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James F. Peacock

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JAMES F. PEACOCK

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AND HERBICIDE ACTIVITY BY OILS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

ENHANCEMENT OF FOLIAR PENETRATION AND HERBICIDE ACTIVITY BY OILS

Abstract

JAMES F. PEACOCK

Under the supervision of Dr. C. Dean Dybing

To learn more about oils and how they affect post-emergence spray applications of herbicides, two major problem areas were investigated. The first dealt with specific physical and chemical properties of oils as they are associated with phytotoxicity and enhancement of herbicidal activity, while the second dealt with foliar penetration as a possible mechanism of enhancement.

Simple correlation and multiple regression analyses were conducted for specific properties of mineral oils applied to green foxtail (Setaria verdis (L.) Beauv.) and grain sorghum (Sorghum bicolor (L.) Moench. 'Northrup King 133') as 5% oil in water emulsions with and without 2.24 kg/ha of atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine). Simple correlations identified individual oil properties important in explaining dry weight variabilities, but these single properties generally accounted for only a small portion of the total dry weight variability. Analyzing several oil properties by multiple regression analyses accounted for 99% of the

variability due both to phytotoxicity and herbicide enhancement. Each of several combinations of eight different oil properties accounted for most of the dry weight variability because the properties were not mutually exclusive. Those properties most highly associated with atrazine enhancement included distillation temperatures, pour point, viscosity, viscosity index, refractive index, and flash point. Phytotoxicity of oil alone was associated with increased aromatic contents and decreased unsulfonated residue values. These findings were slightly altered when natural (crop) oils were included with the mineral oils due to inherent differences in the properties of the two types of oils.

Several oils used as 5% oil/water emulsions significantly increased the herbicidal activity of atrazine on foxtail and sorghum when compared to atrazine in water alone or with 0.1% surfactant. Penetration of ^{14}C from atrazine and dicamba (3,6-dichloro-o-anisic acid) through astomatous leaf surfaces of Tradescantia (Tradescantia fluminesis Vell.) was increased by use of oil-water emulsion as compared to 0.1% surfactant solution. Most of the radioactivity was found below the treated leaves of Tradescantia plants 1 day after treatment but accumulation in the plant parts above treated leaves increased by the

6th day. ^{14}C-labeled mineral oil likewise was found to penetrate and translocate in the Tradescantia plants. Uptake of ^{14}C from the oil increased from 20 to 57% in the first 6 days after treatment. Little additional uptake was noted at 15 days. Measurements of CO_2 uptake by sorghum revealed that photosynthesis was completely inhibited 1.5 hours after treatment with atrazine in oil-water emulsion. Maximum penetration of ^{14}C-labeled herbicides through isolated astomatous cuticles of Prunus armeniaca L. (apricot) leaves occurred (a) at warm rather than cold temperatures, (b) when chloroform extraction was used to remove wax prior to treatment, (c) from the air side of the cuticle to the mesophyll side rather than in the reverse direction, and (d) when oil emulsions were used as the carrier in short treatment periods compared with 0.1% surfactant. Penetration of ^{14}C from dicamba was more rapid than absorption of ^{14}C from atrazine.

Autoradiographic studies using isolated astomatous apricot cuticles showed that cuticle areas over veins were preferred sites of entry for ^{14}C from oils in both pure and emulsified forms and for ^{14}C from dicamba regardless of carrier type. No preferred sites of penetration were found in cuticle areas directly above an individual cell whether the underlying cell was in a vein or non-vein area of the leaf.

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INTRODUCTION

Petroleum oils used in foliar sprays have often been reported to cause injury to plants. This phytotoxicity may be reduced by fractional distillation of the oils to eliminate extremely light or heavy components and by other refinement procedures. Chapman (10) has recommended several ways that less phytotoxic oils might be produced: (a) intensive hydrogenation to eliminate phytotoxic unsaturated structures, (b) addition of antioxidants or similar inhibiting chemicals, (c) production of narrower oil fractions. Such refining processes have promoted modern usage of mineral oils and natural (crop) oils in water emulsions to enhance postemergence herbicidal effectiveness over that provided by water or water carrier with surfactant.

Although properties of the less refined oils that cause phytotoxicity are known, relatively little information is available concerning special properties of the highly refined oils that may contribute toward herbicidal enhancement. Some theoretical mechanisms for oil enhancement of herbicidal activity include: (a) surface tension of the droplet is reduced causing better wetting and spreading, (b) oil remains on the leaf surface longer than

water keeping the leaf surface moist to allow penetration over a longer period of time, (c) oil, being more soluble in the cuticle than herbicides, may help carry the chemical through this apolar barrier (4). Many researchers feel that the enhancing effect of oil is due to increased penetration. It is known that oils and surfactants both reduce the surface tension of water permitting greater penetration through open stomates. However, the effects on cuticular penetration are less clear.

To learn more about oils and how they affect post-emergence spray applications of herbicides, two major problem areas were investigated. The first dealt with specific physical and chemical properties of oils as they are associated with phytotoxicity and herbicidal enhancement. Dry weight analyses of green foxtail (Setaria verdis (L.) Beauv.) and grain sorghum (Sorghum bicolor (L.) Moench 'Northrup King 133') were used to evaluate various oils, while simple correlation and multiple regression analyses were used to evaluate oil properties individually and by groups.

The second area of study dealt primarily with foliar penetration as a possible mechanism of herbicidal enhancement. Uptake of ^{14}C-labeled herbicides and oils through the cuticle of astomatous leaf surfaces of intact plants was determined by radioactive counting techniques, while

photosynthetic measurements indicated the speed at which this penetration occurred and possible involvement of the stomata in foliar penetration by oil emulsions. Isolated cuticles were used to compare penetration rates and quantities of herbicide taken up from various carriers. Finally, penetration sites in isolated cuticles were determined by autoradiography for a herbicide in three different carriers as well as for mineral and natural oils in the pure form and emulsified with water.

LITERATURE REVIEW

Recently, highly refined mineral and natural oils have been used in oil-water emulsions to enhance the effectiveness of foliarly applied herbicides. One of the herbicides most commonly used with oils is atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). With only few exceptions (9) better weed control and higher crop yields are obtained from a postemergence oil in water emulsion of atrazine than with water alone (86). Atrazine in an oil-water emulsion may also give superior weed control compared to herbicide in water plus surfactant (8, 71). Other herbicides sometimes used with oils for increased effectiveness include: chloroxuron (3-[p-(p-chlorophenoxy)phenyl]-1,1-dimethylurea) (62), pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) (20), and linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (65).

Mineral oils are a complex of numerous isomers (85), and it is difficult to identify particular components that affect plants. Two of the most important properties affecting oil phytotoxicity may be unsulfonated residue and distillation temperatures (80). Increasing unsulfonated residues are associated with decreasing phytotoxicity,

while distillation temperatures above 240 C (at 10 mm Hg) are associated with a high degree of phytotoxicity. Other important properties include: viscosity (SUS @ 37.8 C), gravity (API @ 15.6 C), pour point, molecular weight, percent aromatics, sulphur, bromine absorption, acidity, ability to emulsify, and color (10, 16, 28, 30, 47, 57). Studies on types of oils indicate that aromatic types are most toxic (36), followed by naphthenes and olefins, with the straight chain paraffinic oils being least toxic. Inhibition of photosynthesis in plants sprayed with oils becomes more severe as rate of oil deposition increases (58), and plants treated with naphthenic oils may recover more rapidly than those treated with paraffinic oils. Three ways recently recommended to produce less phytotoxic oils include: (a) intensive hydrogenation to eliminate phytotoxic unsaturated structures, (b) additions of anti-oxidants or similar inhibiting chemicals, (c) production of narrower oil fractions (10).

However, phytotoxicity is only one facet of the agricultural use of oils. More important, perhaps, is the enhancement of herbicide effectiveness by oil carriers. Preliminary investigations have indicated that less linseed and sunflower oil are required than a non-phytotoxic mineral oil to give comparable results (48). Viscosity of an oil may be as important in determining herbicidal

enhancement as it is in determining phytotoxicity of the oil alone (66). More detailed knowledge of properties of oil influencing herbicidal enhancement is lacking, although the enhancement appears to be due in part to increased penetration (5, 69, 78). Some theoretical mechanisms for oil enhancement of herbicidal activity include: (a) surface tension of the droplet is reduced causing better wetting and spreading; (b) oil remains on the leaf surface longer than water keeping the leaf surface moist to allow penetration over a longer period of time; (c) oil, being more soluble in the cuticle than herbicides, may help carry the chemical through this apolar barrier (4). Another possibility is that herbicidal enhancement by oil may be similar to the enhancement by surfactants. Surfactants increase the emulsifying, solubilizing, dispersing, spreading, and wetting of herbicide formulations on plant leaves (24). Also, surfactants may orient polarly and become solubilized in the cuticle. This could cause a swelling or loosening of the cuticle structure and result in increased penetration (24).

Penetration of chemicals through leaf surfaces has been widely studied and several excellent reviews are available (12, 23, 24, 26, 61). Penetration through surfaces containing stomates is rapid while penetration through astomatous leaf surfaces or when stomates are

closed is slow (13, 21, 83). Penetration of chemicals through astomatous leaf cuticles is primarily a diffusion process (76) and may be influenced by temperature, concentration, relative solubility of the penetrant molecule in organic solvents, and lipophilic substituents (15).

Penetration of oils into plant parts was early detected by staining methods. Petroleum oils were detected in twigs by using Sudan IV and Unna's polychrome methylene blue (37). Both the large petroleum droplets and smaller droplets of natural oils stained red, so an unsprayed control was required for comparison. A staining method has been developed for temporary mounts that differentiated between petroleum oils and natural plant oils and lipids (63). By an improved staining procedure (56) it was determined that citrus leaf cutin normally stained yellow with Oil Red O, but stained orange to red if oil soaked (45).

The amount of oil retained by citrus foliage has been determined by extracting with ether and recovering the oil in Babcock skim milk bottles (22). Spray oil depositions on citrus have been studied through colorimetric analysis with a spectrometer (18). Similarly, gas chromatographic analysis (34) has been used to measure persistence of oil droplets. Oils tend to become concentrated along leaf midribs and margins (64), and an oil soluble fluorescent

dye was found to penetrate mainly through the midrib and larger veins of grapefruit leaves (17).

From 67 to 75% of lighter oils and 33% of heavier oils disappear from citrus leaves within 3 weeks after application (64), and less volatile portions of oils may remain in citrus leaves throughout their lifetime, often more than 2 years. However, the persistence of these heavier oil fractions often does not appear to lessen leaf vigor. Lubricating oils rapidly penetrate potato leaves and persist for the remainder of the growing season (94). Stomates from citrus leaves resist penetration by water, but may be readily penetrated by oil (46, 82, 95).

Ten percent of the oil that is applied to leaves actually penetrates to the interior (66), and an oil with a SUS @ 37.8 C value of 70 has been found to essentially disappear from citrus and corn leaves within 7 days. However, when this same oil was applied to an inert surface, only 20% evaporated away while 80% remained on the inert surface.

Possible preferred sites of entry for various chemical compounds have been listed as follows: guard cells, inner cuticle of substomatal chambers, exterior cuticle above periclinal walls midway between anticlinal walls (25), cuticle over anticlinal epidermal walls, cuticle over veins, trichomes, open stomates, hydathodes, lenticles,

insect punctures, natural fissures and other imperfections in the cuticle (23). Increased penetration of an oil soluble dye has been observed over veins in astomatous leaves (17), but no generally preferred sites of entry have been determined for urea and inorganic ions (91).

Oils or lipophilic solutes may be absorbed via an apolar (lipid) route through the waxy portions of the cuticle, while polar organic and inorganic compounds may preferentially penetrate through cracks, punctures or leaf areas not completely covered by waxy lamellae (24). These hydrophilic compounds may then follow a polar route through the hydrated cutin or the hydrophilic cellulose and pectic portions of the wall. Substances such as formulated organic herbicides and surfactants may enter through combinations of the pathways listed above. Apple leaf cuticles are composed of discontinuous layers of cutin, cellulose and pectic materials (52), and the pectic substances may form a continuous pathway for polar solutes from the epidermal cells and vein extensions to the outside of the leaf (59). Submicroscopic channels within the cuticle may constrict (53) so that less water is lost from a dry than a moist cuticle. Wax platelets tend to move closer together in cuticles from plants under moisture stress (70), and penetration is hindered. However, permanent or semi-permanent wax canals could not be located

with the electron microscope during surface wax deposition on maize seedlings (68).

During cuticle development, cutin and a small quantity of wax appear first developing a finely layered structure (55, 75), with the main period of wax deposition occurring later. The chemical composition of the wax has been studied (1, 2, 35, 81, 88). The affinity of the cuticle for water depends on amount, physical configuration and chemical composition of the surface wax (73), and waxes containing large amounts of alkanes are least wettable (38). Cuticle permeability to water decreases as the amount of impregnating waxes increases (76). Removal of the wax may enhance both the wettability (39) and penetrability (76) of the cuticle, although the cutin itself inhibits water passage in relation to its thickness. Cuticular transpiration may be increased by rubbing away the wax bloom (19) or brushing a portion of the wax away with a camel's-hair brush (32).

The cuticle has also been reported to contain ectodesmata (27), structures which are thought to be similar to plasmodesmata. Ectodesmata are defined as continuations of the epidermal protoplasts that project through the outer epidermal walls into the cuticle. They do not extend to the outer leaf surface but are covered by the cuticle. Even so, they may be preferred sites for substances penetrating through the cuticle (25), since they are especially

plentiful in leaf structures that are known to be pervious such as guard-cells, conical hairs, anticlinal walls and epidermal cells adjacent to leaf veins.

Several methods for isolating cuticles from leaves and fruits have been reported, and the isolated cuticle membranes have been used in numerous studies of foliar penetration by polar solutes. Cuticles from apple peelings have been removed using a solution containing 1.6 percent ammonium oxalate and 0.4 percent oxalic acid (41). This method has been refined by other research workers (3, 60). In addition, cuticles have been released from various plant tissues that were immersed in filtrates from culture solutions of Bacterium aroideae (89). Intact leaf cuticles were isolated from several plant species using solutions containing pectic enzymes under controlled conditions of pH, concentration and temperature (15, 50, 93). Cuticular membranes have been separated from eight species using a solution containing 50% $ZnCl_2$ in concentrated HCl (40). This method compared favorably with or was superior to both the pectinase and the ammonium oxalate-oxalic acid methods for determining cuticle weights.

In penetration experiments with cuticles, relatively little movement of polar substances in aqueous solutions occurred through astomatous apricot leaf cuticles (49), and surfactants did not influence cuticle permeability (14).

In other studies, penetration of cuticular membranes of Euonymus Japonicus increased as molecular weights of test compounds decreased (43, 44). However, molecular size, electro-kinetic charge and spatial configuration did not influence the penetration of several chemicals through apple cuticles (29). Increasing wax contents of isolated astomatal cuticles by adding previously extracted wax onto the cuticular surfaces decreased penetration of naphthalene-acetic acid (7). Wax on isolated cuticle disks inhibited penetration of mercury from a solution of phenylmercuric acetate (74). Sorption (includes both absorption and adsorption) of various compounds to isolated cuticles was dependent on wax thickness (7), chemical composition of the wax (74) and presence or absence of surfactants (49). Also, sorption of a basic yellow dye was increased at low pH values and decreased at high pH values by a 0.1% solution of the anionic surfactant Vatsol OT (sodium dioctyl sulfo-succinate).

