ADJUVANT EFFECTS ON THE PLANT CUTICLE

By

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ABSTRACT

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Scanning electron microscopy (SEM) studies were undertaken to investigate adjuvant induced changes of cuticular components on cabbage (Brassica oleracea L. var. Copenhagen market), jimsonweed (Datura stramonium L. and velvetleaf (Abutilon theophrasti Medic.). The adjuvants used in these and all subsequent investigations were: crop oil concentrate[1] (a paraffin-petroleum oil blend), d'limonene[2] (1-methyl-4-isopropenylcyclohexane), soybean oil concentrate[3] (85:15 soybean oil to emulsifier ratio) and X-77[4] (alkylarylpolyoxyethylene glycols, free fatty acid and isopropanol mixture).

The SEM investigations showed greater cuticular wax disruption with increased adjuvant concentrations with all of the adjuvants on all three species. These examinations led to the proposal of an additional adjuvant mode of action. These compounds appear to solubilize the leaf outer surface taking cuticular wax components up into solution. Should a herbicide also be present with the adjuvant in solution, its penetration into the plant would be greatly facilitated.

In an attempt to quantify the observed cuticular alterations, a cuticular staining method was developed. Sudan IV lipophilic dye was impregnated onto adult cabbage leaf sections. The stained sections were immersed into various adjuvant solutions and the amount of extracted dye measured photometrically. The more concentrated the adjuvant solution, the more dye was extracted as indicated by greater absorbance. No significant difference was obtained among the adjuvants examined, however, significant differences were found among the concentrations.

The possibility that adjuvants might affect plant evapotranspiration (ET) was also examined. Evaluation of ET was obtained by instantaneous chamber measurement on adjuvant treated field grown soybeans. Chamber measurements were performed at two time intervals, averaging 9.3 and 14.4 min, after adjuvant appliction. Earlier readings indicated an increase in ET following adjuvant solution applications. Later readings indicated less water loss than before spray treatment. Thus, after 10 to 12 min, treated soybean plants were able to compensate for increased ET by decreasing stomatal aperture.

[1]Herbimax, Reg. TM of Union Carbide Corp.

[2]Cide-Kick, Reg. TM of JLB International Chemical Inc.

[3]American Soybean Association Standard

[4]X-77, Reg. TM of Chevron Chemical Co.

To Her Highness

A princess is my Judy,
And gracious, though blood royal,
My heart her throne, her kingdom,
And I a subject loyal.

Long shall you reign, my Judy,
My pet, my darling dearie.
For love, oh, little sweetheart,
Grows never old or weary.

ACKNOWLEDGEMENTS

The author wishes to extend his sincerest gratitude to Dr. William F. Meggitt, his major professor, whose guidance, support and constructive criticism were of invaluable aid throughout the tenure of this project.

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Finally, grateful recognition is made to JLB International Chemical, Inc., Chevron Chemical Co., Union Carbide Corp. and the American Soybean Association for supplying the adjuvants used in these studies.

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INTRODUCTION

In the field of weed science adjuvants are defined as substances which enhance herbicide effectiveness. This enhancement is generally defined to be brought about by the improvement of herbicide emulsifying, dispersing, spreading and wetting characteristics. While these factors undoubtedly contribute to the improvement of herbicide efficacy, the question remains, do adjuvants have other functions? For many years there has been speculation that adjuvants may also be able to dissolve or in some other way physically alter leaf cuticles. Should a herbicide be mixed with the adjuvant in solution, its penetration into the plant might be greatly facilitated.

Past research on this possible adjuvant mode of action has been limited. The use of the scanning electron microscope (SEM), the instrument of choice for examination of specimen surface features, did not come into widespread use until the 1960's. Early SEM work examining pesticides and/or adjuvants used a carbon replica technique which resulted in loss of many fine surface features. Subsequently, most SEM research in this area examined pesticide-adjuvant mixtures making it difficult to discern which chemical caused the observed effects. The effect of varying the amount of adjuvant present in solution was rarely examined as only one adjuvant concentration was generally used.

Our research sought to examine adjuvant effects on the plant both morphologically and physiologically. By means of the SEM careful documentation was made of adjuvant effects on the plant cuticle of three different species. Four adjuvants were examined at three different concentrations. A technique was also developed to quantify the observed alterations. This involved impregnating the cuticle with a lipophilic dye and then measuring the amount stripped off when dipped into various adjuvant solutions. Changes in plant transpiration after adjuvant application were also investigated in both laboratory and field studies.

CHAPTER 1

ADJUVANT EFFECTS ON THE PLANT CUTICLE:
I. ALTERATIONS OF CUTICULAR COMPONENTS BY ADJUVANTS

ABSTRACT

Laboratory and scanning electron microscopy (SEM) studies were undertaken to evaluate the influence of spray adjuvants on plant cuticular components. Aqueous adjuvant solutions of 0.1, 1.0 and 10.0% crop oil concentrate, d'limonene, soybean oil concentrate and X-77 were used in these investigations. Fifty ml of each preparation were irrigated over extracted cuticular components of velvetleaf (Abutilon theophrasti Medic.) and jimsonweed (Datura stramonium L.). The amount of cuticle alteration observed was visually rated. A trend of greater scouring with greater adjuvant concentrations was observed for all four adjuvants tested.

For the SEM studies, cabbage (Brassica oleracea capitita L. var. Copenhagen market), velvetleaf and jimsonweed leaf tissue was sprayed with the adjuvant solutions and prepared by a vapor fixation process for subsequent microscopic examination. This method facilitated excellent fine structure preservation and confirmed greater cuticular alteration with increased adjuvant concentrations with all of the

adjuvants on all three species.

These adjuvants appear to solubilize the plant outer surface taking epicuticular components up into solution. Should a herbicide also be present with the adjuvant in solution, its penetration into the plant would be greatly facilitated. Subsequently, as the solution dries or evaporates, the epicuticular materials are randomly redeposited on the plant surface.

INTRODUCTION

More than 80% of the herbicides marketed in the U.S.A. contain surfactants (18). In addition, many herbicide spray solutions have surfactants added to them before field application. The heavy reliance upon these important chemicals and their increased usage has led to many investigations of their properties over the years.

It has been known for more than 40 years (25) that surfactants increase the activity of organic herbicide sprays, but the exact mechanism of surfactant action remains obscure. It is generally conceded that the nature of herbicidal enhancement is closely associated with penetration (6, 11, 12). However, surfactants could influence the activity of herbicidal sprays at various sites. First, within the actual spray solution itself, second, on the cuticle surface; third, within the cuticular layers; fourth, upon or within the living cells underlying the cuticle and fifth, within plant tissues, possibly quite remote from treated areas (11).

Surfactants facilitate dispersal, wetting, spreading, emulsifying and other surface modifying properties, to enhance herbicidal action (5, 9, 14, 15, 17, 21, 22). Surfactants could accomplish these roles by their combined polar and apolar properties in the same molecule, thus providing compatible aqueous and lipoidal phases (10). These compounds also reduce the contact angle of spray droplets and the

surface tension. So by improving wetting, they may also favor both stomatal and cuticular penetration of the herbicide (2, 5, 6, 7, 8, 9, 10). Personal observations of glaucous cabbage leaf that had come in contact with a dilute surfactant solution indicated a total alteration of the leaf surface to a nonglaucous (shiny) condition. The possibility of surfactants also directly altering leaf surface components was, therefore, investigated.

Furmidge (12) first mentioned the possibility of surfactants solubilizing leaf surface waxes. He theorized that surfactants (at concentrations above the critical micelle concentration, generally > 0.1%) could remove large areas of leaf surface constituents, thereby facilitating penetration of the cuticle. This might account for the increased leaf damage seen in fruit trees with increasing surfactant concentrations.

The earliest significant study of leaf cuticular wax alteration by direct application of an insecticide, a herbicide and a surfactant was that of Wortmann (24). Using a carbon replica technique he conducted electron microscopy studies on the leaf surfaces of rape (Brassica napus L.), wheat (Triticum aestivum L.), and sugarbeet (Beta vulgaris L.) and related the poor wettability of the first two plants to the presence of submicroscopic wax structures. The wettability of sugarbeet leaf was ascribed to its smooth surface. Upon spraying rape or wheat leaves with parathion (diethyl-p-nitrophenyl-thiophosphoacid-ester), MCPA [(4-chloro-o-tolyl)oxy] acetic acid or Rapidnetzer[1], a

[1]Rapidnetzer, Reg. TM of BASF

wetting agent, the wax structures were changed and the wettability increased. Higher concentrations of parathion and MCPA lead to more severe destruction and further increased wettability. MCPA-ester and MCPA-salts had differing effects on the submicroscopic wax structures. The wettability after MCPA-ester treatment was greater than after MCPA-salt teatment. On rape leaf a regeneration of wax structures took place after Rapidnetzer spraying. However, on rape leaves treated with 0.03% parathion, no new fine structure regeneration was seen. Finally, no new structures appeared on wheat leaves after any of the aforementioned treatments.

Sands and Bachelard (20) investigated two eucalypt species examining the effects of herbicide-surfactant solutions on leaf surface morphology. Eucalyptus viminalis Labill. and E. polyanthemos Schau. leaf sections (15 by 15 mm, mid-vein-free, mounted on glass slides) were treated with the mixtures. Three solutions were prepared, each containing 0.2% unlabelled picloram[2] (4-amino-3,5,6-trichloropicolinic acid) in 0.05 M triethanolamine with: a) no surfactant, b) 0.7% Tween-20[3] (polyoxyethylene sorbitan monolaurate), and c) 0.7% Decol T/70 (triethanolamine dodecyl benzene sulphonate). A 6 mm length of 10 mm internal diameter vinyl tubing was adhered to each leaf segment with lanolin and 0.22 ml of one of each solution was applied through the tubing. Enclosed in petri dishes, the segments were left at 20°C under

[2]Tordon, Reg. TM of Dow Chemical Co.

[3]Tween 20, Reg. TM of ICI Americas Inc.

600 ft-candles of light for 8 hr. After the treatment period, the leaves were gently blotted dry and the treated leaf surfaces prepared for either transmission electron microscopy using a modified carbon replica technique or scanning electron microscopy.

Transmission micrographs showed that, in both species, both surfactants, but particularly Decol T/70, removed surface wax. The effects were more pronounced in the more waxy E. polyanthemos. Scanning micrographs displayed relatively little effect of surfactants on the wax of E. viminals except for a slight dissolved appearance of the wax after treatment with Decol T/70. In E. polyanthemos, Tween 20 removed a portion of the surface wax, however, Decol T/70 removed a considerably greater portion of the leaf surface components.

The possible influence of polysorbate surfactants having different HLB (Hydrophilic-Lipophillic Balance) values, and ethanol on the leaf ultra structure of cotton (Gossypium hirsutum L.) and prickly sida (Sida spinosa L.) was investigated by Takeno and Foy (23) using SEM. Both surfaces of cotton and prickly sida leaves were dehydrated when treated with 100% ethanol. The leaf waxes of cotton were severely eroded 72 hr after treatment with 1% (w/w) polysorbate surfactant (HLB 4.3) solution. The surfactant appeared to have an affinity for leaf waxes of cotton and to solubilize them. Cotton leaves treated with a polysorbate surfactant (HLB 8.0) solution became water stressed. Reticulate patterns observed on leaf surfaces treated with water-soluble surfactants (HLB 12.0 and 15.0) solutions may have been the polysorbate surfactants themselves, which had a low affinity for leaf

waxes. Leaf surfaces of prickly sida were less affected than those of cotton by polysorbate surfactants.

Chykaliuk (4) was the first to examine the effects of a surfactant at a concentration (5.0%) considerably above the critical micelle concentration. Using SEM, he viewed the adaxial surfaces of mature and immature field bindweed (Convolvulus arvensis L.) leaves 24 hr after treating one half the leaf with 2 µl droplets of d'limonene[4] (1-methyl-4-isopropenylcyclohexane) while leaving the other half as a control.

D'limonene solutions increased surface disruptions and induced increased ridging on both young and old field bindweed leaf specimens. Chykaliuk concluded that d'limonene caused the epicuticular wax to form into ridges through an undetermined mechanism or stimulated the excretion of new wax in ridge-like formations. Barring additional deposition of new wax onto the leaf surface, thinner waxy areas would exist which should be less of a physical barrier to herbicide penetration.

Careful examination of the micrographs that showed ridging, reveal them to be very reminiscent of leaf folds and shrinkage typically associated with water loss. While evidence for surface disruption exists, the ridges are almost certainly folds due to increased water loss. The disruption may account for the increase in loss of leaf moisture.

Recently, both Kuzych and Meggitt (16) and Bukovac et al. (1) used SEM to examine the leaves of plant species after treatment with

[4]Cide-Kick, Reg. TM of JLB International Chemical Inc.

a number of different adjuvants. In the former study, cabbage leaves were dipped for 3 sec halfway in adjuvant solutions to obtain an 'edge', whereby both treated and untreated areas could be viewed simultaneously. Adjuvants used were a crop oil concentrate[5] (paraffin-petroleum oil blend) , d'limonene and X-77[6] (alkylarylpolyoxyethylene glycols, free fatty acid and isopropanol mixture) at three different concentrations (0.1, 1.0 and 10.0%). In general, the greatest surface disruption was seen to occur with X-77 followed by the crop oil concentrate and then d'limonene. Increasing adjuvant concentrations produced noticeable increases in surface morphological disruptions with all three solutions.

Bukovac et al. (1) studied the effects of selected surfactants on the leaf surface of broccoli (Brassica oleracea italica Plenck), cabbage and pear (Pyrus communis L. var. Bradford), at surfactant concentrations of 0.1 to 1.0% v/v. Droplets of 0.5 or 1.0 µl were applied by means of a syringe or as a spray (about 175 µm) with a spinning disc droplet generator.

Film-like residues were observed over droplet areas after evaporation of aqueous solutions of Tween 20, Regulaid[7] (polyoxyethylenepolypropoxypropanol alkyl 2-ethoxyethanol) and Triton B-1956[8] (modified phthalic glycerol alkyl resin). Surface fine structure was not seen to be altered within the droplet area. Subtle

[5]Herbimax, Reg. TM of Union Carbide Corp.

[6]X-77, Reg. TM of Chevron Chemical Co.

[7]Regulaid, Reg. TM of Colloidal Products Corp.

[8]Triton B-1956, Reg. TM of Rohm & Haas Co.

deformation was noted with X-77, d'limonene and Triton CS-7[9] (blend of alkylaryl polyethoxylate and sodium salt of alkyl sulfonated alkylate). Epicuticular wax fine-structural deformation was observed with Vatsol OT[10] (dioctyl ester of sodium sulfosuccinic acid), Hyamine 2389[11] (40% methyl dodecyl benzyl trimethyl ammonium chloride, 10% methyl dodecyl xylene bis trimethyl ammonium chloride, 50% HOH) and Triton X-100[12] (octylphenoxy polyethoxy ethanol). No evidence was seen of large areas being solubilized or markedly deformed.

Finally, an investigation by Chow and MacGregor (3) examined effects of the herbicide sethoxydim[13] (2-[1-(ethoxyimino) butyl]-5-[2-ethylthio) propyl]-3-hydroxy-2-cyclohexene-1-one) at 0.1 kg/ha on wild oat (Avena fatua L.) leaves with and without the surfactant Atplus 411 F[14] (17% Atplus 300 F (80% polyoxyethylene sorbitan fatty acid ester) in crop oil) at 0.5%. These leaves are covered with a dense network of small plate-like crystals of epicuticular wax. Treatment with sethoxydim alone exhibited distinct circular areas within which the wax structures had been altered. Addition of surfactant to the herbicide gave irregularly shaped affected areas on leaf surfaces, presumably due to increased spreading.

