested a similar procedure while explaining the toxicity of several water-soluble herbicides as paraquat, dalapon, and amitrole. He suggested that short-chain ethylene oxide molecules aligned themselves at the surfaces of cracks or fissures in the cuticle which permitted water-soluble herbicides to diffuse down by this route.

From the data herein, it appears that increased penetration of N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, diphenylacetamide, and diphenylacetic acid is the result of several factors, namely: 1) reduced surface tension, 2) alteration of the physio-chemical properties of the compounds used, 3) increased cuticle hydration, 4) alteration of the cuticle, and 5) formation of micelles with lipoidal properties which solubilize wax. Also, increased uptake by the bean leaf disks is influenced by the metabolic activity of the living cells.

SUMMARY

The sorption and penetration of diphenylacetic acid and its diphenylacetamide, N-methyl-2,2-diphenylacetamide, and N,N-dimethyl-2,2-diphenylacetamide analogs through isolated tomato fruit cuticles, and uptake of these compounds into green bean leaf disks were studied. Sorption, penetration, and uptake of these four different compounds increased with time. At first there was a gradual increase in penetration and uptake for the first 6 to 12 hours, and then a more rapid increase from 12 to 24 hours. There was usually a rapid increase in sorption over the first 3 to 6 hours, with a 2-fold increase noticed at the end of 24 hours. In general, the descending order of sorption, penetration, and/or uptake of the compounds was diphenylacetic acid, N,N-dimethyl-2,2-diphenylacetamide, N-methyl-2,2-diphenylacetamide, and diphenylacetamide.

Donor pH of diphenylacetic acid was a significant influence on sorption, penetration, and uptake. As the hydrogen ion concentration increased, the amount of ^{14}C-carboxyl labeled compound detected increased. After 24 hours, the amount of diphenylacetic acid recovered from bean leaf disks was 21.67 percent at pH 3.0, 20.04 percent at pH 4.0, and 15.44 percent at pH 5.0. As the pH level above pK 3.94 increased, the ionized ($R\text{-}\overset{O}{\overset{\|}{C}}\text{-}O^-$) species also increased, resulting in decreased sorption, penetration, and uptake of diphenylacetic acid.

In explanation, undissociated molecules are usually more lipid soluble and thus more likely to partition into phases such as wax or other lipid components of the cuticle. It is suggested also that pH did not influence the membrane but had a primary influence upon the molecule of the chemical used.

The three diphenylacetamide molecules used in this study were not significantly influenced by donor pH 3.0, 4.0, or 5.0. These molecules are considered to be almost totally undissociated at the levels of pH used, thus no change in lipid solubility occurred. Sorption, penetration, and uptake of the amide molecules increased as the polarity decreased.

In these investigations, there was greater penetration and uptake of the four compounds when a nonionic surfactant (0.1% to 0.4%) was added to the donor solution. The addition of Tergitol 15-S-9 surfactant resulted in as much penetration of a compound after 6 hours as the same treatment without a surfactant after 24 hours. As astomatous cuticles were used, increased penetration and uptake were not a result of reduced surface tension permitting the entrance of molecules into the stomatal cavity. It is suggested that increasing concentrations of surfactant above the critical micelle concentration resulted in the formation of micelles with lipoidal properties which have the ability to dissolve water-insoluble substances including the solubilization of cutin with the subsequent removal of wax. Non-polar (lipophilic) molecules such as those used in this study may have been solubilized in the center of

micelles which consist of the hydrophilic portions of nonionic surfactants. This would permit the apolar compounds to take a more polar route through the cuticle.

Sorption of each compound used in this study was reduced with the addition of a nonionic surfactant (0.1% to 0.4% v/v). The amount sorbed by the cuticles 24 hours after treatment with a surfactant was one and one-half to two and one-half times less than from donors without a surfactant. Nonionic surfactants as Tergitol 15-S-9 may align themselves at the surfaces of fissures or embedded wax platelets in the cuticle. This would permit less binding of the lipophilic compounds used in this study. Thus, molecules were more easily washed out of the cuticle.

In this study, cuticles were immersed in the donor solution; they may have swelled and spread apart the embedded wax platelets. This would have increased permeability of the cuticle to water and to the four compounds studied.

Metabolic activity did not influence sorption or penetration since respiration of isolated tomato fruit cuticular membranes does not occur. Yet, metabolic activity in the green bean leaf disks might explain the increased uptake of compounds used in this study.

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APPENDICES

Appendix A. Influence of donor pH on the sorption of different compounds into isolated tomato fruit cuticles, 24 hours treatment time.

Donor pH	Compound 1/ I	II	III	IV
		Percent Sorbed*		
3.0	3.7	3.2	2.5	6.8 a
4.0	4.2	3.1	2.4	4.9 b
5.0	4.5	3.9	2.4	1.8 c

* Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid

Appendix B. The concentration influence of the nonionic surfactant Tergitol 15-S-9 on the sorption of different compounds into tomato fruit cuticles, 24 hours after treatment.

Percent Surfactant	Compound 1/ I	II	III	IV
		Percent Sorbed*		
None	3.1 a	3.1 a	2.1 a	4.8 a
0.1	2.4 b	1.3 b	0.9 b	2.5 b
0.2	2.4 b	1.1 b	0.7 b	1.4 c
0.3	2.4 b	1.4 b	0.9 b	1.7 c
0.4	2.3 b	1.6 b	0.8 b	1.5 c

*Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid

Appendix C. Penetration of different compounds through tomato fruit cuticles as influenced by time.

Time in hours	Compound 1/ I	II	III	IV
		μMoles x 10^{-5}*		
3	0.25 c	1.63 c	0.08 b	2.19 c
6	2.61 c	2.64 bc	1.02 b	6.55 c
12	8.26 b	7.76 b	4.36 b	20.25 b
24	23.06 a	17.85 a	15.27 a	44.21 a

*Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid

Appendix D. The influence of donor pH on penetration, 24 hours after treatment.

Donor pH	Compound 1/ I	II	III	IV
		μMoles x 10^{-5}*		
3.0	17.51	17.86	10.26	41.07 a
4.0	16.79	18.06	12.55	37.09 a
5.0	17.54	15.19	12.15	30.15 b

*Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid

Appendix E. Influence of surfactant Tergitol 15-S-9 on uptake of N,N-dimethyl-2,2-diphenylacetamide into green bean leaf disks, 6 and 12 hours treatment time.

Percent Surfactant	Time in Hours 6	12
	Percent uptake*	
None	5.58 a	7.10 a
0.1	8.65 b	10.31 b
0.2	8.19 b	11.83 b
0.3	7.25 b	10.31 b
0.4	8.56 b	9.01 b

*Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid

Appendix F. Relation of molecular structure and pH to partitioning into different organic solvents from aqueous donor solutions, 24 hours after treatment.

Organic Solvent	pH	Compound 1/ I	II	III	IV
			Percent*		
Chloroform	4.0	99.16	94.98	95.21	94.47 b
	7.0	99.01	97.61	96.88	66.17 a
Oleic Acid	4.0	93.54	94.85	94.80	92.89 b
	7.0	98.51	95.77	97.50	97.79 b

* Numbers followed by unlike letters are significantly different, HSD (95%).

1/ Compounds:

I. N,N-dimethyl-2,2-diphenylacetamide
II. N-methyl-2,2-diphenylacetamide
III. Diphenylacetamide
IV. Diphenylacetic acid