In other penetration studies with isolated cuticles, glycerine and glucose have been reported to decrease penetration of several compounds (including atrazine and simazine) through astomatous apple leaf membranes (29), and penetration of those compounds was greater from the mesophyll side than from the outer or air side. Other researchers have found different results. Greater

penetration of water occurred from the air side on leaves from Hedera helix L. (67), and greater penetration of organic compounds and inorganic ions occurred from the air side of tomato fruit cuticles (92, 93). In the latter case, enhanced foliar absorption was attributed to greater ion binding on the inside as compared with the air side of the isolated tomato fruit cuticles. Also, the binding of Ca^{++} to the isolated cuticles was stronger and occurred more quickly than the binding of SO_4^{--} (90). However, special binding sites for calcium ions, chloride ions, and urea could not be detected by microautoradiography on either the outer or inner astomatous tomato fruit cuticle (91). Binding sites for calcium and chloride ions on the stomatous green onion leaf cuticles appeared to be associated with the periclinal cell walls and stomatal pores. These point-like binding sites reported in stomated green onion leaves (91) correlated with the locations of ectodesmata (25).

MATERIALS AND METHODS

OIL PROPERTIES

Two experiments dealing with physical properties of oils as related to oil phytotoxicity and herbicide enhancement were conducted in the greenhouse with green foxtail and grain sorghum grown in plastic pots containing equal parts of sand and topsoil. Plants were selected for uniformity and thinned to nine and four plants per pot for foxtail and sorghum, respectively, prior to treatment at the 2.5-leaf stage. Horticultural perlite was placed on the soil surface before spraying and removed after the spray had dried, thus eliminating root uptake. Chemicals were applied in water at a rate of 187 liters/ha and pressure of 2.86 bar (20 gpa at 40 psi). Treatments were always applied uniformly during the afternoon. Shoots from the respective treatments were harvested 6 days after spraying and fresh and oven-dry weights recorded.

The first experiment was a factorial study using both species and four replications. Factors and levels were as follows: (a) eighteen different mineral oils (including 2% Triton X-207 emulsifier) at 9.34 liters/ha, emulsifier alone, and a check with no oil or emulsifier; (b) atrazine (80% wettable powder) at 0, 1.12, and 2.24

kg/ha of active ingredient. Components of the spray mixture were mixed in the following order: water, atrazine, oil and emulsifier. Simple correlation and multiple regression analyses for both foxtail and sorghum were computed on two groups of the oils, the groupings being dependent upon the amount of information available on each oil (see Appendix I for physical and chemical properties of oils). One group included 16 oils and 5 independent variables, while the other included 10 oils with data available on 17 independent variables.

In the second experiment, 19 mineral and 7 natural oils were tested on sorghum using procedures identical to those used in the first experiment. Supplemental lighting provided a 15.5-hour photoperiod to match the natural daylength of the previous experiment. Simple correlation and multiple regression analyses were computed on dryweight data for two groups from the oils. One group included 5 independent variables and 25 oils, while the other included 11 variables from 18 oils.

INTACT PLANT STUDIES

Cuttings from Tradescantia fluminesis Vell. were inserted in vermiculite to permit root establishment and then transferred to containers filled with 1,500 ml of nutrient solution (Hoagland's) in the controlled environment chambers when roots were 8-10 cm long. After the

plants had produced at least six leaves, the youngest fully expanded leaves were treated. These leaves were taped to a flat surface and rubber "O" rings (14.3-mm outside diameter with a 2.4-mm wall) were sealed to the upper leaf surfaces with Dow Corning silicone vacuum grease. The "O" ring on each treated leaf was placed centrally on the leaf and at one side of the midrib. Treatments containing 20 µl of ^{14}C-labeled solution were pipetted into these areas and allowed to penetrate for a given time period on duplicate plants.

Upon harvest, "O" rings and grease were removed, and treated leaves were washed with 10 ml of water to remove excess herbicide from the leaf surface. Where the treatment included atrazine, the leaves received an additional rinse with 10 ml of 50% methanol. Leaves treated with ^{14}C-oil were washed only with excess 2% detergent (Liqui-Nox) prior to drying.

Harvested plants were divided after washing into the following portions: Treated leaves (first fully expanded leaf, and second fully expanded leaf), area above treated leaves, area below treated leaves, and roots. These plant parts were oven dried at 80 C, ground to pass through a 40-mesh screen, placed in a 30-mm diameter planchet, and analyzed in a Packard Model 210 Flo-Window Counter with a 1.4-mg/cm^2 Mylar window. Corrections for self absorption

of samples of intermediate and infinite thickness were determined according to the method described in Chase and Rabinowitz (11). Counting errors and confidence of mean values were determined as described in Overman and Clark (51).

One-ml aliquots of the various rinses were placed in vials containing 10 ml of scintillation solution (100 g of naphthalene; 7 g 2,5-diphenyloxazole; and 50 mg of 1,4-bis-[2-(5-phenyloxazolyl)] -benzene, made up to 1 liter with 1,4-dioxane). These were analyzed in a model 524 Packard Tri-Carb Liquid Scintillation Spectrometer. Radioactivity in the vacuum grease and "O" rings was analyzed by thin window counting in early experiments, and by liquid scintillation counting in later experiments. Radioactivity from the nutrient solutions was determined by both methods.

Photosynthetic responses of plants to atrazine in an oil-water emulsion were measured on grain sorghum grown in controlled environment chambers at 24.4 $\pm$ 3 C in 1090 $\pm$ 70 ft-c of light with a 16-hour day. Treatments including 5% emulsified oil (see oil no 13, Appendix I) and 95% water were applied at the rate of 186.9 liters/ha. Plants were allowed to dry 5-10 minutes after spraying before effects of spray treatment on photosynthetic rates were determined. Procedures and equipment for the CO_2 analyses were the same as those described by Stymiest

(79). Four plants were used per treatment. The third leaf of each plant was removed near the leaf collar and placed in plastic embedding capsules filled with distilled water to maintain turgor while CO_2 measurements were taken. Detached leaves so mounted were placed in glass tubes suspended in a water bath and illuminated from both bottom and top by 4,500 ft-c of light. Air cycled through these glass tubes was analyzed by a Beckman 215 infrared CO_2 gas analyzer. Calculations were expressed either as µl of CO_2 per gram dry weight of tissue per minute, or as CO_2 utilized as a percent of the control. Approximately 1 hour elapsed from the time of spraying until tissues were placed in the glass tubes for analyses. Tissues were in the dark 20-30 minutes while being transported from the spraying room to the CO_2 analyzer in a box.

ISOLATED CUTICLE STUDIES

Penetration

Cuticles from the upper astomatous surface of Prunus armeniaca L. (apricot) leaves were isolated enzymatically and treated with 50% $ZnCl_2$ in concentrated HCl to remove cellulose and attached cell remnants. Preliminary experiments showed pectinase (pectinal 100 D #17, Rohm & Haas Co.) release of cuticles was preferential to three other commercial enzyme preparations and to other published

methods (40, 41). Also, no differences in cuticular penetration of Ficus elastica (Roxb.) or apricot leaves by ^{14}C-dicamba (3,6-dichloro-o-anisic acid) were observed when comparing methods of cuticle isolation or post-isolation treatment for cellulose removal (Table 1). Treatment of isolated cuticles with cuprammonium (41) did not remove all attached cellular debris and left a precipitate on the cuticle. The $ZnCl_2$-HCl treatment removed nearly all constituents except cutin and wax. This was confirmed by staining treated cuticles with Ruthenium Red (54) which gives a red color to materials containing pectin, cellulose starch, and lignin.

To prepare a supply of cuticle membranes for penetration studies, apricot leaf disks 18 mm in diameter were placed in beakers containing 2% pectinase buffered with acetate to a pH of 4.2. The enzyme was kept in partial suspension by mechanically moving the beakers back and forth over a 4-cm linear path 88 times per minute in a water bath at 37 C. Cuticles were released in 10-12 days, and they were then rinsed and stored in deionized water at 5 C. Enzyme-released apricot cuticles weighed 340 $\mu g/cm^2$ (Table 1). Treating with $ZnCl_2$-HCl for 90 minutes caused a 50% weight reduction. Further treatment of these cuticles for 5 minutes reduced dry weight another 17%.

Table 1. Treatments affecting dry weight and penetration of ^{14}C-labeled dicamba through enzyme released apricot leaf cuticles.

Measurement	Cuticle treatment		
	$CuO-NH_4OH$	$ZnCl_2-HCl$	Check
Wt $\mu g/cm^2$	1,240 [a/]	170	340
Wt as percent of check	--	50	100
Wt loss from chloroform soak $\mu g/cm^2$	--	60	60
Wt loss as percent of original treatment wt	--	34	17
Percent ^{14}C-labeled dicamba penetration in .5 hour	3	3	4

a/ Additional dry weight values not included for cuprammonium treatment since the copper precipitate invalidated relationships.

This indicated that the cellulose-free cuticles ($ZnCl_2$-HCl treated) were 34% wax.

Prior to penetration tests, cuticles were immersed for 90 minutes in $ZnCl_2$-HCl, rinsed, and placed in petri-dishes containing deionized water. Two percent agar disks (22 mm diameter X 2 mm thick) were lowered beneath the cuticles and raised so that the cuticles spread out flat on the agar surface. Agar disks + cuticles were placed in 30-mm diameter planchets and the upper surface of the cuticle was allowed to air dry 20-30 minutes before treatment. Isolated cuticles tended to become orientated with the more lipophilic air side exposed upward and the mesophyll side in contact with the water. Also, the edges of the cuticle disks tended to curl inward and away from the water. Thus orientation of the cuticle with the proper surface exposed for treatment was obtained by visual inspection and then confirmed by microscopic observations.

Cuticles were tested for cracks and insect punctures by observing the penetration of fluorescein. Agar disks (14 mm diameter X 2 mm thick) were placed on top of the cuticles. A 10-µl droplet of water containing 3.84×10^{-4} moles of the polar dye was pipetted onto the upper surface of each disk and allowed to penetrate 24-48 hours while agar cuticles and planchets were enclosed in petri-dishes containing a 2-mm layer of agar. This procedure provided

an atmosphere of near 100% relative humidity and arrested desiccation of the agar disks during the penetration period. Upon inspection under an ultraviolet lamp, cuticles which allowed significant amounts of the fluorescent dye to penetrate were discarded. Generally, four to five cuticles were retained from the original 20 prepared for each treatment.

In penetration testing, ^{14}C-labeled dicamba and atrazine were either applied to the top agar block or pipetted directly onto an area of the cuticle enclosed by an "O" ring (14.3-mm outside diameter with a 2.4-mm wall) that was sealed to the cuticle with vacuum grease. Normally, 20-µl droplet applications were used, and cuticles were placed in petri-dishes containing agar during the penetration period to maintain a saturated atmosphere. Treatments including 5% oil all utilized the same paraffinic oil (see oil no 4, Appendix I) with 2% emulsifier (Triton X-207). Surfactant solutions included 0.1% Vatsol OT.

Following the penetration period, cuticles were removed and the lower agar disks placed in vials containing 10 ml of the dioxane scintillating solvent and analyzed as described previously. Counting efficiency and percent penetration were determined from known standards. Prior to statistical analysis the data were inspected and

samples that showed obvious contamination during removal of cuticles from the agar disks were rejected.

Astomatous apricot cuticles were prepared and treated with labeled chemicals as described above to determine sorption to the isolated cuticles. Penetration time was 1.5 hours. Treatments including ^{14}C-dicamba contained 500 nanocuries (one nc equals 10^{-9} curies) in each 20-µl droplet. Oil treatments included 31 nc for ^{14}C-linseed and 6 nc for the ^{14}C-mineral oil applied in a 1-2-µl aliquot in the pure form and combined with 19 µl of water in the emulsified form. Cuticles were then separated from the agar disks and rinsed by immersing three times in each of four beakers containing deionized water. The first two beakers also contained 2% detergent by volume. The cuticles were then mounted on clean glass microscope slides, allowed to dry, and given a final rinsing by immersing each glass slide and attached cuticle three times in each of two beakers of water. Radioactive compounds released in each rinse were recovered and measured by liquid scintillation analysis. Radioactivity retained by the cuticle was measured by thin-window counting.

Autoradiography

Three different methods used to obtain autoradiographs of the cuticles included using nuclear track plates,

stripping film, and the liquid emulsion dipping technique. Nuclear track plates (Kodak type NTB) had a 10-μ emulsion thickness and were positioned against slides supporting treated cuticles so that the cuticles were in contact with the sensitive emulsion. This position was maintained during the period of exposure by securely taping the slide to the nuclear track plate. Stripping film (Kodak type AR. 10) was cut into sections approximately 4 by 5.3 cm and floated in a water bath at 18 C for 2-3 minutes. After the film swelled, and wrinkles started to smooth out, a slide with attached cuticle previously lowered beneath the floating film was raised so that the film covered the cuticle and overlapped on the back of the slide. The slides were air-dried in a vertical position on paper towels for 2 hours before being placed in a light-tight box. Liquid emulsion (Kodak type NTB) kept under refrigeration was in the form of a gel. A quantity of this gel was removed from the stock bottle with a plastic spatula and placed in a 50-ml beaker. This beaker was then placed in a water bath at 41 $\pm$ 2 C. After 1 hour a quantity of the melted emulsion was poured into a container designed especially for coating slides (31). Slides with attached cuticles were held vertically and dipped into the melted emulsion and completely withdrawn three times within a period of 6 seconds. After drying for 30

minutes, the dipping procedure was repeated to build up a thicker layer of photographic emulsion over the cuticle. Approximately 15 ml of melted emulsion were required for the double coating on 21 slides. After the last application of photographic emulsion, slides were air-dried in a vertical position on paper towels for 2 hours before being placed in a light-tight box.

All operations involved in application of emulsions were carried out in the dark or 4 feet away from a 15-watt bulb shielded with a safe-light filter (Kodak Wratten series 6B). After slides were placed in contact with the photographic emulsions, they were put in light-tight boxes containing desiccant and stored at 5 C. After an exposure period of 3 to 59 days, the slides were developed, rinsed, and fixed by standard techniques (31). Developing, rinsing, and fixing of autoradiographs were done in Coplin jars. Pictures were taken through a microscope using Kodak Panatomic-X fine grain black-and-white film.

RESULTS

OIL PROPERTIES

Two experiments were conducted to identify specific properties of oils that enhance herbicidal effectiveness and compare these to properties which induce toxic responses in plants in the absence of herbicides. These properties were determined through the use of simple correlation and multiple regression analyses of the effects of numerous oil properties on the dry weights of sorghum and green foxtail exposed to oils with and without atrazine in 5% oil in water emulsions. Experiment 1 tested both phytotoxic and herbicidal enhancement effects of 18 different mineral oils on foxtail and sorghum plants. Experiment 2 included the same 18 oils with one additional mineral and seven crop oils. These oils were tested for enhancement of atrazine effects on sorghum only. The two experiments will be discussed together since Experiment 2 was a partial repeat of Experiment 1.

Oils used at the rate of 3.78 liters/ha in a 5% oil in water emulsion did not significantly reduce dry weights of foxtail or sorghum compared to untreated controls (Table 2), although a few of the oils appeared more toxic to foxtail than others. A significant reduction in dry

Table 2. Milligrams dry weight of plants sprayed in the 2.5-leaf growth stage with atrazine. Experiment 1.