[9]Triton CS-7, Reg. TM of Rohm & Haas Co.

[10]Vatsol OT, Reg. TM of American Cyanamid Co.

[11]Hyamine 2389, Reg. TM of Rohm & Haas Co.

[12]Triton X-100, Reg. TM of Rohm & Haas Co.

[13]Poast, Reg. TM of BASF Wyandotte Corp.

[14]Atplus 411 F, Reg. TM of ICI America Inc.

Although not specifically mentioned, in the micrographs presenting treatment with the herbicide plus surfactant less remaining crystal structure was shown then with the herbicide alone.

The adjuvant research delineated in this paper is a continuation of that described earlier (16). The investigation was broadened to include the four commercial adjuvants described in Table 1. The objectives of the investigation were: 1) to determine if these adjuvants in any way alter the cuticular components of velvetleaf or jimsonweed; 2) to verify the trend of greater cuticular disruption with increasing adjuvant concentration (16) and 3) to visualize and document any adjuvant induced changes through use of the scanning electron microscope.

MATERIALS AND METHODS

Cuticular components from adult velvetleaf or jimsonweed leaves were extracted by means of immersion in 75 ml chloroform baths (aluminum weighing trays). After the chloroform had evaporated, various 50 ml aqueous adjuvant solutions were allowed to drain over areas of deposited cuticular material from a burette suspended 2 ml above the trays. Examinations were made using the four adjuvants each prepared at concentrations of 0.1, 1.0 and 10.0% v/v. The disruption was rated visually on a scale of 0 to 3 with 0 indicating no change, 1 - a slight amount of scoring, 2 - more advanced disruption and 3 - moderate alteration.

Adjuvant solutions were seen to strip deposited cuticular materials. In order to further confirm and visualize this phenomenon, scanning electron microscopy studies were undertaken on treated areas and subsequently on intact plant leaves.

SEM Specimen Preparation. Square sections (8 by 8 mm) were cut from the 'edge' of treated areas of the pie tins allowing for simultaneous viewing of treated and untreated regions and mounted on aluminum stubs. The stubs were sealed in a glass container, the center of which contained a receptacle with about 10 cc of 2% osmium. The volatile osmium fumes, within the container, fixed the cuticular components within 18 hr. This preparatory procedure is commonly termed the osmium fume or vapor fixation method (19).

Fixed cuticular materials were subsequently gold coated (approximately 200 nm) in a Film-Vac Mini sputter coater and observed on an International Scientific Instruments Super III scanning electron microscope operating at 15 kV. Scanning electron micrographs were taken on Polaroid type 667 positive only film.

Plant culture. The SEM leaf investigations were performed on three broadleaved plant species: cabbage, velvetleaf and jimsonweed. Cabbage, with its prolific and delicate surface features, served as an excellent indicator species for any induced alterations. Seedlings of cabbage were greenhouse grown in vermiculite until the two-leaf stage and then transplanted into 473-cc food ('cottage cheese') containers with a soil mixture containing one-third sand, one-third peat and one-third clay loam. Velvetleaf and jimsonweed plants growing in a silt

Table 1: Adjuvants used in studies.

Common Name	Trade Name & Manufacturer	Chemical Composition
Crop Oil Concentrate (C.O.C.)	Herbimax, Reg. TM of Union Carbide Corp.	A paraffin-petroleum oil blend consisting of: 80 percent Petroleum hydrocarbons 16 percent Surfactant blend 4 percent Formulation aids
d'limonene	Cide-Kick Reg. TM of JLB International Chemical, Inc.	D-(+)-limonene is a monoturpene. Its structure is: 1-methyl-4-isopropenylcyclohexane. An unknown percentage of emulsifiers are also added. It is considered to be a non-ionic surfactant.
Soybean Oil Concentrate (S.O.C.)	American Soybean Association Standard	85 percent Soybean oil 15 percent Emulsifiers Typical soybean oil composition is: 50-60 percent Linoleic acid 20-30 percent Oleic acid 5-11 percent Linolenic acid 6-10 percent Palmitic acid 2-5 percent Stearic acid
X-77	X-77, Reg. TM of Chevron Chemical Co.	Alkylarylpolyoxyethylene glycols, free fatty acid and isopropanol mixture. Classified as a non-ionic surfactant.

loam field were collected, potted and brought to the greenhouse to acclimate for several days before sampling for study. Specimen leaves treated and examined were all approximately 2-months-old for all three species.

Adjuvant application. Aqueous adjuvant solutions were prepared at three different concentrations increasing logarithmically from 0.1 to 1.0 to 10.0% v/v. The range between the first two figures represents the range commonly used in present day foliar applications. The latter concentration is one that had not been previously examined by other researchers. Solutions were applied by means of a flat fan nozzle (Tee Jet 8001 E) delivering 325 ml/min. at the 15 kPa (22 psi). Leaves passed 20 cm (8 in) under the nozzle and were covered in such a way as to insure that only half of the leaf was wetted. In this way an 'edge' was obtained whereby both treated and untreated areas could later be viewed simultaneously under the SEM.

Tissue preparation. Preliminary studies previously reported (16) had been carried out with tissue dipped in adjuvant solutions and fixed with 4% glutaraldehyde, postfixed with 2% osmium, dehydrated in alcohol and critical point dried. Although adjuvant induced modification of leaf surfaces were observed, it was felt that too much surface detail was being lost in the alcohol dehydration. Therefore, subsequent tissue preparation was carried out as follows.

After leaves had been sprayed, rectangular leaf fragments (5 by 7 mm) were excised from 'edge' areas on one side of the midvein near the leaf center. These treated sections were mounted abaxial surface down on aluminum stubs and prepared by the vapor fixation method previously described. This entire process took approximately 10 min from the time the leaves were first sprayed. Tissue coating, viewing and photography were performed in an identical manner to that previously mentioned. Although the vapor fixation method caused some tissue wrinkling, and leaf folding, the fine structure preservation was excellent (Figs 1-6). Since this preparatory procedure did not vary, effects of different adjuvant solutions could be compared.

Adjuvant segregation from solution. Prior to examination of fixed tissue, carbon wafer discs dabbed with 1.0% or 10.0% v/v aqueous solutions of each of the four adjuvants were observed under the SEM. This procedure was initiated to determine if the adjuvants in solution separated out and if so, to determine the extent of this separation and to become familiar with any depositional patterns. One percent treatments showed virtually no difference compared to controls except for a few scattered areas that might have represented slight adjuvant deposition (Figs 7 and 8). Ten percent treatments demonstrated noticeable segregation and deposition with all four adjuvants tested (Fig 9). In all cases, areas of the platy disc surface were draped with a film of adjuvant residue. These coatings displayed similar patterns among all of the surfactants.

Figure 1: Scanning electron micrographs of 2-month-old cabbage leaf (adaxial surface) prepared by immersions in 4% glutaraldehyde, 2% osmium and an alcohol dehydration series. The many tissue baths cause severe cuticular erosion (compare to Figure 2). Views A and B are magnified 800X; C, 2700X and D, 3400X. Bar = 5 μm.

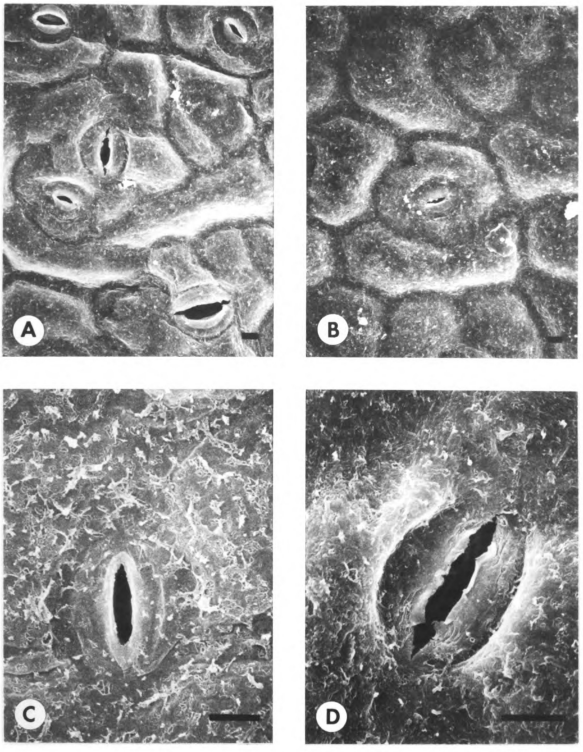
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Figure 2: Scanning electron micrographs of 2-month-old cabbage leaf (adaxial surface) prepared by the vapor fixation method. Since no chemical immersion is involved in this technique, the preservation of fine epicuticular structure is excellent. Characteristic wax rodlets fused in some areas into platelet-like structures are abundantly evident. View A is magnified 1000X, B and C are enlarged 2000X and D, 4000X. Bar = 5 μm.

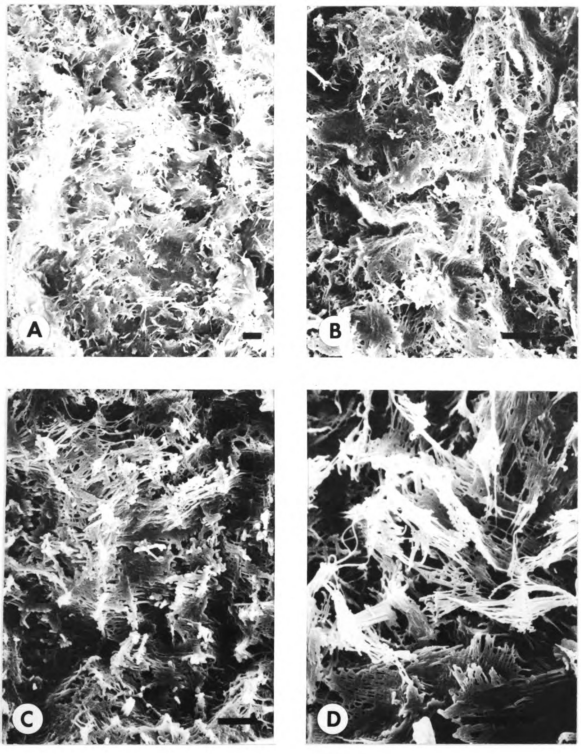
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Figure 3: Scanning electron micrographs of 8-week-old jimsonweed leaf (adaxial surface) prepared by immersions in 4% glutaraldehyde, 2% osmium and an alcohol dehydration series. Since this species contains a relatively thin and smooth cuticle any alterations induced by the fixation method are not readily visible. Fine preservation of epidermal cell walls and trichomes is evident. View A is enlarged 100X; B, 200X; C, 660X and D, 700X. Bar= 25 µm.

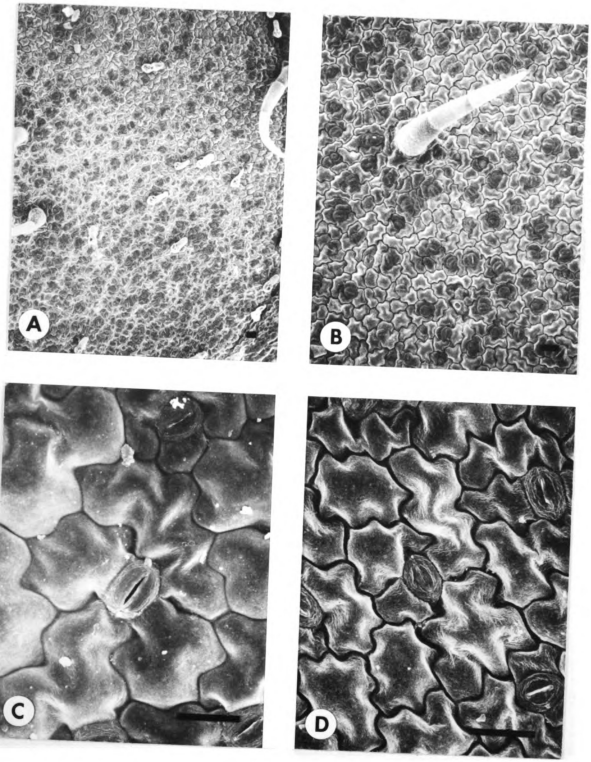
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Figure 4: Scanning electron micrographs of 8-week-old jimsonweed leaf (adaxial surface) prepared by the vapor fixation method. The cuticle consists of a smooth, thin layer overlying the epidermal cell walls. This fixation method generally results in periclinal cell walls that become depressed or sunken, (anticlinal walls stand out as 'ridges'). Thinner cuticle and cell walls (compared to cabbage) account for this phenomenon. Views A and B are enlarged 400X; C, 700X and D, 1000X. Bar = 25 μm.

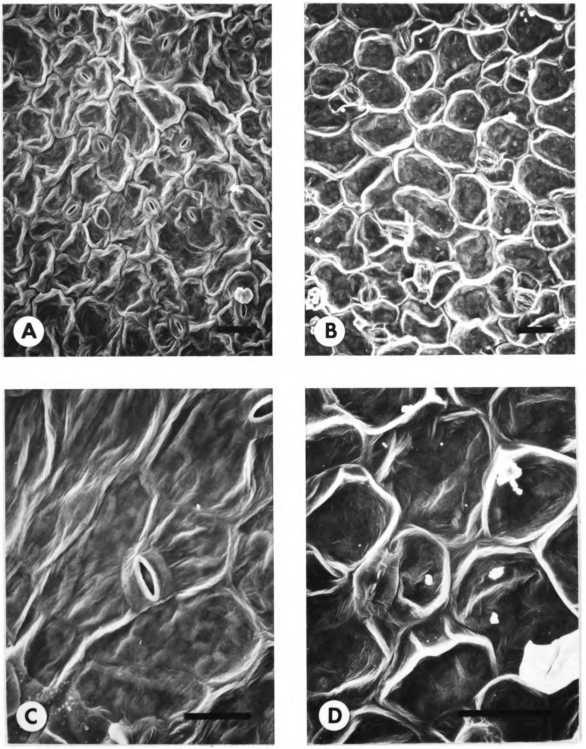
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Figure 5: Scanning electron micrographs of 8-week-old velvetleaf leaf (adaxial surface) prepared by immersions in 4% glutaraldehyde, 2% osmium and an alcohol dehydration series. Possible induced cuticular alterations due to fixation are not apparent as this species too does not display a prominent cuticle. Fine preservation of epidermal cell walls and the various types of trichomes may be seen. View A is magnified 200X; B, 400X and both C and D, 700X. Bar = 25 μm.