Oil number[a/]	Zero atrazine		2.24 kg/ha atrazine	
	Foxtail	Sorghum	Foxtail	Sorghum
Check I	70 abcde[b/]	316 a	57 d	278 g
Check II	73 cde	281 a	53 cd	264 efg
1	78 cde	305 a	30 a	244 defg
2	58 ab	282 a	35 ab	222 bcdef
3	82 de	298 a	35 ab	218 bcde
4	69 abcd	283 a	29 a	217 bcde
6	83 e	292 a	46 bcd	227 bcdef
7	62 ab	311 a	51 cd	270 fg
8	66 abc	275 a	45 bcd	214 bcd
9	69 abcd	282 a	42 abc	264 efg
10	59 ab	315 a	33 ab	230 bcdefg
11	69 abcd	282 a	32 ab	244 defg
12	66 abc	298 a	34 ab	242 cdefg
13	67 abc	292 a	34 ab	264 efg
14	68 abc	317 a	34 ab	155 a
15	56 a	296 a	34 ab	239 bcdefg
16	59 ab	281 a	36 ab	195 abc
17	62 ab	283 a	37 ab	234 bcdefg
18	63 ab	294 a	28 a	196 abcd
19	71 bcde	309 a	40 abc	191 ab

a/ Check I is water; check II is water + 0.1% Triton X-207; for other oils, see Appendix I. Oils numbered 1-5 and 15-17 are naphthenic, oils numbered 8 and 9 are aromatic, while the rest are paraffinic types.

b/ Means within each column having a common letter are not significantly (0.05) different using Duncan's multiple range test.

weight resulted from the inclusion of 2.24 kg/ha of atrazine in oil-water emulsions formulated with many of the oils tested in the two experiments (Tables 2 and 3). Moreover, oil-water emulsions of atrazine produced significantly greater reductions in dry weight than atrazine in water with a surfactant in the case of 13 oils tested on foxtail and five on sorghum in Experiment 1 (Table 2) as well as 11 oils tested on sorghum in Experiment 2 (Table 3). Oils numbered 14 and 16 (Appendix I) provided greater herbicidal enhancement than the 0.1% surfactant in all three instances listed above. Oils numbered 6, 7, and 9 in oil-water emulsions failed to surpass the effectiveness of the 0.1% surfactant carrier for either foxtail or sorghum.

When paraffinic oils were compared to naphthenic types, the two were observed to have opposite effects. Napthenic oils were generally more phytotoxic to foxtail and sorghum than paraffinic types (Table 4). However, when they were used in combination with 2.24 kg/ha of atrazine, paraffinic oils appeared to enhance herbicidal activity more than naphthenic types (Tables 3 and 4).

As rates of herbicide application were increased, progressively greater reductions in fresh and dry weights were observed (Table 5), and sorghum was less affected by atrazine than foxtail (Tables 2 and 5). Weight reductions

Table 3. Milligrams of dry weight of grain sorghum plants sprayed in the 2.5-leaf growth stage with 2.24 mg/ha of atrazine. Experiment 2.

Oil Number [a]	Oil Type	Mean
Control		339 a[b]
Surfactant + atrazine		278 ab
6	Paraffinic	274 abc
H_2O + atrazine		259 bcd
23	Alkali Refined Linseed	259 bcd
22	Crude Linseed	255 bcd
7	Paraffinic	245 bcde
25	Sunflower	240 bcde
9	Aromatic	235 bcde
20	Crude Corn	227 bcde
8	Aromatic	222 bcde
21	Cotton Seed	221 bcde
3	Naphthenic	219 bcde
26	Soybean	219 bcde
24	Peanut	216 bcde
4	Naphthenic	215 bcde
10	Paraffinic	204 bcde
1	Naphthenic	204 bcde
2	Naphthenic	200 cde
5	Naphthenic	200 cde
12	Paraffinic	198 de
16	Naphthenic	196 de
15	Naphthenic	194 de
19	Paraffinic	193 de
18	Paraffinic	193 de
14	Paraffinic	179 e
17	Naphthenic	178 e
11	Paraffinic	172 e
13	Paraffinic	171 e

a/ See Appendix I.

b/ Means with a common letter are not significantly (0.05) different using Duncan's Multiple Range Test.

Table 4. Dry weights of foxtail and sorghum as influenced by various oils in 5% oil in water emulsions with atrazine.

Foxtail			Sorghum		
Oil no[a/]	Type of oil	Dry wt mg	Oil no[a/]	Type of oil	Dry wt mg
		Zero atrazine			
Check I		70	Check I		316
Check II		73	Check II		281
19	paraffinic	71	14	paraffinic	317
11	paraffinic	69	10	paraffinic	315
14	paraffinic	68	19	paraffinic	309
13	paraffinic	67	12	paraffinic	298
12	paraffinic	66	15	naphthenic	296
18	paraffinic	63	18	paraffinic	294
17	naphthenic	62	13	paraffinic	292
10	paraffinic	59	17	naphthenic	283
16	naphthenic	59	11	paraffinic	282
15	naphthenic	56	16	naphthenic	281

Table 4 (continued)

Foxtail			Sorghum		
Oil no[a/]	Type of oil	Dry wt mg	Oil no[a/]	Type of oil	Dry wt mg
		2.24 kg/ha of atrazine			
Check I		57	Check I		278
Check II		53	Check II		264
19	paraffinic	40	13	paraffinic	264
17	naphthenic	37	11	paraffinic	244
16	naphthenic	36	12	paraffinic	242
15	naphthenic	34	15	naphthenic	239
12	paraffinic	34	17	naphthenic	234
14	paraffinic	34	10	paraffinic	230
13	paraffinic	34	18	paraffinic	196
10	paraffinic	33	16	naphthenic	195
11	paraffinic	32	19	paraffinic	191
18	paraffinic	28	14	paraffinic	155

a/ Check I is water; check II is water + 0.1% Triton X-207; for other oils see Appendix I.

Table 5. Effect of atrazine averaged over 18 oils on weights of plants sprayed in the 2.5-leaf growth stage.

Atrazine rate	Foxtail		Sorghum	
	mg	% of check	mg	% of check
		Fresh weight		
check	465	100 %	1715	100 %
1.12 kg/ha	265**	57	1747	102
2.24 kg/ha	223**	48	1618*	93
		Dry weight		
check	68	100 %	295	100 %
1.12 kg/ha	42**	62	252**	85
2.24 kg/ha	38**	56	230**	78

*,** Significant in each column within the fresh or dry weight analyses at the 0.05 and 0.01 levels, respectively, using Dunnett's two-sided test.

expressed as a percent of the control were greater for dry weights than fresh weights of sorghum treated with 2.24 kg/ha of atrazine (Table 5). The opposite effect occurred with foxtail where the greater reductions occurred in the fresh than in the dry weights.

The interaction between levels of atrazine and type of oil was significant, but multiple range tests could not be computed for the foxtail and sorghum dry weights since treatments were not independent. Therefore, simple correlation coefficients were calculated to compare oil properties as related to plant toxicity and herbicidal enhancement. Seven properties were significantly correlated with phytotoxicity of oils to foxtail and one with sorghum in Experiment 1 (Table 6). No oil properties were correlated with atrazine enhancement in this experiment, but nine properties were significantly associated with herbicidal effects as expressed in dry weight reductions in Experiment 2 (Table 6).

Multiple regression analyses were computed in order to account for a larger portion of the dry weight variability than was explained from individual simple correlation coefficients. The independent variables (oil properties) were analyzed by using several computer passes to determine which oil properties best explained experimental variability in the dependent variable (dry weight).

Table 6. Simple correlation coefficients of 17 different properties from 10 different oils (Experiment 1) and 11 properties from 18 oils (Experiment 2) on the dry weight of plants sprayed in the 2.5-leaf growth stage.

Independent variables	Experiment 1[a]				Experiment 2[b]
	No atrazine		2.24 kg/ha atrazine		
	Foxtail	Sorghum	Foxtail	Sorghum	Sorghum
Viscosity (SUS) @ 37.8 C	.17	-.33	.43	-.25	.24
Gravity (API) @ 15.6 C	.61	.57	-.35	-.02	-.69**
Unsulfonated residue	.63*	.60	-.27	.00	-.84**
Percent aromatics	-.59	-.57	.34	-.04	-.56*
Molecular weight	.53	.06	.02	-.21	.81**
50% Distillation temp	.64*	.08	-.05	-.26	--
90% Distillation temp	.43	-.12	.26	-.33	--
10% Distillation temp	.79**	.19	-.03	-.10	--
5% Distillation temp	.80**	.21	-.00	-.08	--
Initial Distillation temp	.80**	.19	.05	-.05	--
Final Distillation temp	.30	-.18	.29	-.42	--
Viscosity (SUS) @ 98.9 C	.36	-.21	.28	-.28	.65**
Viscosity index	.62	.52	-.37	.03	.78**
Flash point	.68*	.13	.03	-.11	.76**
Fire point	.79**	.18	.00	-.21	.79**
Refractive index	--	-.60	.39	-.02	-.09
Pour point	.54	.63*	-.28	-.25	.51*

a/ Included oils numbered 10-19, as listed in Appendix I.

b/ Included oils numbered 1, 2, 10-22 and 24-26, as listed in Appendix II.

*,** Significant at 0.05 and 0.01 levels, respectively, within each column.

For the computer analyses, the oils were classed into various families with the groupings being dependent on the amount of information (independent variables) available on each oil. Values for known properties of these oils are listed in Appendix I. Experiment 1 included two families: (a) five properties measured for 16 different oils and (b) 17 properties for 10 oils. Experiment 2 also included two families: (a) five properties measured for 25 different oils and (b) 11 properties for 18 oils. In Experiment 1, when five properties of 16 oils were analyzed, all five independent variables were considered at one time. The least important variable was dropped and the data reanalyzed. This process was continued until the factors remaining did not account for a major portion of the total variability. Analyses of 17 independent variables for groups of ten oils in Experiment 1 were more difficult. In multiple regression analyses, one degree of freedom is lost for the mean and another for the dependent variable. Since complete data were available for only ten oils, a maximum of eight variables could be computed at one time. With the foxtail data, variables were analyzed in groups of eight. The more important variables from these groups were chosen and recomputed in groups of eight until a total of four or five analyses was completed. With the sorghum data, eight independent variables were analyzed

and the least important variable was dropped. A new variable was then introduced and the new group analyzed again. This process was continued until all 17 oil properties were considered. In the second experiment, two groups of oils were considered. The first group included five properties measured for 25 oils, while the second group included 11 properties for 18 oils. All variables in the multiple regression analyses of these oils from Experiment 2 were considered at the same time. The most important variable was selected and those remaining were reconsidered. This process was continued until selection of other variables did not contribute a significant (0.01) amount of the dry weight variability.

Phytotoxic properties of oils were tested by multiple regression analyses in the first experiment only. Five properties tested over 18 oils accounted for 31% of the dry weight variability for both foxtail and sorghum (Table 7). This was little improvement over that obtained from simple correlations of these properties. Phytotoxic properties for the group of 10 oils were measured with more success. Combinations of eight of these properties were able to explain 99% or more of the dry weight variability (Tables 8 and 9). The procedure for determining oil properties in the group of ten oils was such that the most important oil properties would be present in the last

Table 7. Phytotoxicity of oils to foxtail and sorghum as evaluated by standardized partial regression coefficients for five different properties from 16 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage.[a/][b/]

Independent variables	Foxtail	Sorghum - Pass[c/]				
	Pass 1	1	2	3	4	5
Viscosity (SUS) @ 37.8 C	-.3505	-.0746	-.0620			
Gravity (API) @ 15.6 C	.6535	.9263	.9106	.8419	.5734	.5139
Percent aromatics	.6111	.3846	.3722	.2764		
Flash point	.6856	.4275	.4031	.3274	.1962	
Pour point	.2941	-.0175				
R^2 [d/]	.3094	.3085	.3084	.3075	.2990	.2641

a/ Included oils numbered 1-5, 7, 8, and 10-19, as listed in Appendix I.

b/ Larger values in each column (either positive or negative) account for a greater proportion of the variability within the range of oils tested.

c/ See text for explanation of computer passes.

d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

Table 8. Phytotoxicity of oils to foxtail as evaluated by standardized partial regression coefficients for 17 different properties from 10 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage.[a/]

Independent variables	Pass[b/c/]				
	1	2	3	4	5
Viscosity (SUS) @ 37.8 C	.6115	- .2871			
Gravity (API) @ 15.6 C	.0168				
Unsulfonated residue	-3.1234	- 3.2044			-2.1562
Percent aromatics	-3.8657	- 4.5825			-3.1717
Molecular weight	-1.2235	2.0657			
50% Distillation temp	-1.7169	-12.7258		.2542	-3.8385
90% Distillation temp	1.5816	5.0228			1.6279
10% Distillation temp	2.0170	34.1030		-5.3371	7.9185
5% Distillation temp		-27.1492		10.3994	-4.3353
Initial Distillation temp			2.2843	-1.7338	
Final Distillation temp			-1.5736	-1.6207	
Viscosity (SUS) @ 98.9 C			3.6248	4.5264	1.3240
Viscosity index			- .5670		
Flash point			-4.2247	-5.3015	-1.5590
Fire point			1.0013		
Refractive index			-1.5634	-1.1057	
Pour point			- .1255		
R^2 [d/]	.9540	.9801	.9947	.9954	.9824

a/ Included oils numbered 10-19 as listed in Appendix I.
b/ Larger values in each column (either positive or negative account for a greater proportion of the dry weight variability within the range of oils tested.
c/ See text for explanation of computer passes.
d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

Table 9. Phytotoxicity of oils to sorghum as evaluated by standardized partial regression coefficients for 17 different properties from 10 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage.[a/]

Independent variables	Pass [b/c/]				
	1	2	3	4	5
Viscosity (SUS) @ 37.8 C	- 2.0633	- 3.1389	- 3.4069	- 6.6695	-62.6481
Gravity API @ 15.6 C	.5198				
Unsulfonated residue	4.5625	4.5822	2.0884	.8880	
% Aromatics	3.0606	2.3411			
Molecular Weight	1.8135	4.4171	1.6291		
50% Distillation temperature	- 3.3665	-12.6884	- 8.7693	- 9.4004	-88.5782
90% Distillation temperature	4.2527	7.5771	8.5529	17.1037	89.2534
10% Distillation temperature	- 1.9476	25.4868	8.2147	4.2244	157.1662
5% Distillation temperature		-23.2182	- 1.9150	5.9518	-98.8771
Initial Distillation temperature			- 5.4052	- 9.3346	-32.4260
Final Distillation temperature				- 3.5946	-29.2205
Viscosity (SUS) @ 98.9 C					51.9822
Viscosity index					
Flash point					
Fire point					
Refractive index					
Pour point					
R^2 [d/]	.9469	.9582	.9413	.9625	1.0000

Table 9 (continued)

Independent variables	Pass b/c/				
	6	7	8	9	10
Viscosity (SUS) @ 37.8 C	8.3765	3.3564	5.0135	5.0341	- 2.3916
Gravity API @ 15.6 C					
Unsulfonated residue					
% Aromatics					
Molecular Weight					
50% Distillation temperature	13.8473	3.8475	7.2256	7.6042	- 3.7148
90% Distillation temperature	5.2618	8.1305	7.5198	6.7155	.3603
10% Distillation temperature	-38.3094	-19.1703	-31.1436	-27.8001	-16.5690
5% Distillation temperature	35.6623	21.4596	42.0676	29.3261	27.4255
Initial Distillation temperature	- 3.2863	- 5.8822	-12.8268	- 5.5262	-11.5608
Final Distillation temperature					
Viscosity (SUS) @ 98.9 C	-19.9845	-13.0896	-14.0645	-14.6024	5.8775
Viscosity index	- 0.9690				
Flash point		1.6668			
Fire point			- 2.7383		
Refractive index				0.1582	
Pour point					2.1106
R^2 d/	.9237	.9348	.9549	.9143	.9992

a/ Included oils numbered 10-19, as listed in Appendix I.
b/ Larger values in each column (either positive or negative) accounted for a greater proportion of the dry weight variability within the range of oils tested.
c/ See text for explanation of computer passes.
d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

computer pass. For both species the 5 and 10% distillation temperatures were the most important properties (Tables 8 and 9) for phytotoxicity of the oil. Together, these two properties accounted for 64.12% of the dry weight variability in foxtail and 62.79% in sorghum.