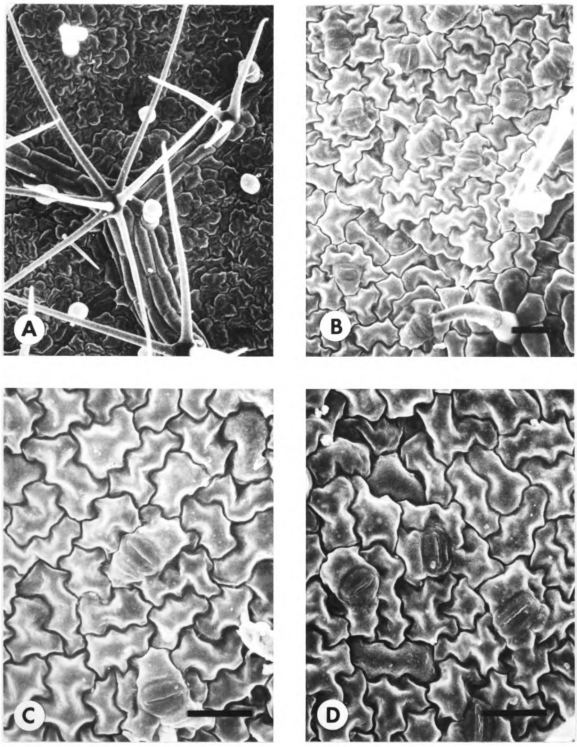
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Figure 6: Scanning electron micrographs of 8-week-old velvetleaf leaf (adaxial surface) prepared by the vapor fixation method. Although this species has a thicker cuticle than jimsonweed, it is still very smooth and difficult to visualize. The periclinal cell wall inversions brought about by this fixation method are not as severe or prominent as those seen in jimsonweed. View A is magnified 320X; B, 360X; C, 380X and D, 650X. Bar= 25 µm.

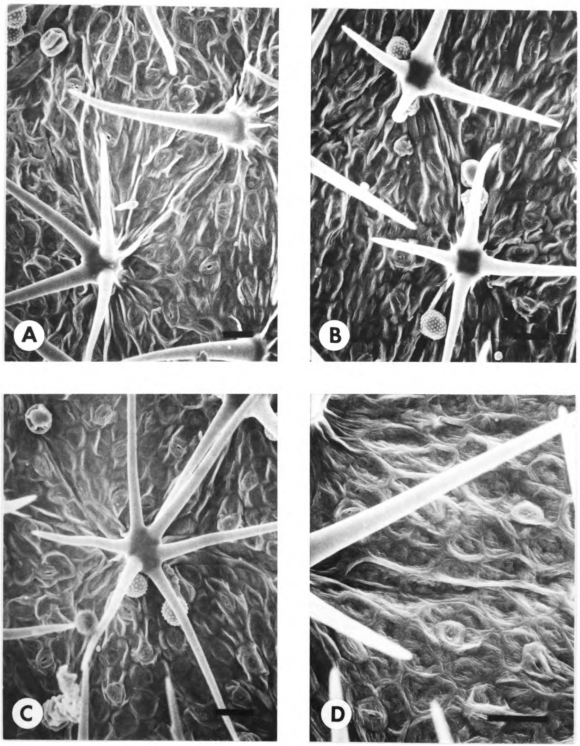
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Figure 7: Scanning electron micrographs showing areas of untreated carbon wafer disc. All views 3000X. Bar = 5 μm.

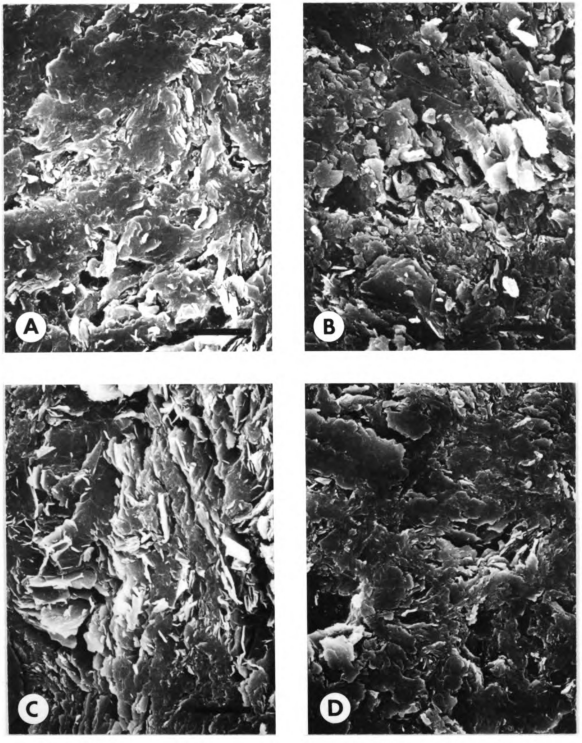
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Figure 8: Scanning electron microscopy views of carbon wafer discs dabbed with 1.0% v/v aqueous adjuvant solutions. Little change can be seen from the previous figure showing untreated aeas. View A was treated with crop oil concentrate, B with d'limonene, C with soybean oil concentrate and D with X-77. All micrographs 3000X. Bar = 5 μm.

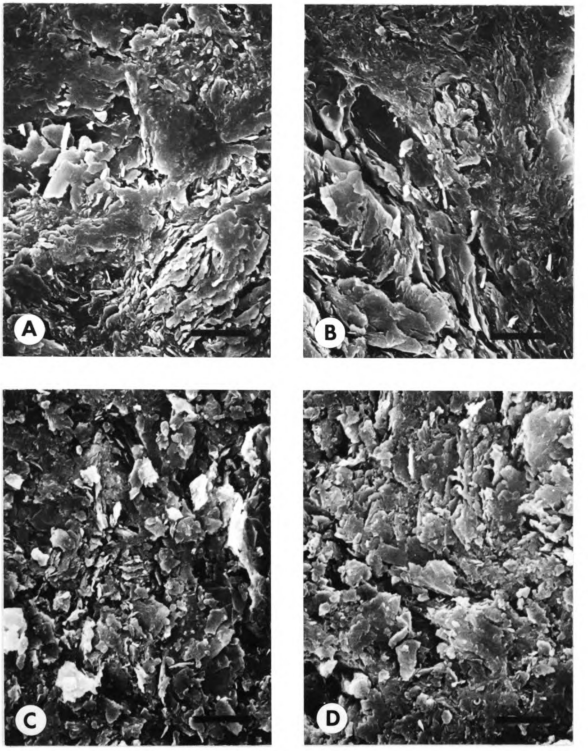
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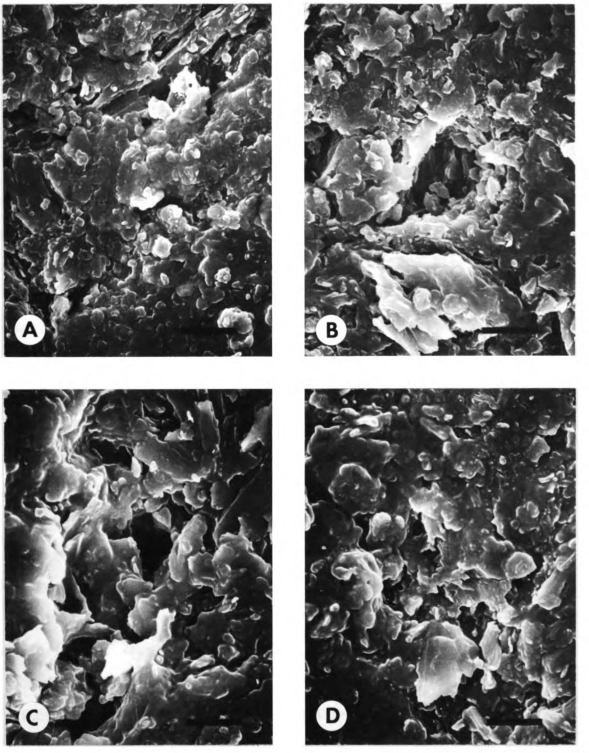
Figure 9: Scanning electron microscopy views of carbon wafer discs dabbed with 10.0% v/v aqueous adjuvant solutions. Some of the distinctive coatings scattered over treated areas may be observed. View A was treated with crop oil concentrate, B with d'limonene, C with soybean oil concentrate and D with X-77. All micrographs 3000X. Bar = 5 µm.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RESULTS AND DISCUSSION

Extracted Cuticular Components. Adjuvant irrigation experiments performed on extracted cuticular components of velvetleaf and jimsonweed demonstrated noticeable alterations of the deposited materials (Table 2). A general trend of increasing disruption with increasing adjuvant concentration is evident (Figs 10, 11). It should be noted that although some irrigation experiments did not demonstrate changes visually, when viewed at higher magnification, invariably alterations were discerned. A good example is 0.1% crop oil concentrate whose visual rating on velvetleaf was zero (i.e. no change). However, when magnified 400X distinctive topographical stripping was noted following treatment at this concentration (Fig 11A).

Cabbage. Alterations of the delicate rods and platelets found on cabbage served as excellent guides to the types and amount of changes that could be produced by adjuvant solutions on leaves. Applied preparations of 0.1% adjuvant concentrations produced some diminution and fusion of rodlets and platelets (Figs 12A-15A, 16D). The degree of alteration on a leaf could vary, however, from very subtle to quite distinct. For all of the adjuvants except X-77, the amount of observed disruption seemed to be about the same. X-77 generally produced more

Table 2: Visual ratings of changes on extracted cuticular components of velvetleaf and jimsonweed by treatment with various adjuvant solutions (average of two replications).

Treatment	Rating[a]	
	Velvetleaf	Jimsonweed
Distilled water	0	0
0.1% Crop oil concentrate	0	0
1.0% Crop oil concentrate	1	1.5
10.0% Crop oil concentrate	2.5	2
0.1% d'limonene	1	0.5
1.0% d'limonene	2	1.5
10.0% d'limonene	2	1.5
0.1% Soybean oil concentrate	0	0
1.0% Soybean oil concentrate	1	0.5
10.0% Soybean oil concentrate	2	2
0.1% X-77	1	1
1.0% X-77	1.5	2
10.0% X-77	2	2.5

[a]Rating scale: 0 - no change observed

1 - careful observation required to distinguish alterations

2 - more distinct scoring visualized with greater ease

3 - fair amount of cuticular components have been stripped, changes easily observed

Figure 10: Scanning electron micrographs of extracted cuticular components of velvetleaf treated with (A) 1.0% d'limonene, (B) 1.0% soybean oil concentrate, (C) 1.0% X-77 and (D) distilled water. Note the extensive alterations that have occurred in each of the adjuvant treatments. All views 400X. Bar = 25 μm.

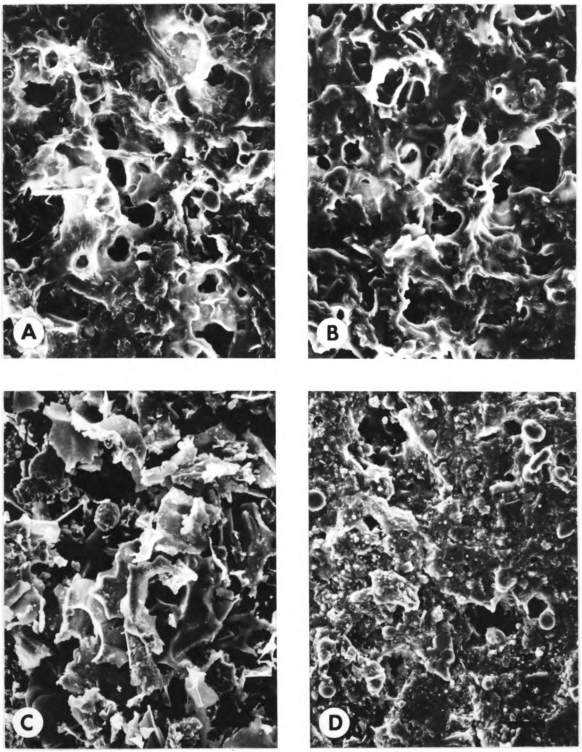
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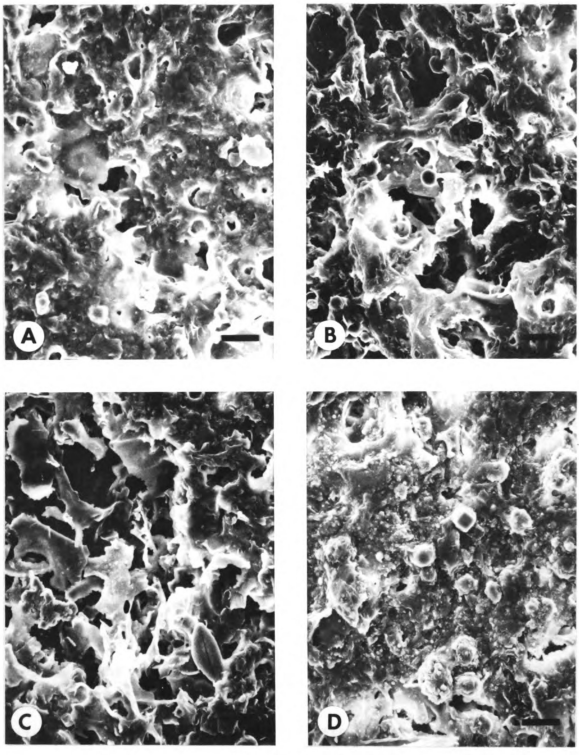
Figure 11: Scanning electron micrographs of extracted cuticular components of velvetleaf treated with (A) 0.1% crop oil concentrate (C.O.C.), (B) 1.0% C.O.C., (C) 10.0% C.O.C. and (D) distilled water. More concentrated treatments display more stripping; the 10.0% treatment, in some areas, stripped deposited materials to the bottom of the container (upper left-hand corner). All views 400X. Bar = 25μm.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extensive changes, of a blocky or globular nature, with most all of the original features drastically transformed (Figs 15A-C, 16D). This pattern of greater alteration with X-77 generally held true at all concentrations.

Applications of 1.0% adjuvant solutions displayed more advanced tissue surface deformation (Figs 12B-15B, 17). Seen here for the first time were areas of dissolved cuticular component redeposition (Figs 15B and 17C). The type of alterations observed with these sprayed treatments in many cases closely resembled those seen on tissue dipped into surfactant solutions and previously reported (16).

One of the methods by which adjuvants may facilitate herbicidal entry into plants, in addition to those commonly cited such as increasing spreading, wetting and sticking, is as follows. The adjuvant treatments examined appear to dissolve the plant epicuticle, at least partially, in areas where contact has occurred. Higher concentrations, 1.0 and 10.0% seem to demonstrate more dissolution. Epicuticular components are likely taken up into the applied solution, leaving behind a thinned barrier. If the solution also contained a herbicide, penetration into the plant would be greatly facilitated. Subsequently, as the solution dries, suspended epicuticular materials are randomly redeposited on the plant surface in amorphous aggregates.

Some of the 'epicuticular' redeposition visualized with higher adjuvant concentration treatments, 1.0 and 10.0%, may be adjuvant settling out and aggregation. However, the amount of deposition seen cannot be solely accounted for by adjuvant segregation.

Figure 12: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with (A) 0.1% crop oil concentrate (C.O.C.), (B) 1.0% C.O.C., (C) 10.0% C.O.C. and (D) distilled water. Note the progressive deformation in fine structure with increasing adjuvant concentration. Views A and D are magnified 3500X; B and C are enlarged 4000X. Bar = 5μm.

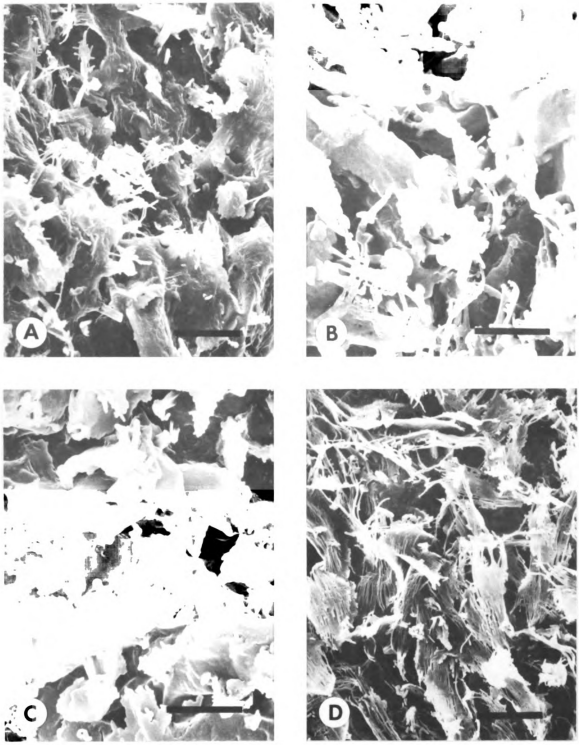
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Figure 13: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with (A) 0.1% d'limonene, (B) 1.0% d'limonene, (C) 10.0% d'limonene and (D) distilled water. Progressive alteration in fine surface structure with increasing adjuvant concentration is readily apparent. Views A and D are enlarged 2800X; B, 2000X and C, 3000X. Bar = 5 μm.

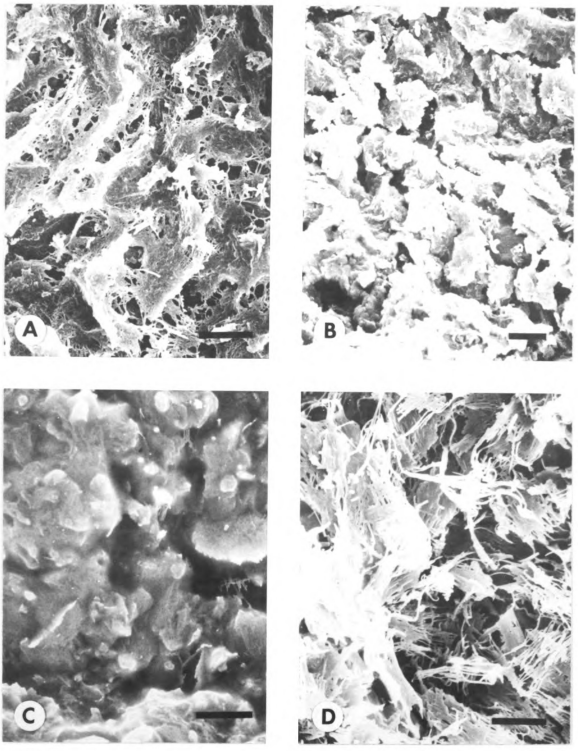
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Figure 14: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with (A) 0.1% soybean oil concentrate (S.O.C.), (B) 1.0% S.O.C., (C) 10.0% S.O.C. and distilled water. Diminution of delicate surface features with increasing adjuvant concentration can be discerned. Views A, B and D are shown 3000X; C, 2000X. Bar = 5 μm.

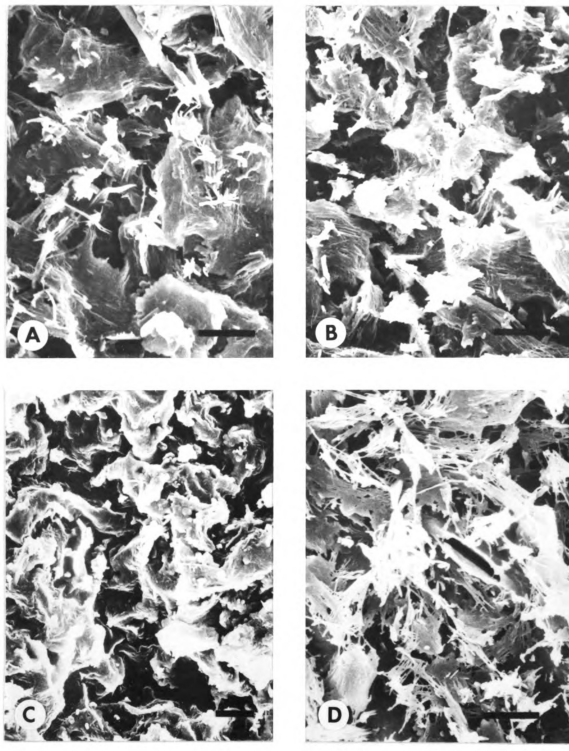
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Figure 15: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with (A) 0.1% X-77; (B) 1.0% X-77; (C) 10.0% X-77 and (D) distilled water. Characteristic epicuticular structures become increasingly more distorted with higher adjuvant concentrations. Views A through C are 2000X; D, 2400X. Bar = 5 µm.

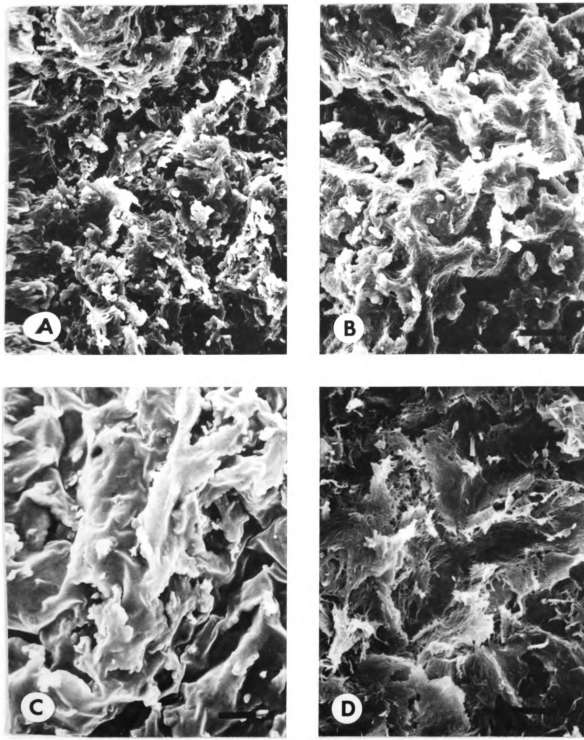
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Figure 16: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with 0.1% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) with X-77. Noticeable fine structure alteration can be seen with all four treatments; changes seem to be especially prominent with X-77 where little of the original features remain. View A is magnified 1400X; B, 1000X; C, 1600X and D, 1000X. Bar = 5 μm.

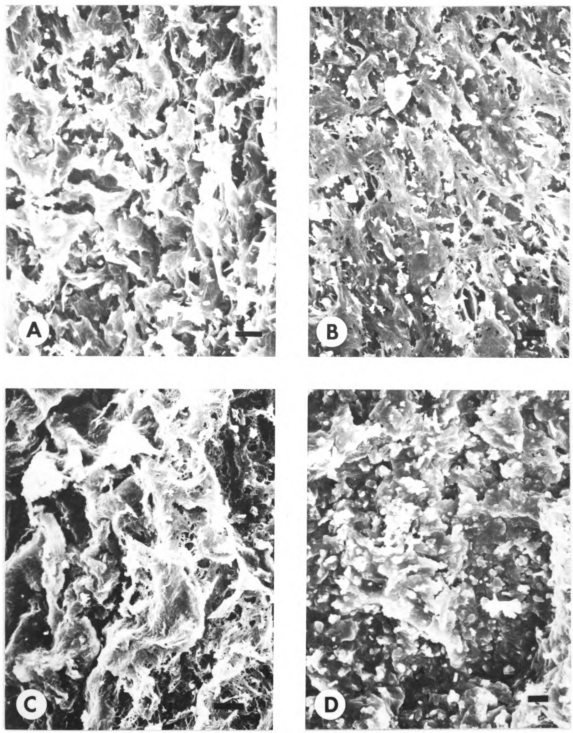
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Figure 17: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with 1.0% aqueous adjuvant solutions of (A) crop oil concentrate (C.O.C.), (B) d'limonene, (C) soybean oil concentrate (S.O.C.) and (D) X-77. Much, if not most, of the original fine surface features have been obliterated with all of the treatments. S.O.C. treatment shows an area of droplet impact with resulting cuticlar wax dissolution and dissolved component(s) redeposition. View A is enlarged 1600X; views B and D 1000X and C, 700X. Bar = 5 μm.

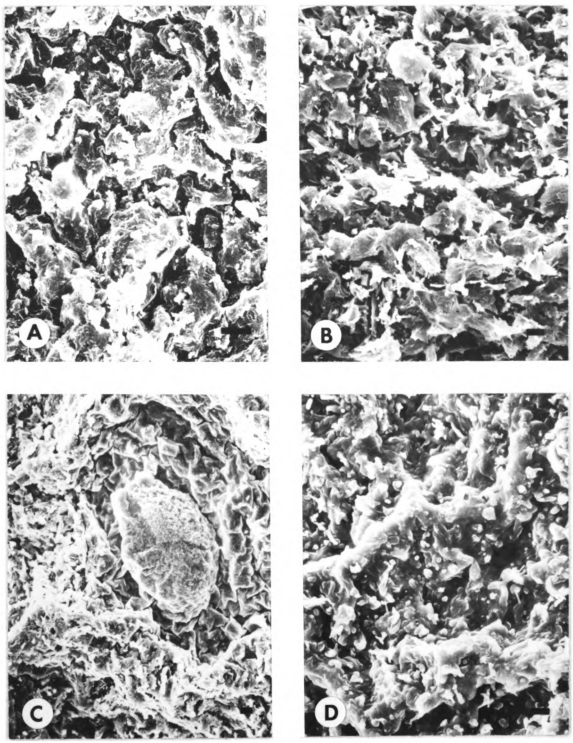
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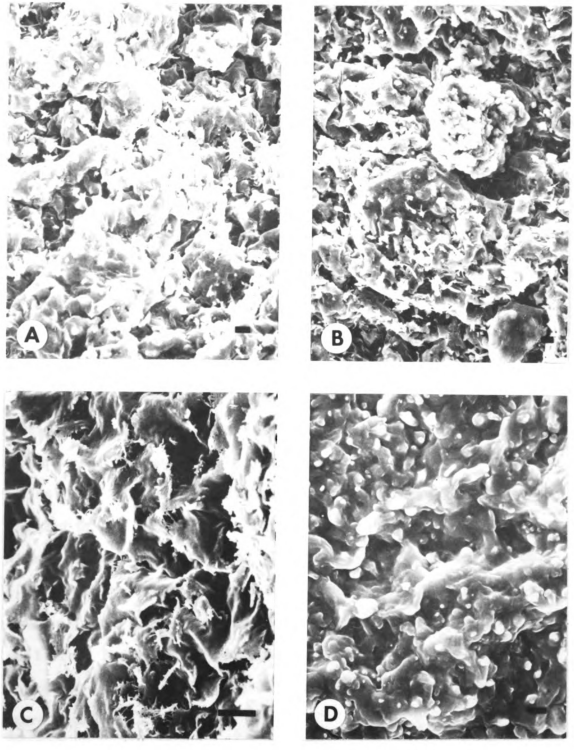
Figure 18: Scanning electron micrographs of 2-month-old cabbage leaves (adaxial surfaces) treated with 10.0% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Extensive alteration of original dendritic structures can be seen to have taken place with all treatments. D'limonene treatment shows aggregates of formerly dissolved cuticular materials redeposited on leaf surface. View A is shown 800X; B, 700X; C, 2000X and D, 1000X. Bar = 5 µm.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Velvetleaf. The cuticular surface of velvetleaf did not display any epicuticular wax structures nor was it as thick or prominent as that seen on cabbage. It consisted of a thin coating adhering to and molding itself over the plant surface. Cuticular changes after adjuvant treatment were not readily observed under the scanning electron microscope. However, dissolved and redeposited cuticular waxes were easily located not only on the leaf surfaces themselves, but also suspended on the many trichomes which give this species its particular texture. The previously mentioned hypothesis, of the possible dissolution of plant epicuticle by adjuvants, was given a substantiating boost by the many observations of affixed materials on trichomes. Since these suspended structures were observed with all adjuvants at all concentrations, they apparently were of cuticular wax composition and not adjuvant segregation (Figs 19, 20 and 23).

Velvetleaf, similar to cabbage, showed more leaf surface disruption with more concentrated adjuvant treatments (Figs 19-25). On occasion more concentrated adjuvant applications resulted in periclinal wall collapse becoming more severe (Fig 20) possibly due to an increase in leaf moisture loss with greater cuticular wax disruption at higher adjuvant concentration. The more extensive periclinal cell wall disruption resembled previous observations made on velvetleaf and jimsonweed leaves dipped for 5 seconds in chloroform, an effective wax solubilizer. These showed dramatic and extensive periclinal wall shrinkage and collapse (Figure 26) due to the dissolution of the moisture retaining barrier.

Figure 19: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with (A) 0.1% crop oil concentrate (C.O.C.), (B) 1.0% C.O.C., (C) 10.0% C.O.C. and (D) distilled water. Larger areas of redeposited cuticular wax material are evident with more concentrated adjuvant treatments. View C is shown 700X, all other views are 400X. Bar = 25 μm.

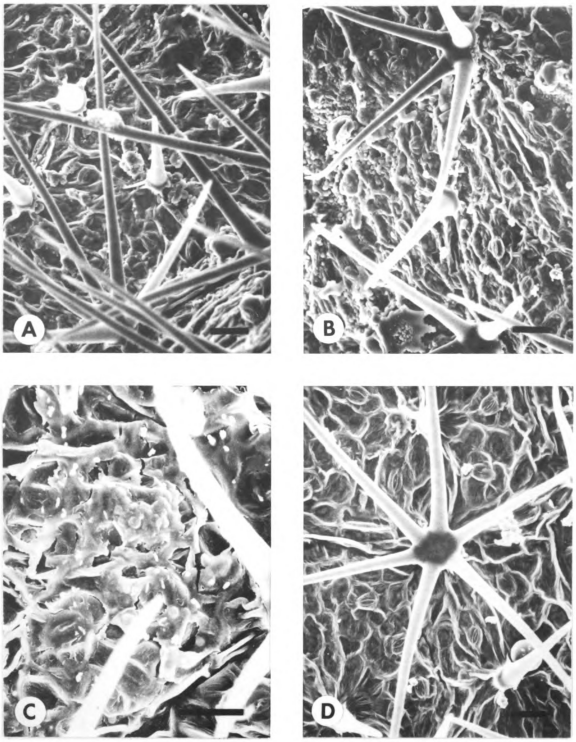
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Figure 20: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with (A) 0.1% d'limonene, (B) 1.0% d'limonene, (C) 10.0% d'limonene and (D) distilled water. Progressively more extensive areas of cuticle wax redeposition (both on the leaf surface and trichomes) and periclinal wall collapse are seen with greater surfactant concentration. All views are 400X. Bar = 25 µm.