Multiple regression analyses were also used to determine combinations of oil properties that were important in herbicidal enhancement. In Experiment 1 these analyses accounted for 75.49% of the foxtail and 31.47% of the sorghum dry weights when five properties were considered over 16 oils (Table 10). Three properties, flash point, gravity (API) @ 15.6 C, and viscosity (SUS) @ 37.8 C, accounted for 75.35% of the dry weight variability in foxtail. Increasing values for flash point were strongly associated with decreased foxtail dry weights.

Multiple regression analyses were able to account for 99% or more of the dry weight variability from the group of 10 oils in Experiment 1 tested for atrazine enhancement on foxtail and sorghum (Tables 11 and 12). Maximum variation in dry weight was accounted for by computer pass no 2 for green foxtail (Table 11) and pass 10 for the grain sorghum (Table 12). The 5 and 10% distillation temperatures were the two most important variables in these high analyses and accounted for 72.79% in the foxtail and 57.70% of the total dry weight variability

Table 10. Enhancement of atrazine effectiveness on foxtail and sorghum as evaluated by standardized partial regression coefficients for five different properties from 16 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage with atrazine at 2.24 kg/ha.[a/][b/]

Independent variables	Foxtail - Pass			Sorghum - Pass	
	1	2	3	1	2
Viscosity (SUS) @ 37.8 C	.5124	.5406	.5856	-.9342	-.4687
Gravity (API) @ 15.6 C	.4545	.4191	.3115	1.3102	.7267
Percent aromatics	.1567	.1290		1.2568	.7988
Flash point	-.8828	-.9379	-1.0210	1.2275	.3181
Pour point	-.0394			-.6516	
R^2 [d/]	.7549	.7544	.7535	.3147	.1760

a/ Included oils numbered 1-5, 7, 8, and 10-19, as listed in Appendix I.

b/ Larger values in each column (either positive or negative) account for a greater proportion of the variability within the range of oils tested.

c/ See text for explanation of computer passes.

d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

Table 11. Enhancement of atrazine effectiveness on foxtail as evaluated by standardized partial regression coefficients for 17 different properties from 10 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage with atrazine at 2.24 kg/ha.[a/]

Independent variables	Pass[b/c/]			
	1	2	3	4
Viscosity (SUS) @ 37.8 C	- .7870	- .7722		
Gravity (API) @ 15.6 C	- .6284			
Unsulfonated residue	.0708			
Percent aromatics	-1.6036	- 1.4621		
Molecular weight	.6781	2.6791		
50% Distillation temp	-6.8752	-12.2659		-3.8205
90% Distillation temp	5.4277	6.3280		1.1719
10% Distillation temp	1.8278	18.3738	3.1955	-4.6114
5% Distillation temp		-15.4218		6.2972
Initial Distillation temp		1.2516		
Final Distillation temp			-2.9503	
Viscosity (SUS) @ 98.9 C			6.9534	3.6964
Viscosity index			-11.6472	-2.5930
Flash point			1.8282	
Fire point			-5.5670	-1.4205
Refractive index			-11.7594	-4.0588
Pour point			.1320	
R^2 [d/]	.9992	.9997	.7261	.9991

a/ Included oils numbered 10-19 as listed in Appendix I.
b/ Larger values in each column (either positive or negative) account for a greater proportion of the dry weight variability within the range of oils tested.
c/ See text for explanation of computer passes.
d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

Table 12. Enhancement of atrazine effectiveness on sorghum as evaluated by standardized partial regression coefficients for 17 different properties from 10 different oils on the dry weight of plants sprayed in the 2.5-leaf growth stage with atrazine at 2.24 kg/ha.[a/]

Independent variables	Pass [b/c/]				
	1	2	3	4	5
Viscosity (SUS) @ 37.8 C	7.6116	7.6386	7.1576	.8201	
Gravity API @ 15.6 C	3.3553	- 4.1134	- 4.0858	- 4.6123	- 4.3668
Unsulfonated residue	- 0.4717				
% Aromatics	2.3688	0.4590			
Molecular weight	2.6443	- 19.2852	- 16.7799	-12.0063	-11.9281
50% Distillation temperature	2.0607	64.5674	56.8257	35.2737	33.7949
90% Distillation temperature	-11.9131	- 24.6660	- 22.6171	- 4.4988	- 4.6167
10% Distillation temperature	.8836	-178.0126	-154.1449	-95.7441	-92.8176
5% Distillation temperature		151.9724	129.9591	81.9629	79.4810
Initial Distillation temperature			1.5534		
Final Distillation temperature				- 5.5250	- 5.4958
Viscosity (SUS) @ 98.9 C					1.7156
Viscosity index					
Flash point					
Fire point					
Refractive index					
Pour point					
R^2 [c/]	.7295	.9655	.9649	.9924	.9941

Table 12 (continued)

Independent variables	Pass b/c/				
	6	7	8	9	10
Viscosity (SUS) @ 37.8 C					
Gravity API @ 15.6 C	- 4.3902	- 4.7220	- 3.7032	- 0.2658	
Unsulfonated residue					
% Aromatics					
Molecular weight	-14.2927	-11.2310	- 7.2416	- 5.3232	- 5.3253
50% Distillation temperature	42.3414	32.4612	20.9704	18.3313	18.1683
90% Distillation temperature	- 3.5666	- 1.9891	.0267		
10% Distillation temperature	-115.6264	-87.7094	-54.3366	-49.1482	-48.3600
5% Distillation temperature	98.5850	75.3692	47.2847	42.4885	41.9159
Initial Distillation temperature					
Final Distillation temperature	- 7.0495	- 6.3906	- 6.0118	- 6.0831	- 5.8050
Viscosity (SUS)@ 98.9 C					
Viscosity index	- 1.2778				
Flash point		- 0.4275			
Fire point			.7220	- 0.7227	- 1.0035
Refractive index				3.1606	3.0689
Pour Point					- 0.2242
R^2 c/	.9843	.9921	.9930	.9980	.9988

a/ Included oils numbered 10-19, as listed in Appendix I.
b/ Larger values in each column (either positive or negative) account for a greater proportion of the dry weight variability within the range of oils tested.
c/ See text for explanation of computer passes.
d/ Value multiplied by 100 gives percent of the total variability accounted for in each column.

in sorghum. Several other properties important in different computer passes for foxtail included the 50% distillation temperature, refractive index, and viscosity index for foxtail (Table 11), and the 90% distillation temperature for sorghum (Table 12).

Properties of oils used in Experiment 1 and Experiment 2 were compared in a final test. Distillation temperatures, found important in previous regression analyses, were not included since these data were not available for the additional oils used in Experiment 2. Five properties accounted for 82.4% of the variability due to oils when tested over the 10 oils used in Experiment 1. These properties were pour point, viscosity index, viscosity SUS @ 37.8 C, refractive index and unsulfonated residue (Table 13). These same five properties accounted for 86.0% of the variability due to oils when tested over 18 oils in Experiment 2. Unsulfonated residue was found important in Experiment 2 but had less value in Experiment 1 where pour point and viscosity index analyzed together were found to be the two most important properties accounting for dry weight variability due to oils.

INTACT PLANT STUDIES

Leaves from Tradescantia do not have stomates on the upper surface, so they were used to study cuticular

Table 13. Simple correlation and multiple regression analyses for five important oil properties.

Oil Property [a]	Correl. Coeff.	Percent of Variability Individual	Cumulative
Experiment 1 [a]			
Pour point	-0.25	6.4	6.4
Viscosity index	-0.02	25.6	32.0
Viscosity SUS @ 37.8 C	-0.25	2.5	34.5
Refractive index	-0.02	27.4	61.9
Unsulfonated residue	-0.00	20.5	82.4
Experiment 2 [a]			
Unsulfonated residue	0.84	69.9	69.9
Viscosity SUS @ 37.8 C	0.24	3.7	73.6
Refractive index	-0.09	1.0	74.6
Viscosity index	0.78	7.0	81.6
Pour point	0.51	4.4	86.0

[a] Experiment 1 included oils numbered 10-19, while Experiment 2 included 1, 2, 20-22 and 24-26, as listed in Appendix I.

penetration of three different ^{14}C-labeled compounds into intact plants. These compounds included the somewhat polar herbicides dicamba and atrazine and a non-polar penetrant, mineral oil. Stomatal penetration was studied with grain sorghum by measuring CO_2 uptake by treated plants that were preconditioned with light or darkness to obtain the desired stomatal condition.

Experiment 1. Twenty µl of a 5% oil carrier containing 51 nc of ^{14}C-dicamba were applied to the "O" ring enclosed areas of Tradescantia leaves and allowed to penetrate for 1-6 days into duplicate plants. Penetration of ^{14}C from dicamba increased with time, although only 16% of the quantity applied was found in the plants after 6 days (Table 14). Most of the ^{14}C which was translocated out of the treated leaf accumulated in the plant portions above the treated leaf. Roots analyzed at both dates of harvest showed only traces of radioactivity. Analysis of the nutrient solution was made difficult because quenching, due to the chemicals in the Hoagland's solution, caused scintillation readings to be below background. However, thin window counting definitely indicated that radioactivity was present in the nutrient solution. Precise determination of the radioactivity was not attempted because of the difficulty of obtaining a representative

Table 14. Amount of ^{14}C from a 5% oil in water emulsion of dicamba remaining in Tradescantia plants after 1 and 6 days.

Area of plant	Percent of applied radioactivity	
	1 day	6 days
Treated leaf	4.38	2.42
Above treated leaf	3.70	8.69
Below treated leaf	2.94	4.99
Roots	0.36	0.07
Total	11.38	16.18
Nutrient solution	a/	a/

a/ Radioactivity was detected but was not accurately measured.

aliquot (1-2 ml) within the 1,500 ml of nutrient solution and the low counting efficiency (7%) of the thin-window method. Thus, leakage of radioactivity into the nutrient solution was not accounted for, and the sum of the radioactivity found in plant parts represented an undetermined portion of the total ^{14}C-dicamba taken up by the plants.

Experiment 2. Twenty-µl aliquots containing 2.6 nc of ^{14}C-atrazine were applied to the first two fully expanded leaves of Tradescantia. Comparisons were made after 1 and 6 days between oil and surfactant carriers using duplicate plants. Carbon^{14} from atrazine was absorbed and translocated in Tradescantia (Table 15) but was not detected in the nutrient solution using thin-window counting. Three times as much ^{14}C was taken up by Tradescantia from the 5% oil emulsion in one day as from the 0.1% surfactant solution. After 6 days the difference due to type of carrier was much smaller.

Generally, radioactivity increased in the various plant regions with prolonged treatment time (Table 15). Radioactivity was essentially absent from the area above the treated leaves after 1 day; however, after a treatment period of 6 days, 6 to 11% of the total radioactivity applied was concentrated in this area. The region between the treated leaves and the roots contained most of the radioactivity that had translocated out of the treated

Table 15. Percent uptake of ^{14}C from labeled atrazine by Tradescantia plants as influenced by penetration time and type of adjuvant.

Area of Plant	0.1% Surfactant		5.0% Oil	
	1 day	6 days	1 day	6 days
First treated leaf	1.9	8.1	11.5	21.2
Second treated leaf	2.7	7.0	15.9	9.8
Above treated leaf	--	6.1	0.2	11.0
Below treated leaf	7.1[1/]	31.7	7.7	19.2
Roots	--	2.6	1.0	0.7
Total	11.7	55.5	36.3	61.9

1/ Composite of the total activity present in the roots and plant parts above and below the treated leaves.

leaves in both treatment periods. Roots contained only a trace of radioactivity.

Analysis of ^{14}C-atrazine that did not penetrate after 6 days indicated that most of it was associated with the "O" rings and vacuum grease, and only slight amounts of radioactivity were obtained from water and methanol rinses of the grease-free leaf surface. More ^{14}C was recovered in the water and methanol rinses after the 1-day treatment periods than after 6 days.

Experiment 3. Oil number four (see Appendix I) was labeled with a reduced ^{14}C-propylene polymer having the following viscosities: 570 SUS @ 37.8 C; 55 SUS @ 98.9 C. This blend was tested for penetration as a 5% oil in water emulsion with treatment periods of 1, 6, and 15 days. Surface oil not absorbed by the treated leaves was removed by washing in excess 2% detergent.

Penetration of ^{14}C from the labeled oil increased with time (Table 16) up to 6 days, but little increase in radioactivity in the plant parts occurred between 6 and 15 days. The non-penetrating portion of the oil lost in the "O" rings, grease, and detergent rinse was not determined in this experiment. The percent of the radioactivity present in the treated leaves remained relatively constant over time. In periods up to 6 days, the area below the treated leaves contained most of the radioactivity.

Table 16. Percent uptake of ^{14}C from labeled oil in a 5% oil in water emulsion by Tradescantia plants.

Area of plant	Penetration times		
	1 day	6 days	15 days
First treated leaf	4.2	6.3	6.0
Second treated leaf	5.6	8.4	7.5
Above treated leaf	1.3	6.2	24.5
Below treated leaf	8.6	33.9	19.9
Roots	0.2	2.1	1.3
Total	19.9	56.9	59.2

However, after 6 days, most of the radioactivity was found in the new growth above the treated leaves. The roots contained very little radioactivity.

It was noted that an oil soaked area on the surface of the leaf extended beyond the boundary of the "O" ring and vacuum grease at the 6-day harvest. The diameter of this area averaged 19.9 mm and did not appreciably increase in size nine days later. It was not determined whether the oil passed through the vacuum grease barrier or diffused laterally through the cuticle or epidermis. However, preliminary visual observations on Tradescantia leaf surfaces and glass indicated that the vacuum grease was an effective barrier to linseed and mineral oils as compared to anhydrous lanolin, stopcock grease, or paraffin wax.

Experiment 4. Photosynthesis measurements were taken on sorghum sprayed with atrazine in a 5% oil in water emulsion to determine the ease of atrazine penetration under conditions favoring the opening of stomates. For this purpose, the plants were divided into two groups. Group I was preconditioned in darkness for 49.5 hours and sprayed immediately upon removal from the dark. Group II was preconditioned in darkness for 46.5 hours and then transferred to the light (1,400 ft-c) for 3 hours. Plastic bags were placed over the plants to further enhance opening

of the stomates. Treatments were replicated four times and included the 5% oil in water emulsion plus atrazine at 224, 448, and 673 mg/ha. Carbon dioxide measurements were taken 1.5 hours after spraying as well as 2 and 5 days later.