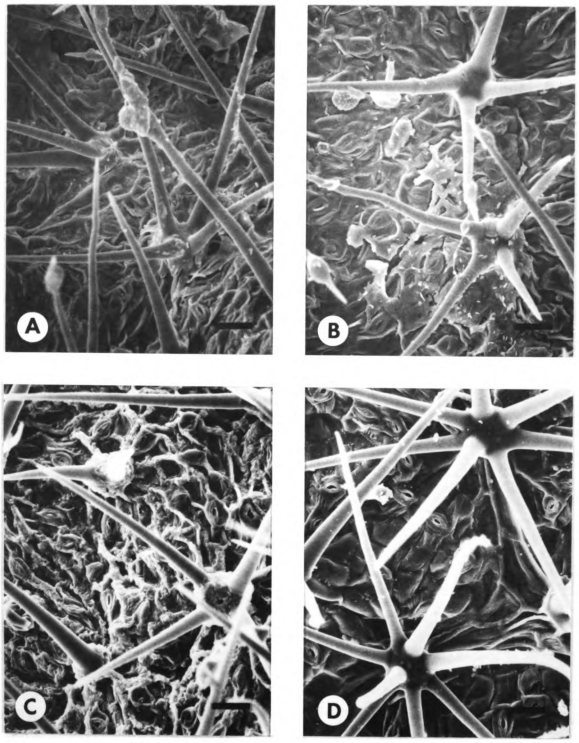
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Figure 21: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with (A) 0.1% soybean oil concentrate (S.O.C.), (B) 1.0% S.O.C., (C) 10.0% S.O.C. and (D) distilled water. As treatment concentrations increase, so too do patterns of cuticular wax redeposition. All views 400X. Bar = 25 μm.

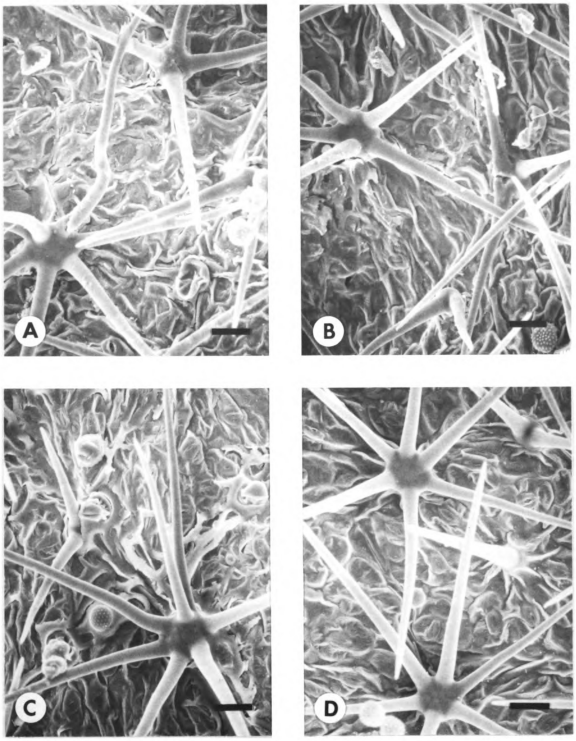
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Figure 22: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with (A) 0.1% X-77, (B) 1.0% X-77, (C) 10.0% X-77 and (D) distilled water. Patterns of cuticle wax redeposition become more prominent with greater surfactant concentrations. All views 650X. Bar = 25 μm.

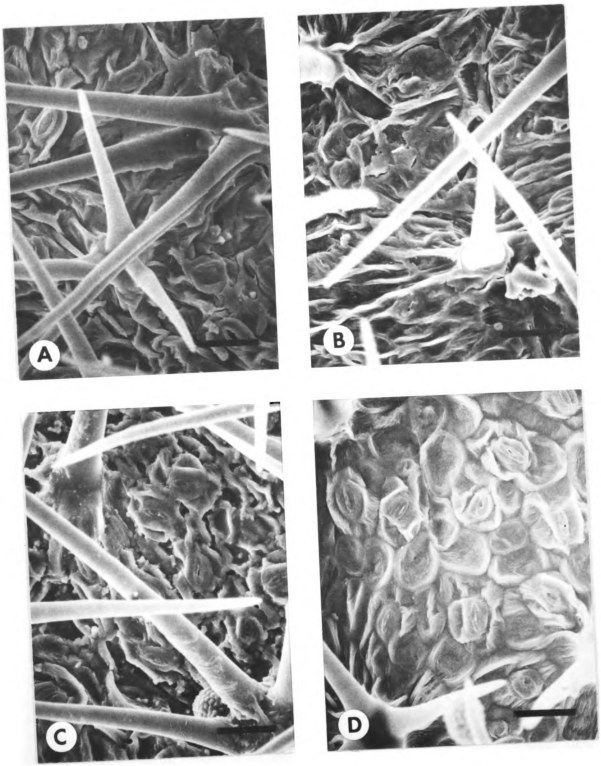
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Figure 23: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with 0.1% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Scattered areas of light cuticular wax redeposition may be seen. All views 400X. Bar = 25 μm.

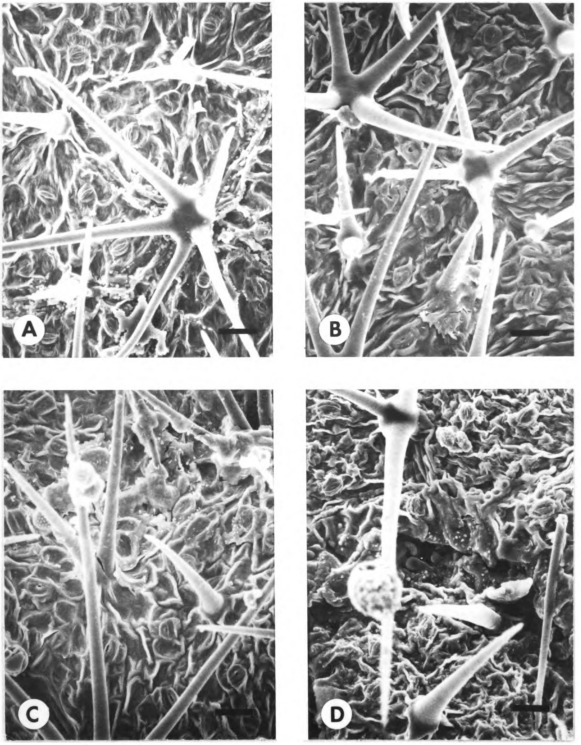
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Figure 24: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with 1.0% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Areas of cuticular wax disruption and redeposition may be discerned. All views 400X. Bar = 25 μm.

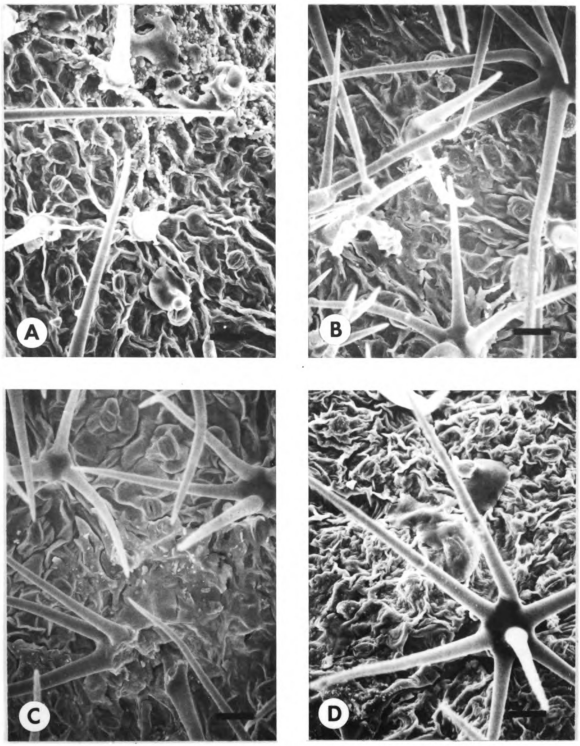
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Figure 25: Scanning electron micrographs of 8-week-old velvetleaf leaves (adaxial surfaces) treated with 10.0% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Extensive patterns of cuticular wax and adjuvant redeposition may be visualized. All views 400X. Bar = 25 μm.

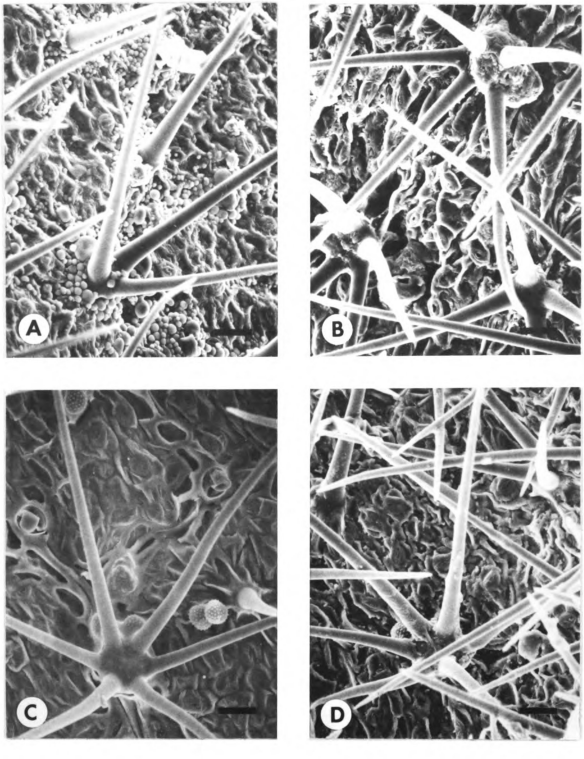
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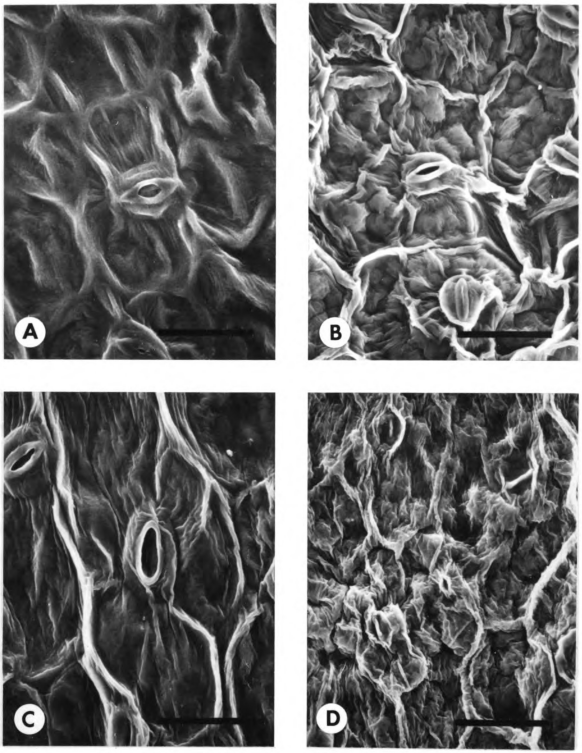
Figure 26: Scanning electron micrographs of: (A) 2-month-old velvetleaf (adaxial surface) control; (B) portion of same leaf dipped in distilled chloroform. Note the extreme shrinkage under periclinal wall areas of treated tisue. View C, 2-month-old jimsonweed leaf (adaxial surface) control; view D, portion of same leaf dipped in distilled chloroform. Once again, extensive collapse is evident along treated periclinal surfaces. All leaf sections vapor fixed, all views 1000X. Bar = 25 µm.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Jimsonweed. Trends and observations previously described for cabbage and velvetleaf were also found with jimsonweed. As with velvetleaf, the cuticle consisted of a thin featureless sheath closely following the contours of the plant surface. Once again as adjuvant treatments became more concentrated they demonstrated more apparent cuticular wax redeposition (Figs 27-33). Progressive periclinal wall structural failure with more concentrated treatments of adjuvants was again found in some instances (Fig 30).

SUMMARY

It is hypothesized that an additional possible adjuvant mode of action exsts in addition to those generally cited, such as increasing wetting, spreading and sticking characteristics of a solution. Scanning electron microscopy studies indicated adjuvants are able to alter and partially dissolve plant cuticular components. Herbicides present in solution with adjuvants would therefore be facilitated in their entry into plants. SEM observations also exhibited a trend of more leaf surface alteration with greater adjuvant spray concentration applied. This pattern held true for all three species examined. Laboratory studies corroborated the ability of adjuvants to not only disrupt cuticular components, but also to do so with increasing capacity at higher concentrations.

Figure 27: Scanning electron micrographs of 2-month-old jimsonweed leaves (adaxial surfaces) treated with (A) 0.1% crop oil concentrate (C.O.C.), (B) 1.0% C.O.C., (C) 10.0% C.O.C. and (D) distilled water. Areas of cuticular wax redeposition become more prominent with more concentrated treatments. Views A and B are 600X; views C and D 400X. Bar = 25 μm.

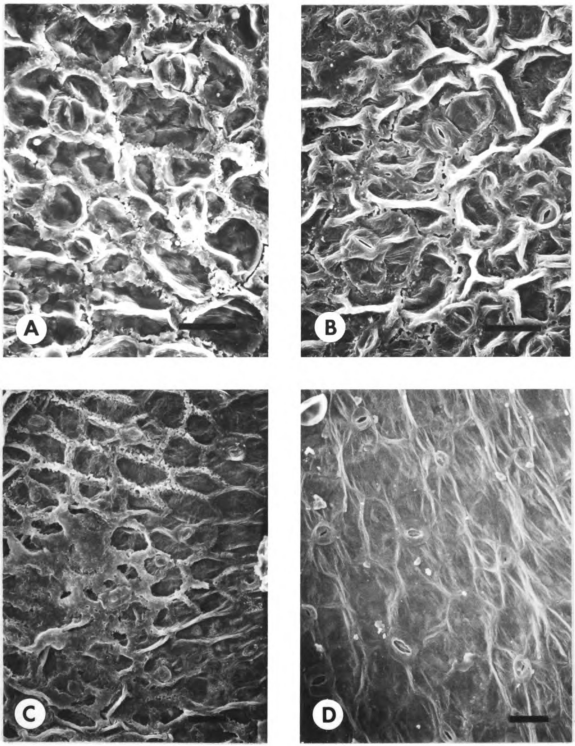
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Figure 28: Scanning electron micrographs of 2-month-old jimsonweed leaves (adaxial surfaces) treated with (A) 0.1% d'limonene, (B) 1.0% d'limonene, (C) 10.0% d'limonene and (D) distilled water. Redeposition occurred along sulci over anticlinal walls. When the periclinal walls sank back, the web-like pattern remained. View A is enlarged 700X, all others 1000X. Bar = 25 μm.

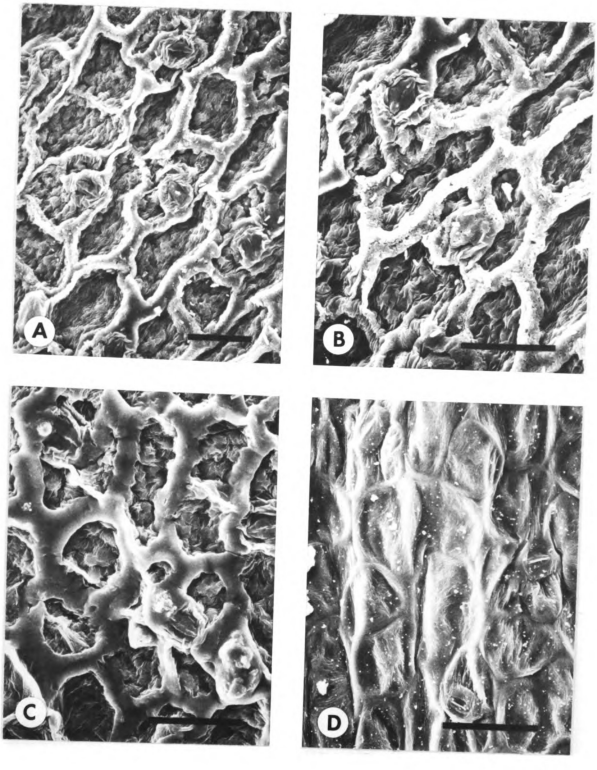
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Figure 29: Scanning electron micrographs of 2-month-old jimsonweed leaves (adaxial surfaces) treated with (A) 0.1% soybean oil concentrate (S.O.C.), (B) 1.0% S.O.C., (C) 10.0% S.O.C. and (D) distilled water. Sheetlike redepositional patterns are characteristically produced by this adjuvant. All views 400X. Bar = 25 μm.

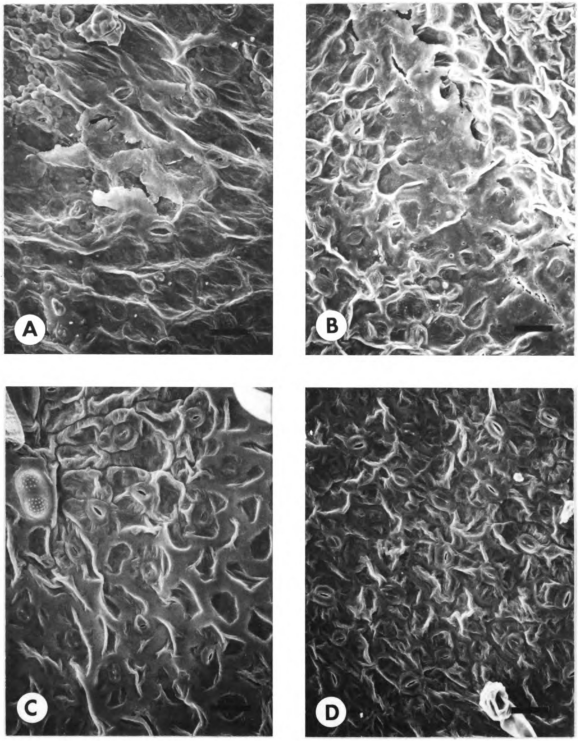
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Figure 30: Scanning electron micrographs of 2-month-old jimsonweed leaves (adaxial surfaces) treated with (A) 0.1% X-77, (B) 1.0% X-77, (C) 10.0% X-77 and (D) distilled water. Larger patterns of cuticular wax redeposition and more prominent sinking of periclinal walls are two evident trends seen with more concentrated surfactant treatments. View C is magnified 650X, all others 700X. Bar = 25 μm.

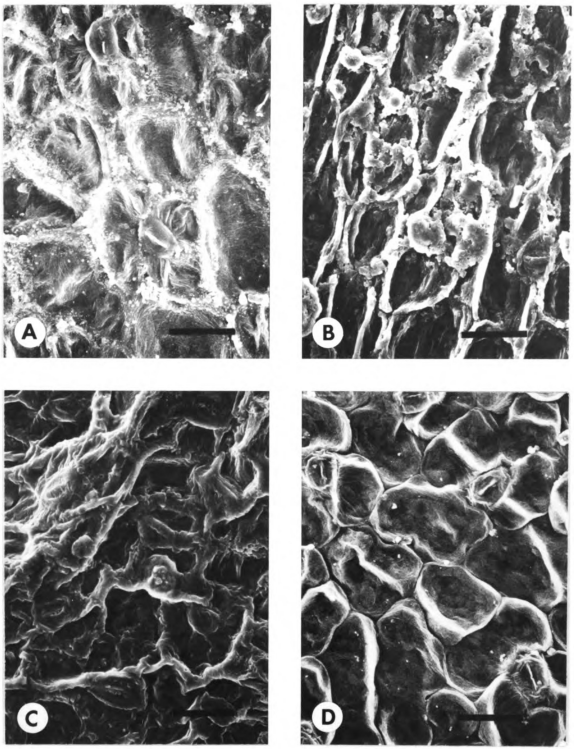
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Figure 31: Scanning electron micrographs of 8-week-old jimsonweed leaves (adaxial surfaces) treated with 0.1% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Random cuticular wax redeposition may be seen. Views A and B are shown 700X, views C and D, 400X. Bar = 25 μm.

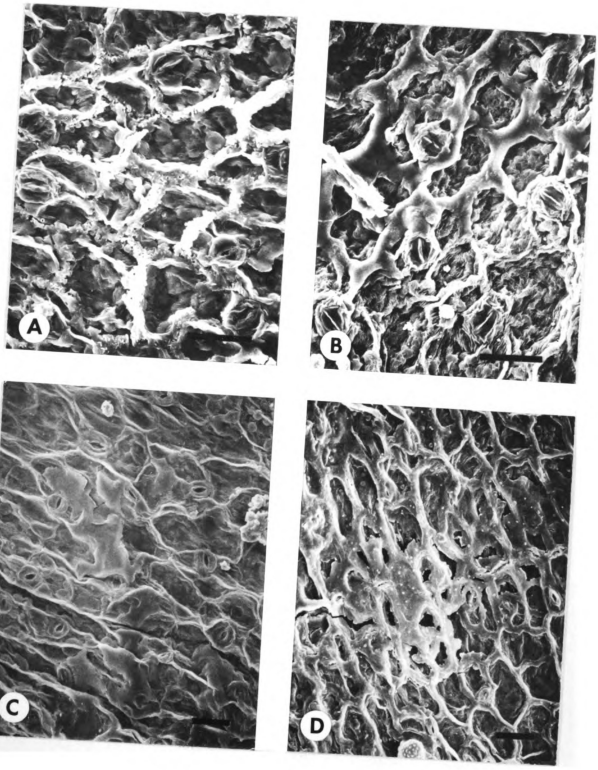
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Figure 32: Scanning electron micrographs of 8-week-old jimsonweed leaves (adaxial surfaces) treated with 1.0% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Distinct areas of cuticular wax redeposition are apparent. Views A and C are enlarged 400X; view B, 650X and view D, 700X. Bar = 25 μm.

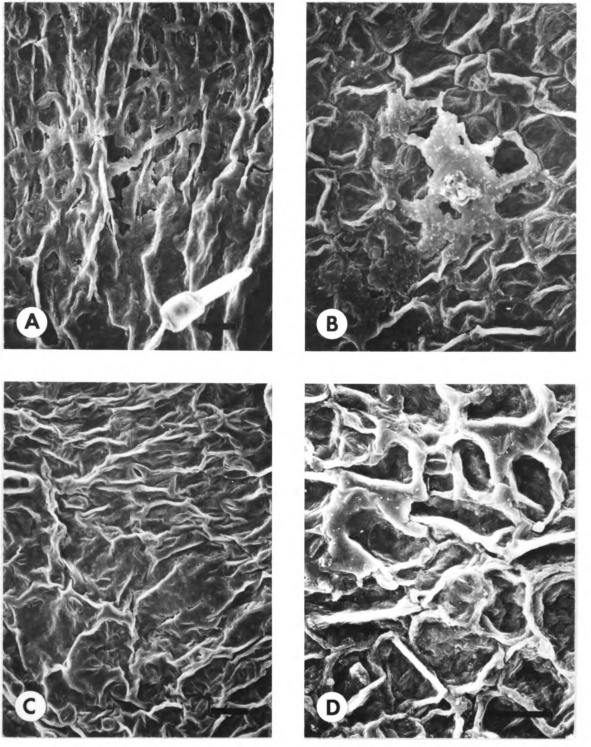
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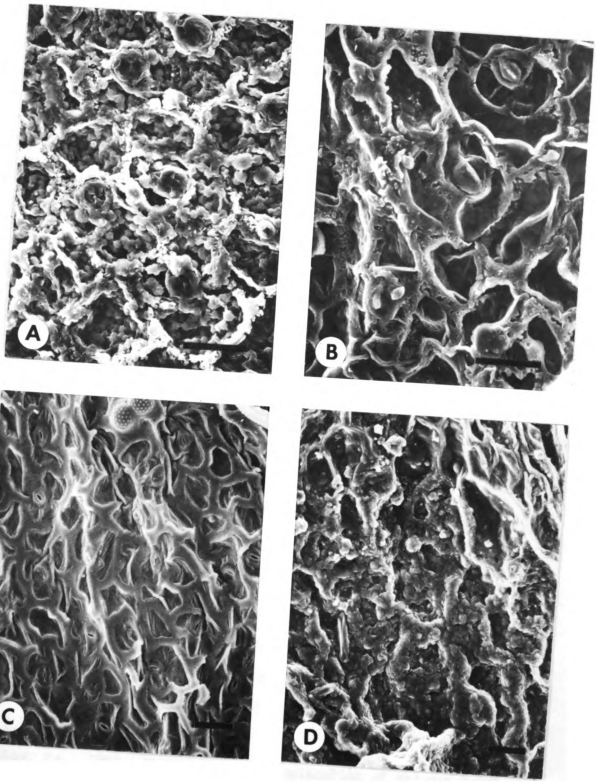
Figure 33: Scanning electron micrographs of 8-week-old jimsonweed leaves (adaxial surfaces) treated with 10.0% aqueous adjuvant solutions of (A) crop oil concentrate, (B) d'limonene, (C) soybean oil concentrate and (D) X-77. Profuse redeposition of cuticular components and adjuvants is evident. Views A and B are 700X; views C and D are 400X. Bar = 25 μm.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CHAPTER 2

ADJUVANT EFFECTS ON THE PLANT CUTICLE: II. QUANTIFICATION OF CUTICULAR DISRUPTIONS INDUCED BY ADJUVANTS

ABSTRACT

A cuticular staining method was developed to quantify cuticular alterations induced by aqueous adjuvant solutions (0.1, 1.0 and 10.0% solutions of crop oil concentrate, d'limonene, soybean oil concentrate and X-77). Sudan IV lipophilic dye was impregnated onto adult cabbage (Brassica oleracea capitita L.) leaf sections by means of an atomizer. These stained sections were immersed into various adjuvant solutions and the amount of extracted dye measured photometrically. At higher adjuvant concentrations more dye was extracted as indicated by greater absorbance. No significant differences were observed among the adjuvants examined, however, their effects were concentration dependent. Trend analysis indicated that the relationship between absorbance values and adjuvant concentrations was linear.

INTRODUCTION

Previous investigations (1, 2, 3, 5, 6, 7, 8) have demonstrated that adjuvants are capable of disrupting plant leaf surface components. The scanning electron microscopy work of the author has shown increasing disruption with increasing adjuvant treatment concentration. A cuticular staining method was developed to quantify these changes and to compare the effects of the various adjuvants.

MATERIALS AND METHODS

A Sudan IV lipophilic dye solution was prepared using the method described by Clark (4). The dye powder was dissolved in 95% ethanol then mixed with an equal amount of glycerol and filtered. The dye solution was applied by means of an atomizer onto mature cabbage head leaf sections (trimmed to uniform 2.5 by 5.0 cm sizes). Sixty mls of dye proved sufficient to stain 180 leaf pieces.

Fifty ml aqueous adjuvant solutions (of 0.1, 1.0 and 10.0% v/v, as well as distilled water controls) were prepared for all four adjuvants tested: crop oil concentrate[1] (a paraffin-petroleum oil

[1]Herbimax, Reg. TM of Union Carbide Corp.

blend), d'limonene[2] (1-methyl-4-isopropenylcyclohexane), soybean oil concentrate[3] (85:15 soybean oil to emulsifier ratio) and X-77[4] (alkylarylpolyoxyethylene glycols, free fatty acid and isopropanol mixture). Ten dyed cabbage sections were then dipped into each solution; each piece was dipped for 10 seconds. The ten sections were suspended over the solutions for five minutes after dipping and allowed to dry.

After the dipping process was completed, 20 ml aliquants of each of the adjuvant-cuticular wax-label mixture solutions were sampled and added to 120 ml separatory funnels with 35 ml of xylene to obtain a colored, cloudy (dye containing) layer. Subsequently, 35 ml of ethanol were added to this colored portion to precipitate out impurities and leave a clear, colored layer. The dyes extracted were measured and compared photometrically to the water-dipped control sample on a Beckman DB-G grating spectrophotometer (maximum absorption range: 524-546 nm). The entire procedure was replicated four times.

[2]Cide-kick, Reg. TM of JLB International Chemical Co.

[3]American Soybean Association Standard

[4]X-77, Reg. TM of Chevron Chemical Co.

RESULTS AND DISCUSSION

As adjuvant concentrations increased more dye was extracted as indicated by greater dye absorbance (optical density, O.D.) values (Table 1). Analysis of variance showed no significant difference in dye extraction among the four adjuvants. However, there were significant differences among the concentrations, in going from the 0.1% to the 1.0% to the 10.0% solutions (Table 2). Trend analysis indicated that the relationship between absorbance values and adjuvant concentrations was strictly linear and that two of the adjuvants, X-77 and soybean oil concentrate, exhibited significant linear increases (Table 3). These linear trends are shown in Figure 1.

The results obtained with these four adjuvants confirm previous SEM observations by the author, that more concentrated aqueous adjuvant solutions exert a more pronounced disrupting effect on plant cuticles. This effect, however, is not as outstanding or dramatic as might have been expected. Logarithmic increases in adjuvant solution concentrations were required to bring about significant increases in the amounts of cuticular dye extracted (and then only for two of the four adjuvants tested). An arithmetic progression in the concentrations of adjuvant solutions might not have resulted in any significant differences in the amounts of wax displaced. X-77 may be an exception as its slope increased markedly over the other three materials tested. Nevertheless, the overall general trend of greater

Table 1: Average absorbance (O.D.) values of Sudan IV dye extracted from stained cabbage leaf sections, (all values x 10^3).

	Adjuvant Concentration		
	0.1%	1.0%	10.0%
Crop oil concentrate	19	28	39
D'limonene	9	33	45
Soybean oil concentrate	14	25	58
X-77	24	42	96

(Maximum Absorption Range: 524-546 nm)

Table 2: Analysis of variance performed on absorbance data.