Measurements of CO_2 uptake at 1.5 hours after treatment revealed that photosynthesis was completely inhibited in plants exposed to conditions favoring stomate opening prior to treatment (Table 17). Plants presumably having stomates closed due to exposure to complete darkness prior to treatment utilized small quantities of CO_2 1.5 hours after treatment but rapidly responded to atrazine treatment with cessation of CO_2 utilization. Results 2 days later showed that light-treated plants still used less CO_2 than did the plants preconditioned with darkness. After 5 days, both treatments still had reduced CO_2 uptake, although leaves receiving light prior to spraying appeared to use more CO_2 than those from the dark. Plants receiving 673 mg/ha of atrazine were heavily damaged in comparison to those treated with lower rates and were not suitable for analyses at the 5-day period.

Experiment 5. The final experiment with intact plants was conducted to determine whether the quick response of sorghum sprayed with atrazine and oil could be prevented by washing the plants immediately after spraying

Table 17. CO_2 uptake (µl/g dry wt) of grain sorghum as affected by light and dark preconditioning periods and rates of atrazine.

Time after spraying	Atrazine (mg/ha)[a] 224 D[b]	224 L[c]	448 D	448 L	673 D	673 L	Check D	Check L	Minutes in CO_2 chamber[d]
1.5 hr.	78	0	45	0	46	0	656	436	12-28
	0	0	0	0	0	0	536	426	28-44
2 days	700	217	778	0	0	0	1580	1330	38-54
5 days	199	335	267	499	-	-	1186	1208	114-130

a/ Rates of atrazine (mg/ha) in oil-water emulsion.

b/ Plants received 49.5 hours of continuous darkness and were sprayed immediately upon removal from the dark.

c/ Plants received 46.5 hours of continuous darkness, were covered with plastic bags and transferred to light (1400 ft-c) for 3 hours before spraying.

d/ CO_2 measurements required 2 minutes for each treatment and were taken in order as treatments appear from left to right in the table.

by immersing first in a wetting agent (2% Tronic; Colloidal Products Corp.) and then in distilled water. Treatments included an untreated control; control plus the washing treatment; 1.12 kg/ha of atrazine in the 5% oil in water emulsion; and 1.12 kg/ha of atrazine in the emulsion plus the washing treatment. Treatments were replicated four times, and readings were taken 1.5 hours after spraying and washing.

The washing of unsprayed plants with 2% Tronic and distilled water reduced photosynthesis to approximately 80% of the level of the controls (Table 18). This reduction was constant over the 1-hour period of time that measurements were taken. Atrazine applied at a rate of 1.12 kg in the emulsion completely inhibited photosynthesis in about 30 minutes. Washing immediately after herbicide treatment did not prevent the reduction of photosynthesis but delayed the onset of complete inhibition.

ISOLATED CUTICLE STUDIES

Penetration

Isolated cuticles were used to determine rate, amount, and site of penetration of ^{14}C-labeled compounds through astomatous apricot leaf cuticles. These experiments had the following advantages for testing cuticular penetration over intact plant studies: (a) temperature

Table 18. CO_2 uptake of grain sorghum as influenced by herbicide and leaf-washing treatments.

CO_2 used as a percent of the control				Minutes in CO_2 chambers[c]
control	control + rinse[a]	atrazine in oil[b]	atrazine in oil + rinse	
100	84	31	67	3-11
100	79	3	29	28-36
100	81	0	7	58-66

a/ Plants immersed 3 times in 2% Tronic and 3 times in distilled water within 1 minute after spraying.

b/ 1.12 kg atrazine plus 9.34 liters of emulsified oil per hectare basis.

c/ CO_2 measurements required two minutes for each treatment and were taken in order as treatments appear from left to right in the table.

and humidity were accurately controlled; (b) large quantities of cuticle membranes were stored and used as needed; (c) the tests were relatively simple to conduct and were analyzed by scintillation counting (80% efficiency) rather than thin window counting (less than 7% efficiency); (d) cuticles were mounted directly on glass slides for autoradiographic studies eliminating difficult and tedious microtechniques necessary in intact plants.

Experiment 1. As a preliminary experiment 20-µl aliquots of ^{14}C-dicamba in three different carrier solutions were applied to upper agar disks and allowed to penetrate 48 hours. Carriers and nc of ^{14}C-dicamba applied were as follows: water, 205 nc; 5% oil in water emulsion, 189 nc; 0.156% acetone in the oil-water emulsion, 239 nc. Percent penetration through the isolated apricot cuticles ranged from 3.5 to 3.7%, and no significant difference due to type of carrier was observed.

Experiment 2. The effects of treatment duration and type of carrier were measured on the penetration of dicamba. Fifty-µl aliquots from a 0.1% surfactant solution and 5% oil emulsion were pipetted directly onto apricot cuticles into an area enclosed with an "O" ring. Both carriers contained 94 nc of ^{14}C-dicamba in the 15-minute and 20 nc in the 3-hour penetration trials. Since penetration times were short and desiccation of

agar not a problem, the experiment was conducted in the open air of the laboratory. Use of an oil emulsion carrier significantly enhanced penetration of ^{14}C from dicamba as compared to penetration of the 0.1% surfactant solution at both the 15-minute and 3-hour time periods (Table 19). Also, the total amount of herbicide penetrating the cuticles increased with prolonged penetration time.

Experiment 3. The next experiment measured the cuticular penetration of ^{14}C from atrazine as influenced by duration of treatment and type of carrier. Twenty-µl aliquots of each carrier containing 2.6 nc were pipetted directly onto the cuticle inside areas enclosed by "O" rings. The experiment was conducted in a water saturated atmosphere for 3-hour and 24-hour time periods. Significantly more ^{14}C penetrated the cuticles from the 5% oil than the 0.1% surfactant carrier in 3 hours (Table 20). In the 24-hour penetration trials, two additional carriers (water and 5% oil emulsion containing 0.156% acetone in water) were included. Orthogonal comparisons showed that water was significantly more effective than the other three carriers compared together at 24 hours (Table 20).

Experiment 4. Treatments containing 0.1% surfactant and 5% oil emulsion were repeated in a similar ^{14}C-atrazine experiment using a 24-hour penetration period. As an

Table 19. Effects of carrier and treatment time on the penetration of ^{14}C from dicamba through isolated apricot leaf cuticles.

Carrier	Percent of applied radioactivity	
	15 min	3 hrs
water + 0.1% surfactant	2.06	41.84
5% oil-95% water emulsion	5.44*	65.92**

*,** Differences are significant at 0.05 and 0.01 levels, respectively, within each column.

Table 20. Effects of carriers and treatment time on the penetration of ^{14}C from atrazine through isolated apricot leaf cuticles.

Carrier	Percent of applied radioactivity	
	3 hours	24 hours
water + 0.1% surfactant	0.71	5.27
5% oil - 95% water emulsion	1.26**	8.17
water + 5% oil + .156% acetone	--	6.85
water	--	12.66[a/]

** Difference is significant at 0.01 level within the column.

a/ Orthogonal comparisons indicated that the 12.66 mean was significantly (0.05) greater than the 5.27, 8.17, and 6.85 means compared together.

additional treatment, 5% oil was applied to cuticles mounted upside down. Orthogonal comparisons showed that significantly greater penetration occurred from the air side to the mesophyll side of the isolated cuticles (Table 21). In fact, very little penetration occurred when cuticles were mounted upside down. Also, for those cuticles mounted right side up, the 5% oil carrier appeared to allow more ^{14}C-atrazine penetration than the 0.1% surfactant carrier, although this difference was not significant at the 0.05 level.

Experiment 5. Another experiment was performed in a manner similar to the previous ^{14}C-atrazine study except that prior to treatment, the cuticles were extracted with chloroform for 30 seconds to remove wax. This caused a 15% reduction in dry weight and, judged by further extraction in chloroform, removed about 50% of the wax. Herbicide carriers were water and a 5% oil emulsion. Greater penetration was again obtained when the herbicide was applied to the air side rather than the mesophyll side of the cuticles (Table 22). No significant difference due to type of carrier was found when wax was removed from the cuticles.

Experiment 6. The effects of temperature and partial wax removal on the penetration of ^{14}C-atrazine through apricot cuticles were tested. A number of the cuticles

Table 21. Effects of carrier and side of cuticle treated in a 24-hour penetration study using isolated apricot leaf cuticles treated with ^{14}C-atrazine.

Carrier	Cuticle side treated	% of applied radioactivity[a/]
water + 0.1% surfactant	air	3.93
5% oil - 95% water emulsion	air	7.33
5% oil - 95% water emulsion	mesophyll	0.81

a/ Orthogonal comparisons showed that 3.93 and 7.33% were significantly (0.01) greater than 0.81%.

Table 22. Effects of carrier and side of cuticle treated in a 24-hour penetration study using isolated apricot leaf cuticles previously extracted with chloroform to remove wax and treated with ^{14}C-atrazine.

Carrier	Percent of applied radioactivity	
	Air side	Mesophyll side
5% oil in water emulsion	18.88	7.24
Water	16.80	8.70
Average	17.84**	7.97

** Difference between the two averages is significant at the 0.01 level.

were soaked in chloroform for 30 seconds, while others received no extraction. Half of the cuticles from each of these two groups above were kept at 5 C, and the remainder were held at 24 C during penetration periods. The 20-µl treatment aliquots contained 2.2 nc of ^{14}C-atrazine instead of the 2.6 nc used previously. Significantly greater penetration occurred at 24 C than at 5 C (Table 23). Also, penetration of ^{14}C from atrazine at 24 C was greater when the wax was partially removed than occurred in the case of the unextracted cuticles. The reverse occurred at 5 C, although the differences were not significant (0.05). However, penetration at 5 C was greater in all replications when the wax was intact than when wax was partially removed with chloroform.

Experiment 7. In the final quantitative experiment with isolated cuticles, compounds labeled with ^{14}C were tested for sorption as differentiated from penetration. This experiment differed from the previous studies in that radioactivity remaining in or bound to the cuticle was assayed instead of determining radioactivity passing through the cuticle into an agar disk below. The technique employed to measure sorption involved recovery of applied radioactivity in a series of washes. Sorption of ^{14}C-dicamba was low irrespective of carrier type (Table 24), although more ^{14}C was removed in the early rinses

Table 23. Effects of temperature and partial removal of wax in a 24-hour penetration study using isolated apricot leaf cuticles treated with ^{14}C-atrazine.

Additional cuticle treatment	Percent of applied radioactivity		
	5 C	24 C	Mean
None	3.09[b/]	4.55[b/]	3.82
Wax extracted	0.88[b/]	28.74[a/]	14.81**
Mean	1.99	16.65**	---

** Differences between means for cuticle treatment and temperature are significant at the 0.01 level. Values for individual treatments followed by the same letter do not differ significantly (0.05).

Table 24. Percent of applied radioactivity recovered in various rinses, from ^{14}C-labeled compounds allowed to penetrate 1.5 hours through isolated apricot leaf cuticles.

Rinse no.	Dicamba[1]			Oil Alone		Emulsions	
	water	surfactant	oil	mineral[1]	linseed[1]	mineral[1]	linseed[1]
1[2]	12.07	32.29	15.60	3.31	0.18	9.20	3.45
2[3]	29.72	47.25	44.58	23.88	25.40	15.25	11.36
3[3]	27.15	15.13	27.09	35.38	15.86	21.24	21.78
4[3]	12.12	2.81	5.59	14.13	4.22	18.15	16.54
5[3]	7.39	0.59	1.94	4.41	3.79	13.07	8.91
6[4]	9.13	0.44	3.53	3.66	2.93	12.98	8.59
7[5]	1.05	0.31	0.41	0.28	0.01	0.59	0.59
8[5]	0.17	0.04	0.09	0.26	0.04	0.59	1.18
Total	98.80	98.86	98.82	85.32	52.45	91.08	72.41
Cuticle[6]	1.20	1.14	1.17	14.68	47.55	8.92	27.59

1/ Compound was labeled with ^{14}C.
2/ Cuticle separated from agar by immersing agar in water.
3/ Isolated cuticles immersed three times in deionized water. Rinses 3 and 4 contained 2% detergent.
4/ Isolated cuticles mounted on glass slides.
5/ Cuticles on glass slides immersed three times in deionized water.
6/ Percent of the radioactivity remaining on cuticles after rinsing treatments.

from the 0.1% surfactant solution than from the other two carriers. On the other hand, sorption of oil was relatively high compared to herbicide. Moreover, three times more ^{14}C-linseed oil was bound to the cuticles than ^{14}C-mineral oil regardless of form of application (Table 24). Sorption of oils applied in pure form was 1.7 times greater than when applied in the emulsified form.

Autoradiography

Experience in autoradiography with isolated cuticles indicated that a total of 20,000 disintegrations per mm^2 was necessary as a minimum level of radioactivity to obtain a good autoradiograph. Therefore, a number of autoradiographs were prepared from cuticles exposed to varied penetrant molecules and carrier solutions with exposures ranging from 8,300 to 15,144,000 disintegrations per mm^2 of cuticle tissue. Time of exposure was dependent on the amount of radioactivity in each cuticle and varied from 3 to 59 days. Fewer disintegrations per mm^2 were required to give a good image on nuclear track plates than on liquid emulsion or stripping film. However, the latter two emulsions were coated directly on the cuticles and developed in place. This allowed more intimate contact between cuticle and emulsion, and localization of emitted radiation could be defined more accurately than with the nuclear track plates.

Autoradiographs showed differences in patterns of accumulation of ^{14}C from dicamba in apricot cuticles. Large, dense areas of silver deposition revealed random accumulations of radioactivity distributed uniformly over the treated area of cuticles exposed to dicamba applied in water (Figures 1-a, b, and 2-a, b). Similar areas were also observed in the case of cuticles treated with 5% oil emulsion, but they were much smaller in size (Figures 1-c, d, and 2-c, d). Treatment with the 0.1% surfactant solution produced a relatively even distribution of radioactivity and few areas of extremely dense silver deposition on the autoradiograph (Figures 1-e, f, and 2-e, f). Heavy concentrations of ^{14}C from dicamba tended to be associated with the cuticle areas directly over veins in the leaf regardless of the carrier solution used with the herbicide.

Both the ^{14}C-mineral and linseed oils tended to accumulate in areas over the veins whether in pure or emulsified form (Figures 3 and 4). In areas between the veins, the radioactivity was randomly distributed, and no particular areas of ^{14}C accumulation were observed (Figure 5-c, d).

Patterns of radioactivity above individual cells revealed no preferred sites of entry for ^{14}C from linseed oil either over cells above the veins (Figure 5-a, b), or over cells in areas between veins (Figure 5-e, f).