Source of Variation	df	SS	MS	Observed F
Replications	3	3297	1098.92	1.36
Adjuvants	3	5318	1772.81	2.19
Concentrations	2	15074	7537.15	9.30**
Adjuvants x Concentrations	6	3886	647.70	0.80
Error	33	26758	810.85	

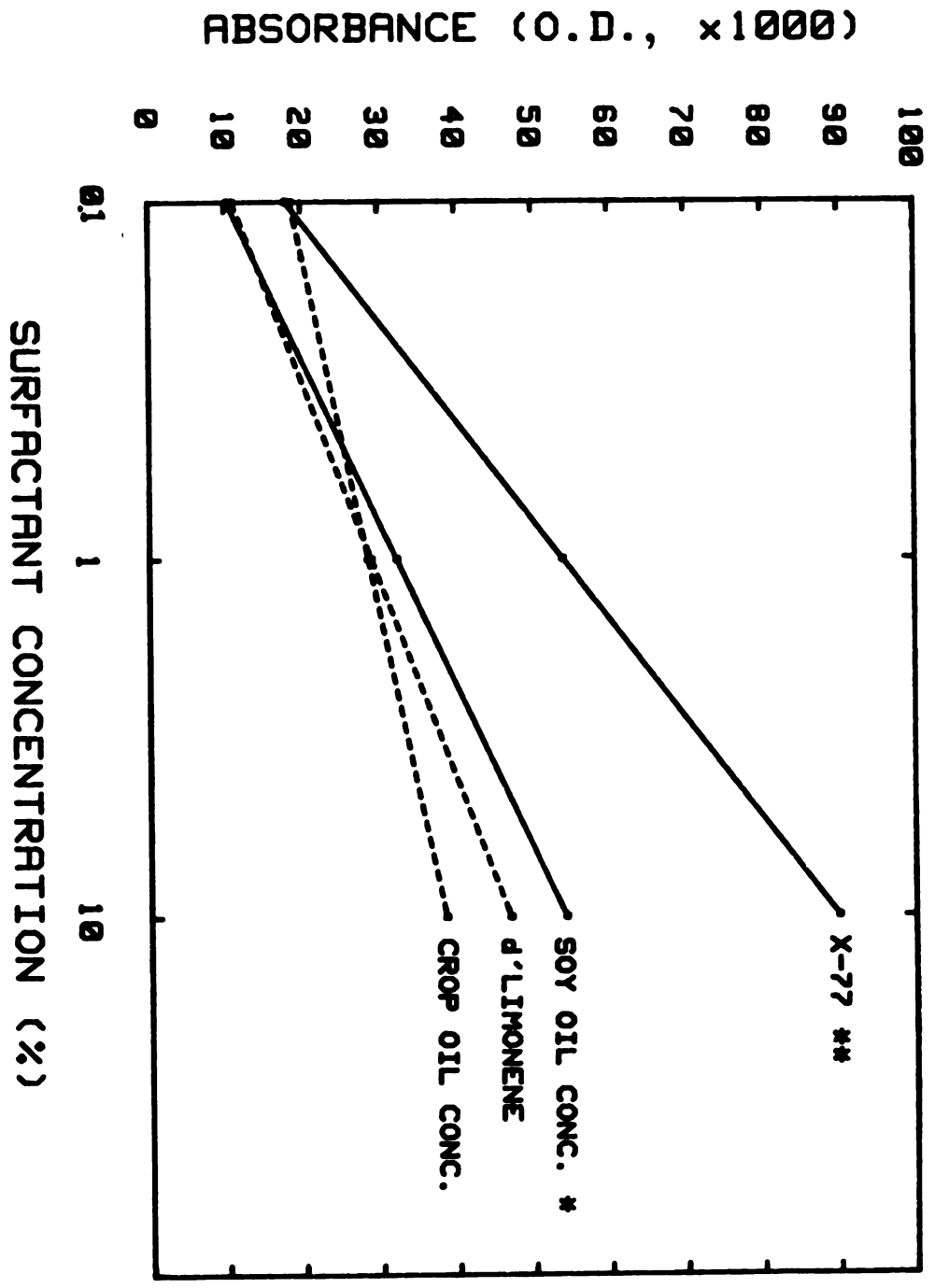
Tabular F values: $F(2, 33)_{.05} = 3.28$ $F(2, 33)_{.01} = 5.32$

Table 3: Trend analysis of Sudan IV absorbance data.

Source of Variation	df	SS	MS	Observed F
Replications	3	3297	1098.92	1.36
Adjuvants	3	5318	1772.81	2.19
Concentrations				
X-77 linear	1	10368	10368	12.79**
X-77 quadratic	1	864	864	1.07
S.O.C. linear	1	3872	3872	4.78*
S.O.C. quadratic	1	322.67	322.67	.40
d'limonene linear	1	2592	2592	3.20
d'limonene quadratic	1	96	96	.12
C.O.C. linear	1	800	800	.99
C.O.C. quadratic	1	2.67	2.67	.003
Error	33	26758	810.86	

Tabular F values: $F(1, 33)_{.05} = 4.14$ $F(1, 33)_{.01} = 7.47$

Figure 1: The effect of logrithmic increases in adjuvant concentration on the amount of dyed cuticle removed from cabbage as indicated by absorbance of Sudan IV at 540 nm.


ABSORBANCE (O.D., ×1000)
0
10
20
30
40
50
60
70
80
90
100
0.1
1
10
SURFACTANT CONCENTRATION (%)
X-77 **
SOY OIL CONC. *
d'LIMONENE
CROP OIL CONC.

cuticular alteration resulting from application of higher adjuvant concentrations has been demonstrated.

SUMMARY

A method was developed whereby the cuticle disrupting properties of various aqueous adjuvant treatments (0.1, 1.0 and 10.0% solutions of crop oil concentrate, d'limonene, soybean oil concentrate and X-77) could be compared and measured. Sudan IV lipophilic dye was inpregnated onto mature cabbage leaf sections by means of an atomizer. The stained sections were immersed into the various adjuvant solutions; after an extraction process the recovered dye was measured photometrically. With higher adjuvant concentrations, more dye was extracted as reflected by greater dye absorbance values. No significant differences were discerned among the four adjuvants examined, however, significance was shown between the various concentrations. Trend analysis indicated a linear relationship between absorbance values and adjuvant concentrations.

Although a pattern of greater cuticular alteration with application of more concentrated adjuvant solutions was demonstrated, the effect was not as pronounced as might have been expected. Logarithmic increases in adjuvant solutions were necessary to bring about significant increases in the amounts of extracted cuticular dye. An arithmetic progression in the concentrations of adjuvant solutions might not have resulted in significant differences in the amounts of cuticular components displaced.

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CHAPTER 3

ADJUVANT EFFECTS ON THE PLANT CUTICLE: III. THE EFFECTS OF ADJUVANTS ON THE EVAPOTRANSPIRATION OF SOYBEAN

ABSTRACT

The effects of various adjuvants (crop oil concentrate, d'limonene, soybean oil concentrate and X-77) on water loss from soybean (Glycine max (L.) Merr.) were evaluated. Preliminary investigations on excised leaflets dipped into or sprayed with either 1.0% or 10.0% aqueous adjuvant solutions resulted in accelerated moisture loss in all cases except for the 1.0% adjuvant sprayed treatments. Immersion produced greater water loss than spraying at both concentrations. In general, 10.0% adjuvant treatments caused greater transpiration rates than 1.0% adjuvant treatments.

Instantaneous chamber measurement on soybeans grown in the field and sprayed with either 0.1, 1.0 or 10.0% aqueous adjuvant solutions from a tractor mounted spray system provided sensitive evaluation of transpiration. As soon as the spray dried, chamber measurements were performed at two time intervals averaging 9.3 and 14.4 min after treatment. The earlier reading indicated an increase in

evapotranspiration (ET) following adjuvant solution applications. Increasing adjuvant concentrations generally produced greater ET rates. The later reading indicated less water loss than before the spray treatment. Thus, after 10 to 12 min, treated soybean plants were able to compensate for increased ET by decreasing stomatal aperture.

INTRODUCTION

There are three main sites of water loss within a leaf; one is the hydathode pores, another is the mesophyll cells lining the substomatal cavity, and the third is the outer epidermal cells themselves. Water vapor, or sometimes liquid water, flows to the leaf surface through the hydathodes, through the stomates, or through the cuticle. Since the number of hydathodes in comparison to the number of stomates is small, the contribution of the hydathodes to vapor flow is generally ignored. When stomates are open, there is little resistance to vapor flow (i.e. evapotranspirational flow) and a large proportion of the vapor lost from a plant flows through them. When stomates are closed, no evapotranspirational flow takes place and the only remaining pathway is through the cuticle.

Previous investigations by the author (16) indicated that adjuvants were capable of altering cuticular components. Since cuticles are able to limit evapotranspirational flow, application of adjuvant solutions to plant leaves might be expected to increase water loss and/or evapotranspiration. Many past investigators have shown the importance of the cuticle in conserving water (7, 10). Pieniazek (24) studied the physical characteristics of apple (Malus sylvestris Mill.) skins in relation to transpirational loss. No correlation was found

between the thickness of the cuticle and transpiration rate, but surface russeting greatly increased water loss. Wiping the fruit increased transpiration rate; this was not reduced by the new layer of wax, which formed on the surface. Hall (12) found that removal of cuticular waxes from apples, by any method, even contact with wrapping paper, caused an increase in transpiration rate. Exposure to petroleum ether vapors disorganized the wax structures on the leaves and fruit of grapes (Vitis vinifera L.) and markedly increased transpiration (25). Physical disruption of leaf surface waxes of white clover (Trifolium repens L.) with a camel-hair brush enhanced cuticular transpiration even when the stomates were closed (11).

Pfeiffer et al. (23) found that treatment of the soil with TCA (trichloracetic acid) suppressed the formation of wax on pea (Pisum sativum L.) plants and, as a consequence, transpiration from the leaves was greatly increased. More recently, Flore and Bukovac (9) working with EPTC (S-ethyl dipropylthiocarbamate), another known herbicide suppressant of wax formation, incorporated into soil in which cabbage plants were grown, also found significant increases in cuticular transpiration. Larger doses of EPTC caused correspondingly higher rates of transpiration.

Limited work on the affects of adjuvants on transpiration has been performed to date. Okanenko et al. (21) reported increased transpiration in sugar beet (Beta vulgaris L.) following treatment with Sulfanol (sodium-dodecylbenzenesulfonate). Interestingly, photo-

synthesis was also increased and the ratio of water use to carbon fixed remained constant. This contrasts with the results of Coats and Foy (3), who reported that 0.1% Atlox 209-FG[1] (non-ionic polysorbate surfactant) reduced water loss from soybean plants. Differences in surfactants used might account for differences in response. Sun 11E[2] oil was also seen to suppress transpiration in soybean, but had no effect on corn (3).

Indirect evidence of water loss from plants due to application of adjuvants has come from researchers investigating the solubilization effects of these chemicals on leaf surface waxes. Takeno and Foy (33) noted that cotton leaves treated with a polysorbate surfactant (HLB 8.0) solution became water stressed. Chykaliuk (5) in treating field bindweed (Convolvulus arvensis L.) with droplets of a 5.0% aqueous solution of d'limonene[3] (1-methyl-4-isopropenylcyclohexane) noted that the surfactant increased surface disruptions and induced increased ridging. He concluded that the d'limonene caused the epicuticular wax to either form into ridges through an undetermined mechanism or that the surfactant stimulated the excretion of new wax into ridges. Careful examination of the micrographs indicating ridging, reveal them to very closely resemble leaf folds and shrinkage commonly associated with water loss. The ridges appear to be folds brought about by water loss stimulated by surfactant induced epicuticular alteration.

[1]Atlox 209-FG, Reg. TM Atlas Chemical Division, ICI America Inc.

[2]Sun 11E, Reg. TM Sun Oil Co.

[3]Cide-kick, Reg. TM of JLB International Chemical Inc.

In order to expand on previous research that measured adjuvant effects on plant transpiration, investigations were conducted in both the laboratory and field. Initial studies examined the effect of four adjuvants on the transpiration rate of excised leaflets from potted soybeans. Subsequently, a more sensitive 'chamber' technique was adopted to measure evaportranspiration from soybean plants growing in the field.

Instantaneous Chamber Measurement

Chambers to measure evaptotranspiration (ET) and canopy apparent photosynthesis (CAP) have been utilized by researchers for about the last 25 years. Most of the work has been done wih chambers placed in the field for several hours, days or weeks (1, 4, 6, 8, 15, 17, 20, 26, 27, 31). The energy exchange in this type of chamber is similar to that of a greenhouse and has been described by Bussinger (2) and Lee (18). The presence of enclosed chambers affects the crop environment altering it from the surrounding environment. Thus, researchers have attempted to artificially simulate 'outside' conditions by controlling the humidity, temperature, and CO_2 concentration of the chamber atmosphere. Without exception, however, they have been forced to accept conditions different from those of the ambient environment (15, 18, 20, 31).

Another type of chamber used for determining ET and/or CAP in the field is the instantaneous measurement chamber, a version of which was used for our measurements. It is termed "instantaneous" because the measurements are made over approximately a 1 min time period. The goal for the use of this type of chamber is to obtain accurate and absolute point measurements of ET and/or CAP in the field. To achieve this goal, the chamber is lowered over or placed around a group of plants prior to measurement of evapotranspiration, typically by means of aspirated thermistor psychrometers. The short duration of the measurement serves to minimize plant response to the presence of the chamber.

Pioneering efforts with instantaneous chambers were undertaken by Peters et al. (22), Reicosky and Peters (28), Schulze (32), Reicosky et al. (29), Mason et al. (19), Harrison et al. (14), Reicosky (30) and Wells et al. (34). These investigations are summarized by Harmsen (13). He also undertook a number of studies at the Agricultural Engineering Department of Michigan State University, to optimize chamber design, physical (ambient) and physiological (measured plant) parameters (13). His portable chamber design recommendations have been adapted, expanded, and refined by G. Peterson and T. Loudon (M.S.U. Agric. Engin. Dept.) who continue research in this area. A joint venture was undertaken with these researchers to measure possible alterations in ET after adjuvant applications at various concentrations.

MATERIALS AND METHODS

Laboratory studies were undertaken on 'Forrest' soybeans to examine some of the transpirational changes that might be induced by adjuvants. Soybean plants used were in the fifth trifoliolate stage (V 5) grown outdoors in pots. Fully-expanded leaflets of fourth trifoliolate leaves were excised and their petiolules sealed with lanolin. The change in leaflet osmotic pressure after excision is known to induce stomatal closure; so after lanolin application, leaflet moisture loss was greatly curtailed.

Aqueous solutions of four adjuvants: crop oil concentrate[4] (a paraffin-petroleum oil blend), d'limonene, soybean oil concentrate[5] (85:15 soybean oil to emulsifier ratio) and X-77[6] (alkylarylpolyoxyethylene glycols, free fatty acid and ispropanol mixture), were prepared at 1.0% and 10.0% v/v along with a distilled water control. Sets of three leaflets, approximately equal in size but not from the same trifoliolate leaf, were weighed and fully immersed for 3 seconds into 1.0% adjuvant solutions. This procedure was repeated with additional leaflets using the 10.0% adjuvant

[4]Herbimax, Reg. TM of Union Carbide Corp.

[5]American Soybean Association Standard.

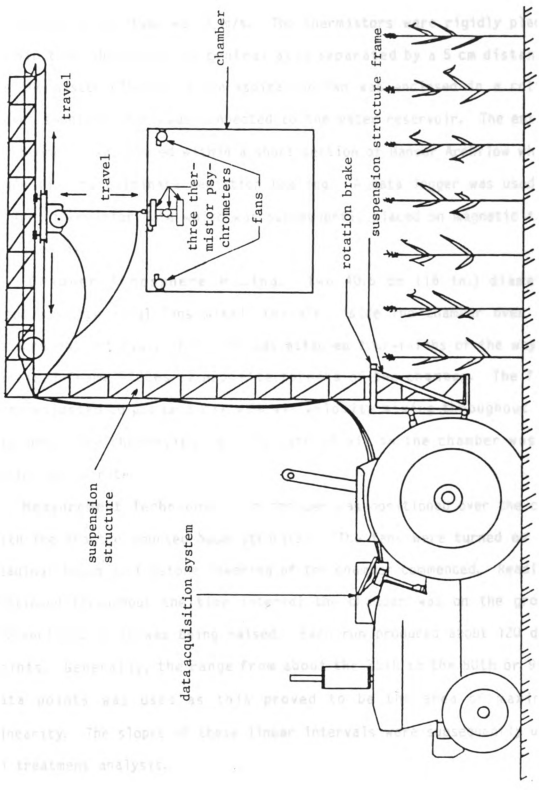
[6]X-77, Reg. TM of Chevron Chemical Co.

preparations. The leaflets were then placed into a dark environment at 22°C and 50% relative humidity and weighed at intervals of 1, 2, 3, 6, 9, 12 and 24 hr to determine water loss. An identical procedure was also carried out on leaflet sets sprayed with the same surfactant solutions. Application was by means of a flat fan nozzle (Tee Jet 8001 E) suspended 20 cm (8 in) over the tissue and delivering 325 ml/min at 150 kPa (22 psi). The entire process was replicated three times.

Chamber and Suspension Structure. Field investigations on possible ET changes induced by adjuvants were carried out in cooperation with G. Peterson (M.S.U. Agric. Engin. Dept.) on July 7, 1983 at M.S.U. soybean research fields (silt loam) located in Ingham Co., MI. The ET measurement system was similar to that described by Harmsen (13), but with a few modifications. Chamber dimensions were 1.22 m (W) by 1.52 m (L) by 0.91 m (H) (4 ft by 5 ft by 3 ft); the frame was constructed of lightweight 2.54 cm square modular aluminum pieces. A tractor mounted suspension structure was built to suspend the chamber above the crop and lower it into place for measurement. This structure is shown in Figure 1 along with the chamber and farm tractor used for support and mobility.

Data Acquisition Equipment. Determination of the vapor density within the chamber was accomplished by means of three aspirated thermistor psychrometers suspended at 15 cm intervals from the top center of the chamber. Each psychrometer consisted of an aspiration tube with a 2 cm inside diameter to which was attached a small water

Figure 1: Chamber and suspension structure in field.
Adapted from Harmsen (1983), p. 73.


travel
travel
chamber
three thermistor psychrometers
fans
rotation brake
suspension structure frame
suspension structure
data acquisition system

reservoir. At the rear of the tube the intake of a 2500 cm^3/s DC fan was attached. The resulting wind velocity over the two thermistors positioned in the tube was 8 m/s. The thermistors were rigidly placed within the tube along its central axis separated by a 5 cm distance. The thermistor closest to the aspiration fan was enclosed in a cotton jacket shoelace which was connected to the water reservoir. The entire psychrometer was placed within a short section of Hancor Archflow white drain tile to eliminate radiation heating. A data logger was used to collect thermistor data which was subsequently placed on magnetic tape for storage.

Chamber Atmosphere Mixing. Two 40.6 cm (16 in.) diameter propellor-type axial fans mixed the air inside the chamber over the measurement interval. Each fan was attached four-fifths of the way up vertical frame pieces at opposite corners of the chamber. The fans were adjusted to produce maximum air velocity mixing throughout the chamber. The theoretical mixing rate of air in the chamber was 20 cycles per minute.

Measurement Technique. The chamber was positioned over the crop with the tractor mounted boom structure. The fans were turned on and readings begun just before lowering of the chamber commenced. Readings continued throughout the time interval the chamber was on the ground (90 sec) and as it was being raised. Each run produced about 120 data points. Generally, the range from about the 25th to the 80th or 90th data points was used as this proved to be the area of maximum linearity. The slopes of these linear intervals were subsequently used in treatment analysis.

Measurements were made on 'Corsoy' and 'Wells' soybean plants in the sixth trifoliolate stage (V 6) before and after treatment with various adjuvant solutions. Four adjuvants previously described, were prepared at three different aqueous concentrations: 0.1%, 1.0% and 10.0% v/v, resulting in twelve different applications. Adjuvant solutions were distributed by means of flat fan nozzles on a tractor mounted spray system, operating at 324 kPa (47 psi) and delivering 253 L/ha (27 gpa). Three chamber measurements were made at each treated location as soon as applied sprays dried. Meteorological conditions were very favorable as the day was bright and sunny.

The layout of the rows and field were such that one group of readings were obtained soon after treatment (averaged 9.3 min after adjuvant application), while the measurement on others were slightly delayed (averaged 14.4 min after application). This proved to be a fortuitous arrangement, as will be subsequently discussed.

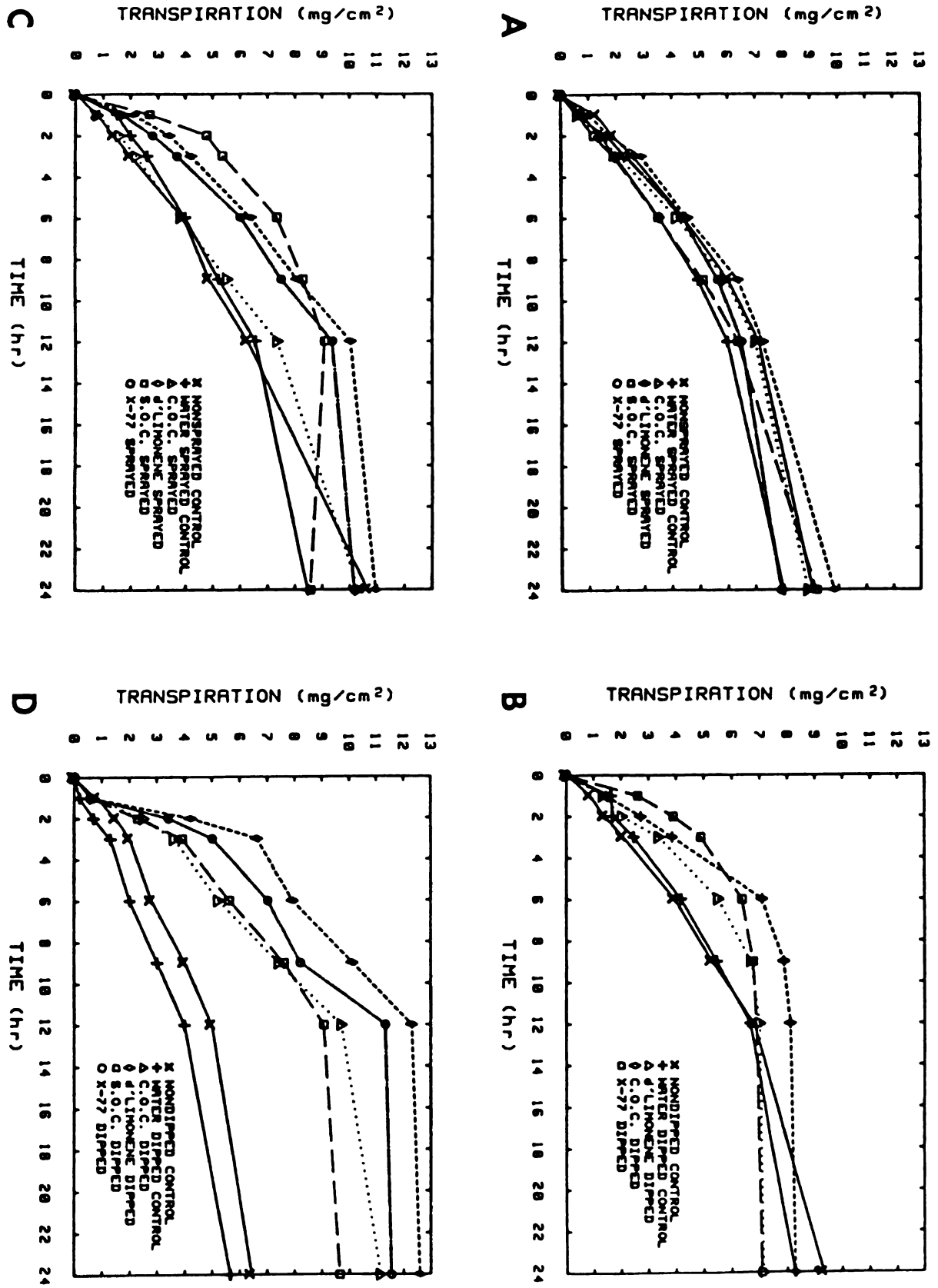
Data Interpretation. Raw data stored on tape was transferred to a microcomputer for derivation of treatment slopes as previously mentioned. The calculations performed on each discrete sample and linear interval are manifold and complex and do not fall within the scope of this paper. The interested reader is referred to Harmsen (13). The resulting slopes represent the increase in water vapor in the chamber over the measurement time in centimeters per hour.

RESULTS AND DISCUSSION

Treatment with adjuvants markedly accelerated soybean moisture loss in both laboratory and field investigations. In the former study adjuvants increased water loss from excised leaflets in most all cases, except where 1.0% solutions were sprayed onto leaflet surfaces (Figure 2). Greatest moisture loss occurred within the first 3 hr of treatment with transpiration rate almost linear. Slope values from linear regressions performed on coordinates fom 0 to 3 hr are summarized in Table 1. At both concentrations, leaflets that had been immersed, showed greater moisture loss than their sprayed counterparts. Also, the 10.0% sprayed treatments exhibited greater transpiration rates than 1.0% sprayed treatments. A similar pattern was also shown by the dipped tissue.

In the chamber study, slope values obtained before and after treatment with adjuvant solutions were compared and the percent change calculated. A positive value indicated an increase in chamber water vapor over the initial reading; a negative value indicated a lessening of the amount of water vapor given off by plants compared to the initial measurement. Near the beginning of the data acquisition, one of the three psychrometers malfunctioned. Only two sets of readings were, therefore, obtained with every lowering of the chamber. Since three chamber measurements were made at every treated area, six

Figure 2: Effect of aqueous adjuvant solutions on transpiration from soybean leaflets (A) sprayed with 1.0% solutions, (B) dipped in 1.0% solutions, (C) sprayed with 10.0% solutions and (D) dipped in 10.0% solutions.


A
TRANSPIRATION (mg/cm²)
TIME (hr)
X NONSPRAYED CONTROL
+ WATER SPRAYED CONTROL
Δ C.O.C. SPRAYED
◇ d'LIMONENE SPRAYED
□ S.O.C. SPRAYED
O X-77 SPRAYED
B
TRANSPIRATION (mg/cm²)
TIME (hr)
X NONDIPPED CONTROL
+ WATER DIPPED CONTROL
Δ d'LIMONENE DIPPED
◇ C.O.C. DIPPED
□ X-77 DIPPED
C
TRANSPIRATION (mg/cm²)
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X NONSPRAYED CONTROL
+ WATER SPRAYED CONTROL
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□ S.O.C. SPRAYED
O X-77 SPRAYED
D
TRANSPIRATION (mg/cm²)
TIME (hr)
X NONDIPPED CONTROL
+ WATER DIPPED CONTROL
Δ C.O.C. DIPPED
◇ d'LIMONENE DIPPED
□ S.O.C. DIPPED
O X-77 DIPPED

Table 1: Slopes of linear regressions performed on first four values obtained (at 0 to 3 hr) during soybean transpiration experiments.

	1% Solution sprayed	1% Solution dipped	10% Solution sprayed	10% Solution dipped
	----------(slope)----------			
Crop oil concentrate	0.71	1.28	0.74	1.26
D'limonene	0.94	1.08	1.39	2.33
Soybean oil concentrate	0.63	-	1.81	1.34
X-77	0.81	1.59	1.22	1.33
Water treated control	0.84	0.68	0.66	0.67
Non-treated control	0.66	0.75	0.83	0.44

readings were obtained. As previously mentioned, measurements were made at two discrete times following treatment with adjuvants. Two chamber placements were conducted soon after treatment, the third was delayed. The values summarized in the first column of Table 2, therefore, are the average percent change of four readings, while the figures in the the second column represent the average percent change of two readings.

In the early measurements the percent change in chamber moisture became more positive (i.e. more water vapor was lost by the plants) as the adjuvant concentrations increased. In contrast to the earlier measurements, the delayed measurements (averaged about 5 min later) show no relationship to adjuvant concentration and most are negative. The plants lost less water than before treatment. The soybeans compensated for the increased water loss brought about by the adjuvant solution by decreasing stomatal aperture. This compensation occurred about 10 to 12 min after treatment.

The 10.0% treatments of two of the adjuvants, d'limonene and crop oil concentrate, at the early time interval, caused a decrease in water loss compared to treatments with these adjuvants at lower concentrations. The greater percent of change than exhibited at the same treatment concentrations with the other two adjuvants may explain this observation. When treated with 10.0% solutions of d'limonene or crop oil concentrate, soybean plants may lose water so rapidly that the trigger mechanism for stomatal closure may be set off more quickly, thus, showing lower evapotranspiration values.

Table 2: Average percent change in evapotranspirational slope obtained from soybeans after treatment with various aqueous adjuvant solutions.

Adjuvant concentration	Measurements made ≤12 minutes after adjuvant application ($\bar{x}$ = 9.3 min)	Measurements made >12 minutes after adjuvant application ($\bar{x}$ = 14.4 min)
0.1% X-77	+ 9.5	+ 13.2
1.0% X-77	+ 8.2	+ 11.3
10.0% X-77	+ 28.9	- 23.4
0.1% Soybean oil concentrate	- 6.0	- 17.4
1.0% Soybean oil concentrate	+ 2.6	- 21.3
10.0% Soybean oil concentrate	+ 3.5	- 10.8
0.1% d'limonene	+ 20.1	- 0.5
1.0% d'limonene	+ 21.9	- 60.9
10.0% d'limonene	+ 5.5	- 1.1
0.1% Crop oil concentrate	+ 15.3	+ 12.2
1.0% Crop oil concentrate	+ 29.6	- 35.1
10.0% Crop oil concentrate	- 12.5	- 4.5

SUMMARY

The spray adjuvants, crop oil concentrate, d'limonene, soybean oil concentrate and X-77 were evaluated for their possible effects on water loss from soybean. Initial investigations on excised leaflets dipped into or sprayed with either 1.0% or 10.0% aqueous adjuvant solutions resulted in accelerated moisture loss in all instances but the 1.0% sprayed treatments. Immersion resulted in greater water loss than spraying at both concentrations. Generally, 10.0% adjuvant treatments exhibited higher transpiration rates than 1.0% treatments.

More sensitive field investigations were subsequently done by instantaneous chamber measurement on soybeans sprayed with either 0.1, 1.0 or 10.0% aqueous adjuvant solutions from a tractor-mounted spray delivery system. As soon as the spray dried, chamber measurements were carried out at two time intervals averaging 9.3 and 14.4 min after treatment. Earlier readings indicated an increase in evapotranspiration (ET) following the application of adjuvant solutions. Generally, greater ET rates were recorded with higher adjuvant concentrations. Later readings indicated less water loss occurred than before any treatment. The soybeans were able to compensate for the increased water loss due to adjuvant treatment by decreasing stomatal aperture. This adjustment took place 10 to 12 min after treatment.

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SUMMARY AND CONCLUSIONS

Many properties are often assigned to adjuvants to explain their herbicide enhancing abilities. Those most frequently cited are the enhancement of the emulsifying, dispersing, spreading, and wetting characteristics of liquids. The foregoing chapters present substantial evidence for yet another adjuvant mode of action, the ability to solubilize and alter leaf cuticular components. This property has been speculated upon for many years, however, only limited previous investigations had hitherto been performed. Altered cuticles brought about by adjuvant application on plants may present commingled herbicides with a reduced entry barrier, thus explaining many of the enhancing properties of these additives. Increasing adjuvant concentrations were found to increase cuticle disrupting abilities. However, logarithmic increases were generally needed to bring about statistically significant alterations. Adjuvant applications were also found to increase the rate of transpiration from treated plants. The increases were directly proportional to applied adjuvant concentrations. Treated soybeans were able to adjust to increases in water loss after about 10 to 12 min by decreasing stomatal apperture.

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