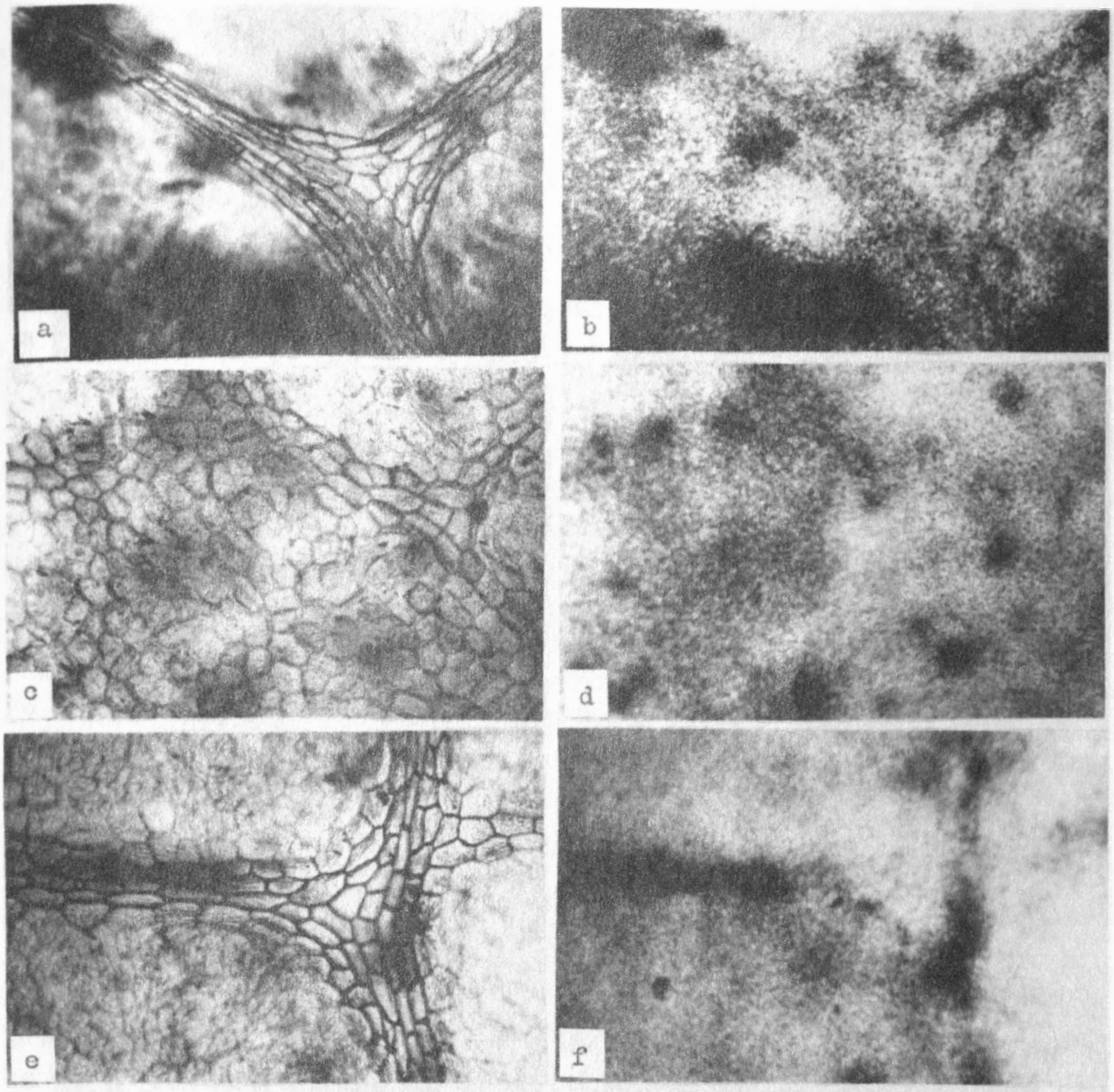

Figure 1. Focus is on astomatous apricot leaf cuticles treated with ^{14}C-dicamba in various carriers (a, c, e) and autoradiographs on nuclear track plates from these cuticles (b, d, f) showing accumulations of ^{14}C from dicamba. Carriers were as follows: a and b, water; c and d, 5% oil-95% water emulsion; e and f, 0.1% surfactant. About 380 X.

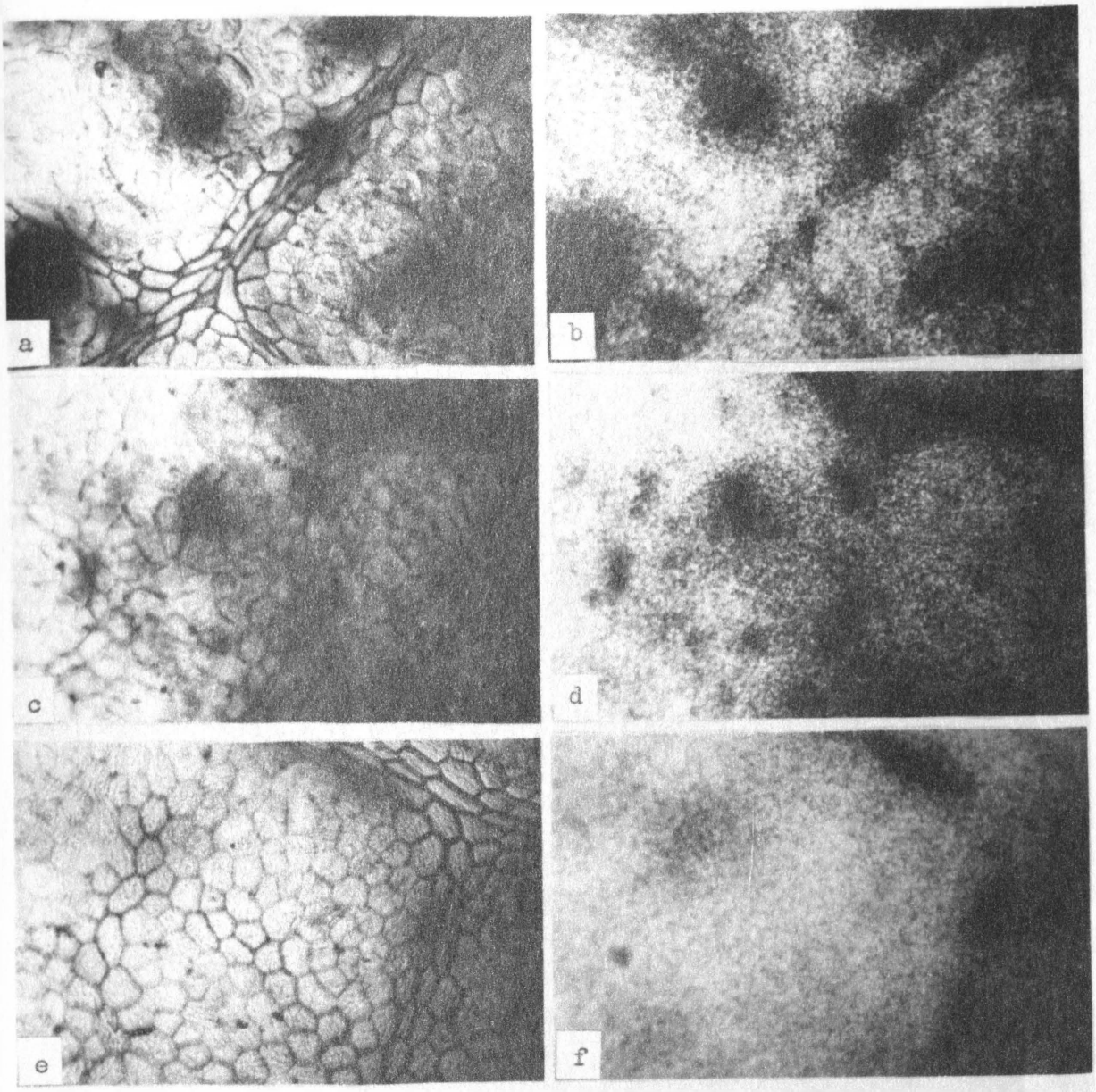

Figure 2. View of the treatments of figure 1 showing another region of each cuticle. Focus is on astomatous apricot leaf cuticles treated with ^{14}C-dicamba in various carriers (a, c, e) and autoradiographs on nuclear track plates from these cuticles (b, d, f) showing accumulations of ^{14}C from dicamba. Carriers were as follows: a and b, water; c and d, 5% oil-95% water emulsion; e and f, 0.1% surfactant. About 380 X.

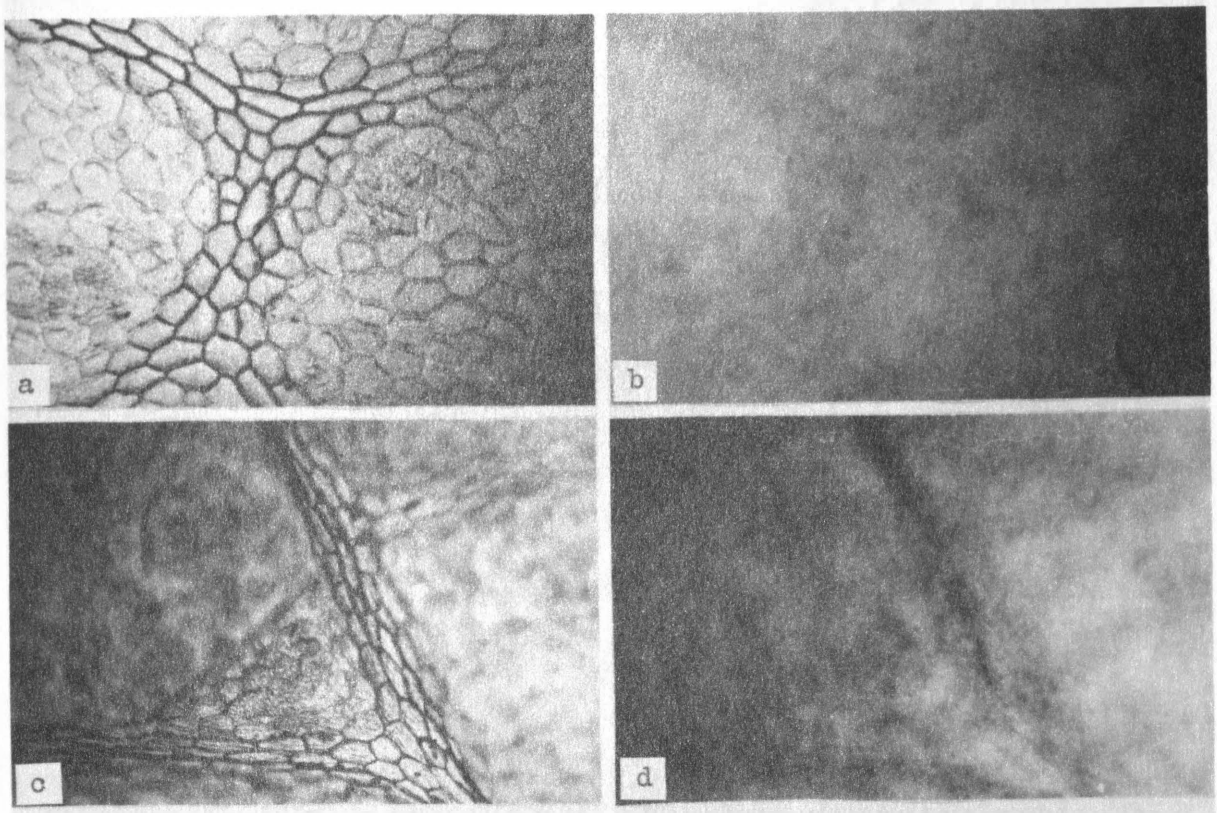

Figure 3. Astomatous apricot leaf cuticles treated with ^{14}C-mineral oil in the pure form (a, b), and emulsified with 95% water (c, d). Nuclear track plates b and d correspond to cuticles a and c, respectively. About 380 X.

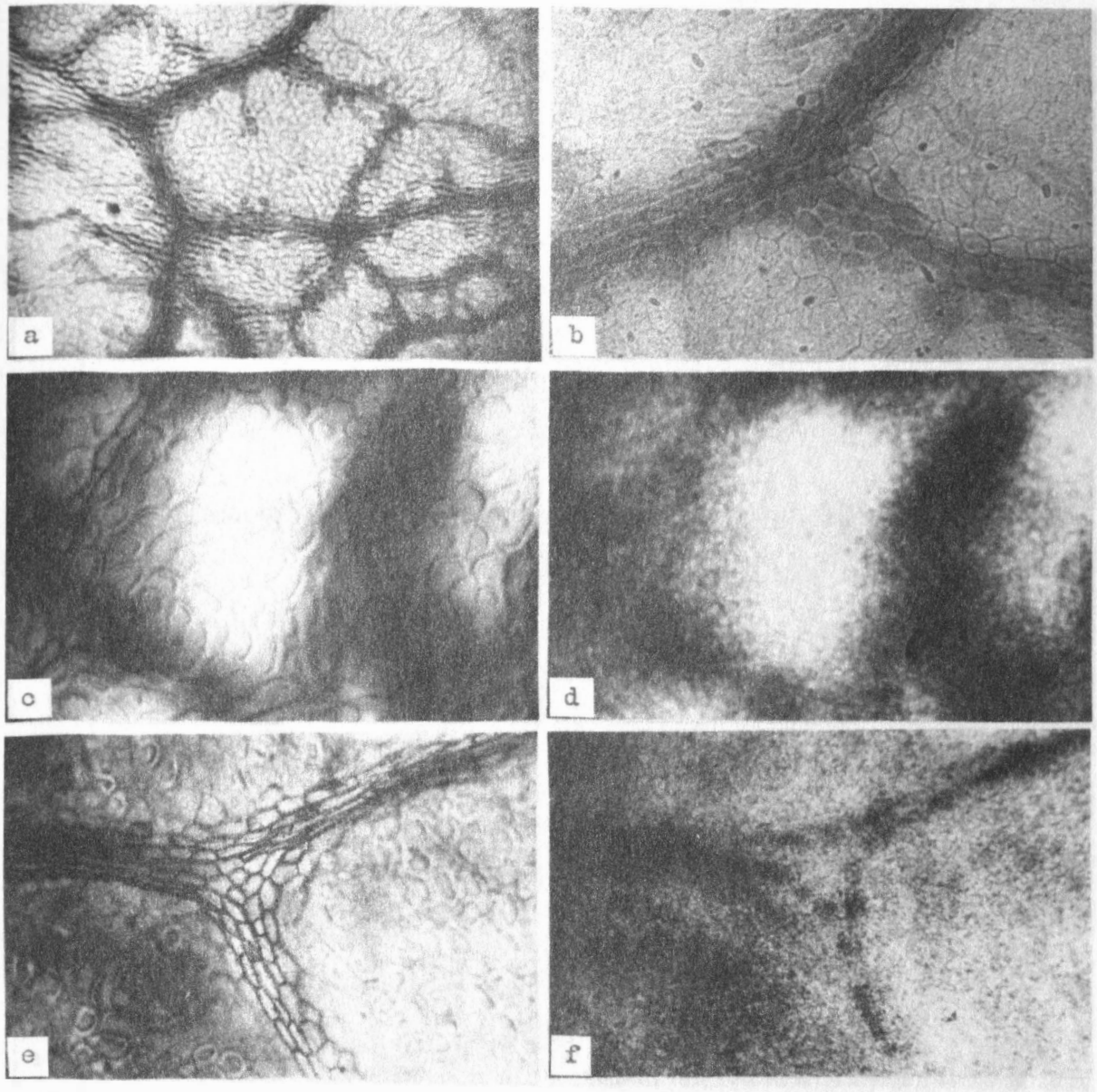

Figure 4. Astomatous apricot leaf cuticles treated with ^{14}C-linseed oil in the pure form (a-d) and emulsified with 95% water (e, f). Nuclear track plates d and f correspond to cuticles c and e, respectively. Approximate magnifications: a, 130 X; b-f, 380 X.

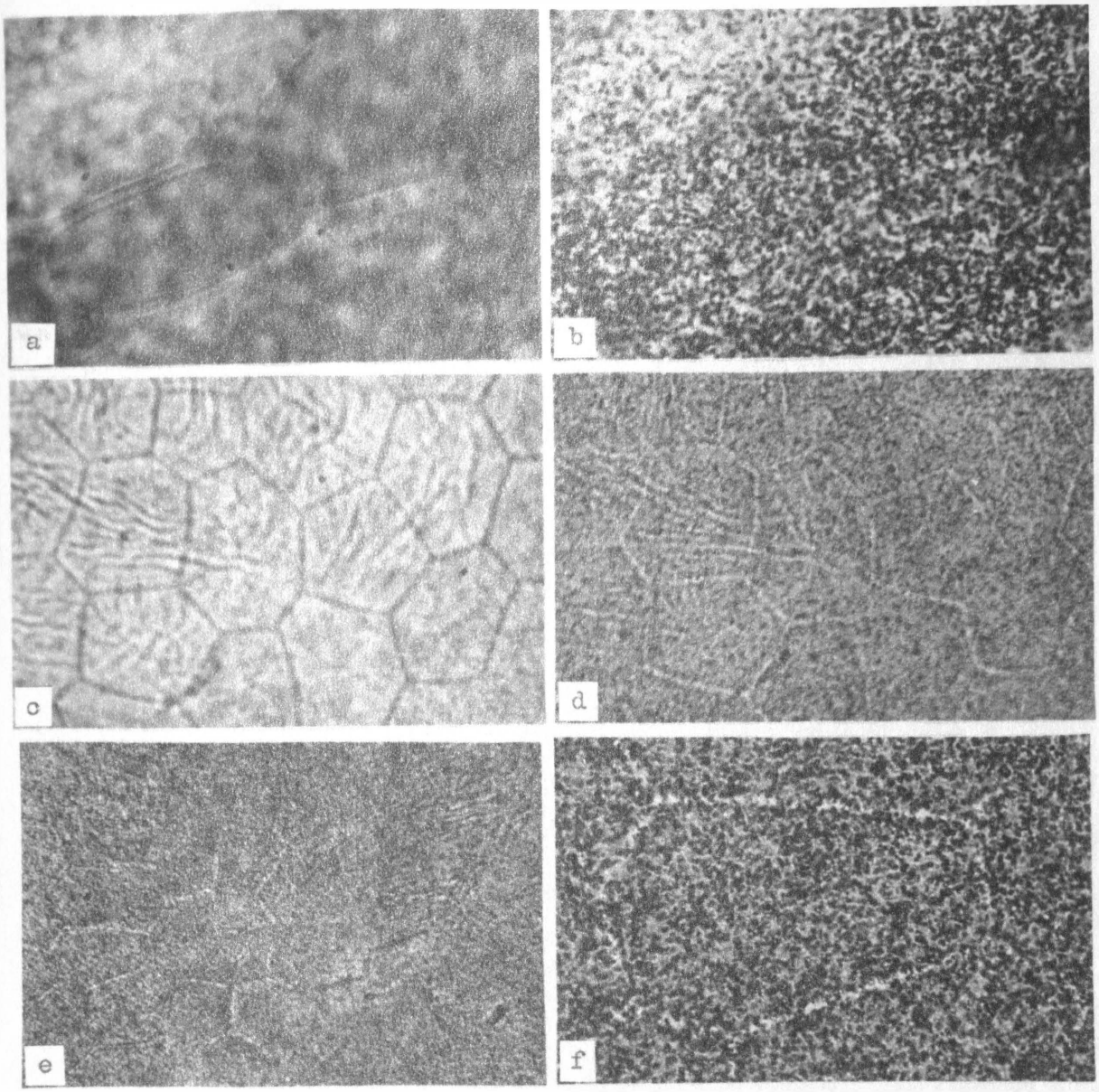

Figure 5. Astomatous apricot leaf cuticles treated with ^{14}C-linseed oil in the pure form. Cuticles are in focus in a and c, while ^{14}C tracks are in focus in b and d, respectively. Cuticles are coated with NTB liquid emulsion. Approximate magnification: a and b, 3,610 X; c and d, 1,520 X; e, 950 X; f, 3,610 X.

Patterns for ^{14}C from dicamba were randomly distributed over individual cells above veins for all three carriers (e.g., surfactant carrier, Figure 6-d). Moreover, areas above individual cells between vein areas showed a random distribution of radioactivity for ^{14}C-dicamba applied in water (Figure 6-a), 0.1% surfactant (Figure 6-b), and 5% oil (Figure 6-c) carriers.

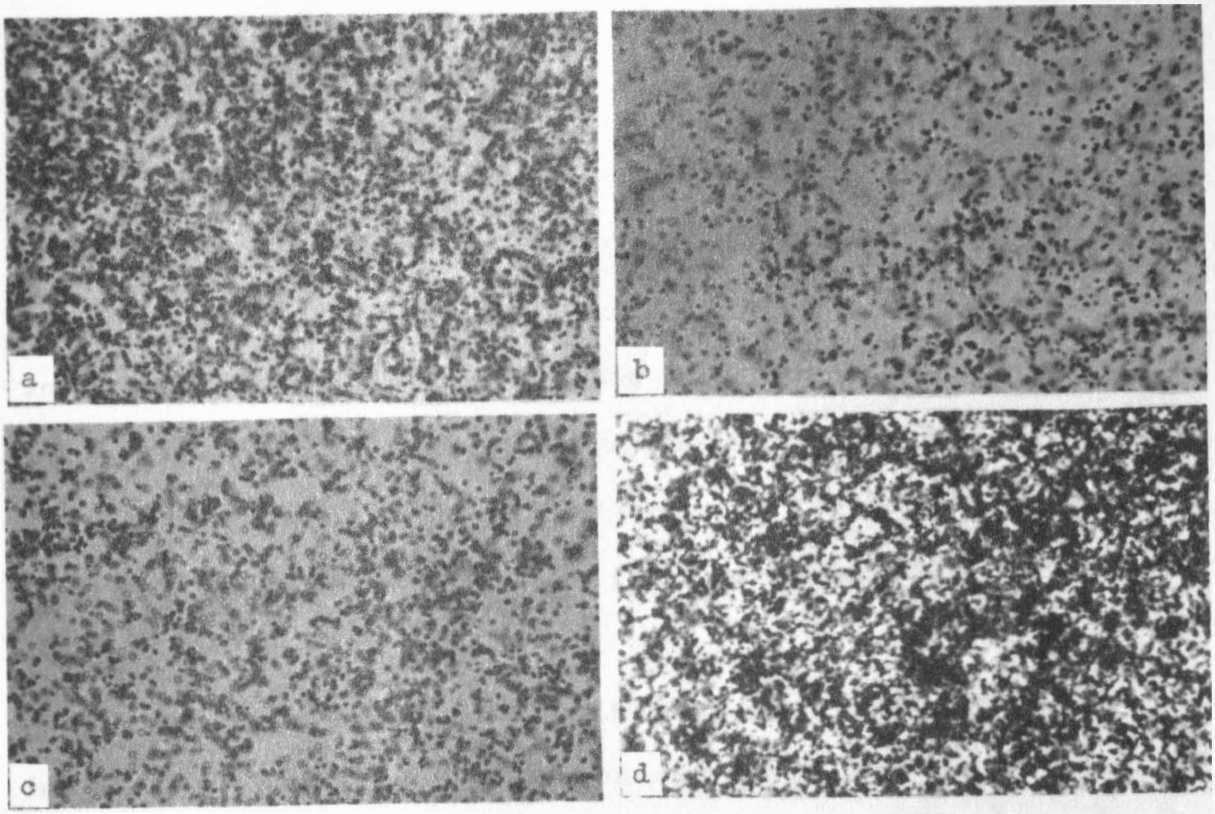

Figure 6. Nuclear track plates showing tracks of radiation emitted from astomatous apricot leaf cuticles treated with ^{14}C-dicamba in various carriers. The following carriers are shown on areas between veins: a, water; b, 5% oil-95% water emulsion; c, 0.1% surfactant. An area over a vein, as shown by d, received 0.1% surfactant carrier. About 3,610 X. See figure 5-a, b, and f for comparison.

DISCUSSION

OIL PROPERTIES

Atrazine in a 5% oil in water emulsion often reduced dry weights of foxtail and sorghum more than atrazine in water without oil. In addition, 11 of the different oils in a 5% emulsion of atrazine reduced sorghum dry weights more than the atrazine in 0.1% surfactant solution. Thus, the observation that oils may enhance the herbicidal activity of atrazine was verified for some oils in this work.

When weights of plants treated at varied levels of herbicide (Table 5) were expressed as a percent of the respective controls, an interesting difference between species was observed. Greater percent weight reductions were observed in dry than wet weights of sorghum for each herbicide rate. However, for foxtail the reverse relationship was evident, and greater percent weight reductions occurred in the dry as compared to the wet weight analysis. This relationship may be linked to the degree of tolerance each species possess to atrazine. Sorghum is moderately tolerant, while green foxtail is somewhat susceptible to the herbicide. This matter was not pursued, but it would be an interesting relationship to study.

Green foxtail appeared to be more sensitive to oil treatments than sorghum both for oil alone and oil with herbicide. Individual properties responsible for these variances were often difficult to identify. However, when multiple regression analyses were computed for both species in the presence and absence of the herbicide, little difference generally was observed between species regarding the total amount of variability in dry weight. In the regression analyses, the greatest difference appeared to be between groups of oils.

Generally, only a small portion of the dry weight variability was accounted for by calculation of simple correlations, although several of the individual correlation coefficients were statistically significant. The two groups of oils used in Experiment 1 did, however, react differently in the multiple regression analyses. Total amount of the dry weight variability explained in the group of 16 oils ranged from 30 to 75% while 99% or more was explained in the smaller group. There are several reasons for this difference. Included in the group of 16 were several oils low in unsulfonated residue, viscosity, and high in percent aromatics. These oils were not included in the smaller group since specific data on certain variables being tested were not available. Thus, the smaller group of oils was less variable in physical and

chemical properties, and a more sensitive analysis resulted. Also, more independent variables (eight) were analyzed at one time with the smaller group of oils than for the larger group (five) and including three extra independent variables resulted in more of the dry weight variability due to oils being explained.

Within the two groups of oils a few oil properties or combinations of properties were outstanding in explaining variability of dry weights. Viscosity (SUS) @ 37.8 C, gravity (API) @ 15.6 C, and flash point were able to account for 75.35% of the foxtail dry weight variability in the presence of 2.24 kg/ha of atrazine for the 16 oils. In the smaller group of oils, numerous combinations of eight variables were able to adequately explain dry weight variability for oil and oil-with-herbicide analyses. However, the most sensitive analyses usually included several of the distillation temperature values.

Specific oil properties that were related to toxicity of oils to foxtail and sorghum were difficult to determine since most of the oils used were relatively non-phytotoxic. None of the properties were statistically associated with decreasing dry weights of foxtail and sorghum.

Paraffinic types of mineral oils tended to enhance atrazine effectiveness over that of naphthenic oils. This

is desirable from the point of view of spraying practice since naphthenic oils in the absence of atrazine were phytotoxic to foxtail and sorghum. Other researchers (33) have found similar results. The less viscous oils were probably ineffective since they did not remain on the plants long enough to enhance atrazine toxicity. Crop oils were generally less effective than paraffinic or naphthenic types in enhancing atrazine effects (Table 3). Emulsions of the crop oils were generally less stable than those of the mineral oils, and a different emulsifier could possibly have increased the effectiveness of the former.

Considering all of the groups of oils, some of the same properties or types of oil properties appear important in explaining dry weight variability. These include flash point, pour point, molecular weight, refractive index, viscosity measurements, and distillation temperatures. It is evident these properties are related and refer to the heaviness of the oil.

Both simple correlation and multiple regression analyses appear important in defining oil properties. The correlations indicate the importance of each individual oil property in explaining dry weights. However, the individual correlations generally do not explain a large share of the dry weight variability. On the other hand,

combinations of oil properties analyzed together are able to explain a much greater percent of the total variability. In fact, it appears that nearly any group of eight properties out of the total of 17 properties as listed in Tables 11, 12, 14, and 15 will account for most of the dry weight variability. This indicates that there is broad overlapping of the chemical and physical characteristics of the various properties. This overlapping was verified by simple correlations computed on all combinations of two properties. Both simple correlations and multiple regressions may give deceiving results if completely different types of oils are included. The negative correlation of unsulfonated residue to dry weight observed in the data of Experiment 2 (Table 6) does not normally occur. Lesser toxicity in mineral oils is associated with higher unsulfonated residue values. However, in crop oils there is no unsulfonated residue. Since the crop oils exerted less influence in decreasing weights than the mineral oils, the unsulfonated residue values of these mineral oils were associated with decreasing dry weights. In Experiment 1, when natural oils were not included, no significant negative correlations of unsulfonated residue to dry weight were observed. Thus, this oil property should not be considered in simple correlation and multiple regression

analyses when both crop and mineral oils are tested together.

Current recommendations from available literature indicate that mineral oils used in selective weed control in postemergence spray applications should have high unsulfonated residues and low aromatic contents to have low toxicity to desired plants. This agrees with the results from this study. Viscosity (SUS) @ 37.8 C is often used in recommendations as an oil property associated with herbicidal enhancement. Results from this study indicate that distillation temperatures would be more satisfactory. Theoretically, if the properties of a given oil are known, it should be possible to determine through a multiple regression equation what effects this oil would exert on weed control from a herbicide mixture when compared with a large number of oils previously tested under similar circumstances.

INTACT PLANT STUDIES

Penetration of labeled herbicides and oil increased with time. Approximately 60% of ^{14}C from atrazine and mineral oil was taken up by Tradescantia plants in 6 days. Most of the remaining activity was in the grease and "O" rings from the atrazine treated plants. The percent of ^{14}C absorbed from mineral oil did not increase from the

6 to 15-day period. This indicates that the ^{14}C-activity found in the vacuum grease at 6 days was not taken up by Tradescantia plants and that the vacuum grease itself was not functioning as a penetrating carrier.

One of the plants treated with ^{14}C-atrazine in the 0.1% surfactant carrier grew rapidly from lateral branches below the treated area, while most other plants did not grow markedly during the 6-day penetration period. Comparisons of all plants indicated a positive association of radioactivity with regions of rapid growth. This in part may account for the large increase in percent radioactivity in the surfactant treated plants of Table 15 at 6 days.

Atrazine normally translocates acropetally from the point of application (42). To explain counts below the treated leaf, it may be assumed that the herbicide and/or its degradation products was translocated basipetally in the laticiferous ducts in Tradescantia, although this hypothesis was not tested. The exact location of the ^{14}C-activity in the "below treated leaf" section was not defined. It may have accumulated near the base of the treated leaves where the bundle traces join the main bundles, randomly over the lower area, or in some of the rapidly developing side branches. At 6 days treatment time a greater proportion of the ^{14}C from atrazine was

found to have translocated acropetally to the new growth area above the treated leaves than was found at 1 day.

Photosynthesis measurements provided a rapid method for determining effects of atrazine in a 5% oil in water emulsion. When sorghum plants were preconditioned to favor stomate opening, less CO_2 was utilized initially than was the case of plants with stomates presumably closed. This relationship appeared to reverse by the 5th day. If one assumes that decreased CO_2 consumption is associated with greater penetration, then more atrazine was able to penetrate the light preconditioned plants initially, and the plant may have started to recover after 5 days. Since less penetrated the dark preconditioned plants initially, more atrazine may have been absorbed during the next few days. However, µl CO_2 consumed/mg of dry weight for both treatments were low at 5 days. This may have been due to three factors: (a) older leaves utilized less CO_2 per mg dry weight than younger leaves; (b) readings were delayed after sorghum leaves were placed in the CO_2 measuring chambers which resulted in less CO_2 consumption; (c) residual atrazine may have inhibited photosynthesis.

Rinsing treated sorghum leaves in a 2% Tronic solution and then in distilled water immediately after spraying did not remove the CO_2-inhibiting effect of treatment with

atrazine in a 5% oil emulsion. Thin window counting of ^{14}C-atrazine in a separate experiment indicated that 5.37% of the atrazine (60 mg/ha) remained on the leaves after a similar rinsing procedure. This amount may have been larger in the photosynthesis experiment since whole plants were immersed rather than a leaf section. It has been found that atrazine causes stomatal closure in plants which causes a cessation of CO_2 uptake (87). Either enough atrazine was able to penetrate into the plant before the rinsing treatment, or the oil was able to keep some of the herbicide in contact with the plant through the rinsing procedure for later penetration so that photosynthesis was later inhibited.

Carbon dioxide measurements were more sensitive to rates of atrazine application than were the methods involving dry weight reduction. However, it was necessary to use rates much lower than those currently used in field applications since the normal rates completely inhibited CO_2 uptake. Carbon dioxide measurements were usually taken 0 to 2 days after treatments were applied, while dry weight analyses measured atrazine and oil effects over a longer period of time. Thus, for a determination of immediate effects of rates of atrazine in various carriers, the photosynthesis method might be preferred, while longer range effects would probably be better measured by dry weight analyses.

ISOLATED CUTICLE STUDIES

Penetration

Applying a ^{14}C-labeled herbicide directly on the cuticles or applying it to an agar disk and letting it diffuse through to the cuticle apparently provided different results. In one study involving penetration of ^{14}C from dicamba, no significant effect of type of carrier was observed when treatments were applied to an agar disk and allowed to diffuse through the disk and cuticle for 48 hours. In other studies, significant differences were found resulting from treatment with different carriers when treatments were applied directly to the cuticle in water saturated (using ^{14}C-atrazine) and unsaturated (using ^{14}C-dicamba) atmospheres. These results, showing no differences in penetration when treatments were applied to an agar disk instead of directly on the cuticle, agree with findings of Darlington and Barry (14), who reported that several surfactants had no effect on the permeability of isolated apricot leaf cuticles. The probable reason that no differences were observed among carriers applied to the agar disks is that of dilution factor. The 20-µl droplet would be diluted 15 times since the agar block contained 308 ul of water. This would lower the concentration gradient of the herbicide from one side of the cuticle to the other. In addition, the emulsion may have

been broken, making it possible that the oil did not even reach the cuticle but remained on top of the agar disk as the emulsion separated.

Penetration of ^{14}C from both atrazine and dicamba increased with time. However, the quantity of ^{14}C accumulated as a result of application of dicamba exceeded that accumulated from atrazine. This difference could not be explained on the basis of molecular weights or concentration gradients. Both compounds have similar molecular weights (atrazine 215.7, and dicamba 221.0) and atrazine treatments were 38 to 180 times as concentrated as dicamba treatments. It should be noted that 2.5 times the amount of carrier was used with ^{14}C-dicamba as was used with ^{14}C-atrazine. However, it was observed that at least a portion of the original 20-µl droplet containing atrazine was visible on the cuticle surface even after the 24-hour penetration period. Treatment solutions were visible on dicamba treated cuticles after 15 minutes, and cuticles were still moist after 3 hours. Thus, the effect of amount of carrier should not have been a large factor in explaining this difference.

More ^{14}C from atrazine and dicamba penetrated through the apricot cuticles in the 5% oil than the 0.1% surfactant solution. However, more herbicide penetrated the cuticles when water alone was used as the carrier. This

is in agreement with Bester, Meynhardt, and Strydom (6) who found that two surfactants inhibited urea absorption in peach leaves, and Sharma and Vanden Born (72) who found that surfactants were less effective at high humidities. Smith (77) found that more ^{14}C-atrazine was taken up by water solutions than 10% oil in water emulsions when leaf sections from yellow foxtail (Setaria glauca (L.) Beauv.) were placed in the respective solutions. He felt that the emulsion was unstable and that the oil, containing twice the concentration of atrazine compared to the water phase, layered on the top surface of the water and was not in contact with the plant. This concept could not be tested using isolated cuticles since the water would have to penetrate or disappear before the oil would make direct contact. The penetration tests had to be carried out under moist conditions to prevent agar desiccation and a portion of the water droplets remained on cuticle surfaces at the end of 24 hours.

More penetration of ^{14}C from atrazine occurred when the direction of movement was from the air side to the mesophyll side of the isolated apricot cuticles. This is in agreement with other reports of tests dealing with the permeability of astomatous leaf surfaces to water (67) and astomatous tomato fruit cuticles to organic compounds and inorganic ions (91, 92). Greater penetration of ^{14}C

from atrazine was also observed when cuticles were treated with chloroform to remove wax. Percent penetration through the isolated cuticles increased 2.5 times when treatments were applied to the air side and 9 times when applied to the mesophyll side. However, even with the chloroform treatment, twice as much ^{14}C from atrazine penetrated the isolated cuticles when applied to the air side rather than the mesophyll side.

Warm temperatures were associated with increased penetration as compared to cool temperatures. This effect was much more pronounced with the de-waxed cuticles. Chloroform extracted cuticles at 5 C permitted less penetration of ^{14}C-atrazine than cuticles not treated with chloroform at the same temperature. This may have been because the chloroform removed only half of the wax. If minute pores, cracks, or other passageways previously existed through the wax, the chloroform treatment probably destroyed their structure and the portion of wax remaining may have smoothed over and filled in any imperfections. The cold temperature may have then contracted this thin layer of wax causing it to become less permeable. At higher temperatures the physical structure may have been open enough to allow penetration through the thin layer.

^{14}C-labeled mineral and linseed oils, in pure and emulsified forms, were more strongly sorbed to the cuticles

than the ^{14}C-dicamba. This may have been due to the lipophilic properties of the oils causing a tighter binding to the relatively non-polar wax and cutin, while little attraction occurred between the polar type herbicide molecules and non-polar cuticle. Considering the rigorous rinsing procedure used, it was felt that most of the ^{14}C-activity remaining was absorbed into the isolated apricot leaf cuticles rather than adsorbed to the surface. In a separate test, the same amount of ^{14}C-dicamba that was applied to individual cuticles was put directly in a beaker containing 50 ml of deionized water (the same volume as used in each rinse). A cuticle was first immersed in this solution, placed on a glass slide and blotted dry. No radioactivity was detected by thin-window counting. This indicates that little ^{14}C-dicamba was sorbed to the surface of cuticles and that the radioactivity measurements depicted absorption rather than adsorption.

Autoradiography

The autoradiographs prepared in this work revealed actual penetration of ^{14}C-labeled compounds and not merely deposits adsorbed to the cuticle during washing since adsorption to the cuticle surfaces could not be detected in the test discussed above. Cuticles not receiving the ^{14}C-treatment were developed to determine background

radiation and to determine artifacts caused by pressure or handling procedures. Artifacts due to pressure occasionally occurred on nuclear track plates at the perimeter of the cuticle disk. Other areas including regions over veins did not give autographic images in the absence of radioactivity. Attached cell remnants were not sinks for accumulation of tracer since they were removed in the 50% $ZnCl_2$ in concentrated HCl treatment.

Tracks from ^{14}C-labeled compounds were much more easily detected on the nuclear track plates than on the other emulsions. However, the observer had to focus down to determine where the radioactivity was located in relation to the cuticle. Closer contact was made between cuticle and emulsion with the stripping film and liquid emulsion. This gave a more accurate location of the source of the emitted ^{14}C-labeled particles. Two difficulties were associated with the coating methods. Both the stripping film and liquid emulsion tended to split on the slides when drying after the developing process was completed, and the stripping film occasionally unwrapped from the slide after development and drying. Unwrapping of the stripping film could possibly have been avoided if the slides had first been dipped in a subbing solution

(5 g gelatin + 0.5 g chrome alum in 1,000 ml distilled water) as recommended by Gude (31).

The finding that large areas of radioactivity accumulated randomly over the cuticle when water was used as the herbicide carrier but not with 0.1% surfactant solution was not surprising. Surfactants lower the surface tension of water and increase spreading of the droplet. This rapid spreading undoubtedly provided a uniform distribution of the ^{14}C from dicamba. With water alone, less spreading of the droplet was visually observed; presumably large quantities of the radioactivity accumulated and settled randomly on the cuticle as the droplet dried. The 5% oil emulsion was intermediate between the other two carriers in ability to distribute the radioactivity uniformly over the cuticle surface. This may have been related to the stability of the emulsion.

Radioactivity from both ^{14}C-labeled oils in pure and emulsified forms tended to accumulate in areas over the veins. This is in agreement with previous findings (64, 17). Distribution of the radioactivity from oil and dicamba appeared to be random in areas between veins. No preferred sites of entry for the labeled compounds were located above an individual cell whether in a vein or non-vein area. This is in agreement with Yamada, Rasmussen, and Bukovac (91) who found no localization of binding

sites in astomatous tomato fruit cuticles for calcium and chloride ions and urea. However, these authors did report binding sites for calcium and chloride ions on green onion leaf cuticles above the periclinal cell walls midway between the anticlinal walls and in the substomatal chamber. Franke (25) noted that these areas contained large concentrations of ectodesmata, whereas ectodesmata have not been found in tomato fruit cuticles. He suggested that cuticular binding sites are located directly on top of the ectodesmata.

The mechanism of oil enhancement of herbicide activity appears to be due to increased penetration of herbicide into treated plants. These conclusions are based on results showing greater uptake of ^{14}C-labeled herbicides into Tradescantia plants and more penetration through isolated apricot leaf cuticles when oil-water emulsions were used as the carrier rather than a surfactant solution.

SUMMARY

To learn more about oils and how they affect post-emergence spray applications of herbicides, two major problem areas were investigated. The first dealt with specific physical and chemical properties of oils as they are associated with phytotoxicity and enhancement of herbicidal activity, while the second dealt with foliar penetration as a possible mechanism of enhancement.

Simple correlation and multiple regression analyses were conducted for specific properties of mineral oils applied to green foxtail and grain sorghum as 5% oil in water emulsions with and without 2.24 kg/ha of atrazine. Simple correlations identified individual oil properties important in explaining dry weight variabilities, but these single properties generally accounted for only a small portion of the total dry weight variability. Analyzing several oil properties by multiple regression analyses accounted for 99% of the variability due both to phytotoxicity and herbicide enhancement. Each of several combinations of eight different oil properties accounted for most of the dry weight variability because the properties were not mutually exclusive. Those properties most highly associated with atrazine enhancement included

distillation temperatures, pour point, viscosity, viscosity index, refractive index, and flash point. Phytotoxicity of oil alone was associated with increased aromatic contents and decreased unsulfonated residue values. These findings were slightly altered when natural (crop) oils were included with the mineral oils due to inherent differences in the properties of the two types of oils.

Several oils used as 5% oil/water emulsions significantly increased the herbicidal activity of atrazine on foxtail and sorghum when compared to atrazine in water alone or with 0.1% surfactant. Penetration of ^{14}C from atrazine and dicamba through astomatous leaf surfaces of Tradescantia was increased by use of oil-water emulsion as compared to 0.1% surfactant solution. Most of the radioactivity was found below the treated leaves of Tradescantia plants 1 day after treatment but accumulation in the plant parts above treated leaves increased by the 6th day. ^{14}C-labeled mineral oil likewise was found to penetrate and translocate in the Tradescantia plants. Uptake of ^{14}C from the oil increased from 20 to 57% in the first 6 days after treatment. Little additional uptake was noted at 15 days. Measurements of CO_2 uptake by sorghum revealed that photosynthesis was completely inhibited 1.5 hours after treatment with atrazine in oil-water emulsion. Maximum penetration of ^{14}C-labeled

herbicides through isolated astomatous cuticles of apricot leaves occurred (a) at warm rather than cold temperatures, (b) when chloroform extraction was used to remove wax prior to treatment, (c) from the air side of the cuticle to the mesophyll side rather than in the reverse direction, and (d) when oil emulsions were used as the carrier in short treatment periods compared with 0.1% surfactant. Penetration of ^{14}C from dicamba was more rapid than absorption of ^{14}C from atrazine.

Autoradiographic studies using isolated astomatous apricot cuticles showed that cuticle areas over veins were preferred sites of entry for ^{14}C from oils in both pure and emulsified forms and for ^{14}C from dicamba regardless of carrier type. No preferred sites of penetration were found in cuticle areas directly above an individual cell whether the underlying cell was in a vein or non-vein area of the leaf.

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APPENDIX

Appendix I. Physical and chemical properties of oils.[a/]

Oil no.	Source[b/]	Company designation	Type Oil	SUS @ 37.8 C
1	Texaco	773	naphthenic	73
2	Texaco	783	naphthenic	101
3	Texaco	769	naphthenic	77
4	Texaco	754	naphthenic	77
5	Texaco	774	naphthenic	85
6	Texaco	308	paraffinic	--
7	Texaco	422	paraffinic	31
8	Texaco	454	aromatic	35
9	Texaco	7545	aromatic	32
10	Sun Oil	R419-45	paraffinic	73
11	Sun Oil	R419-48	paraffinic	103
12	Sun Oil	X819-9	paraffinic	63
13	Sun Oil	X819-10	paraffinic	100
14	Sun Oil	X819-11	paraffinic	74
15	Sun Oil	X819-12	naphthenic	69
16	Sun Oil	X819-13	naphthenic	157
17	Sun Oil	X819-14	naphthenic	207
18	Sun Oil	R519-50	paraffinic	161
19	Sun Oil	R519-51	paraffinic	210
20	Cargill	crude corn oil		171
21	J.D.Nalewaja	crude cotton seed oil		176
22	Cargill	crude linseed oil		130
23	J.D.Nalewaja	alkali refined linseed oil		131
24	J.D.Nalewaja	crude peanut oil		189
25	Cargill	sunflower oil		155
26	Cargill	crude soybean oil		158

a/ See Appendix II for definations of terminology. These properties were ascertained according to standardized testing techniques approved and adopted by the American Society for Testing and Materials. The publication of this Society should be consulted for complete descriptions of all analytical techniques used to measure oil properties.

Appendix I (continued)

Oil no.	SUS @ 98.9 C	Visc. Index	Gravity API	Pour Point C	Unsulf. Residue
1	36	55	30.5	-15.0	85
2	39	61	28.4	- 9.4	90
3	--	--	33.5	- 9.4	96
4	--	--	33.5	- 9.4	96
5	--	--	34.6	- 9.4	--
6	--	--	48.0	-51.1	--
7	--	--	43.3	-40.0	--
8	--	--	31.3	-34.4	--
9	--	--	17.1	-28.8	--
10	37	100	34.1	-12.2	95
11	40	101	33.8	-26.1	93
12	36	112	35.9	-12.2	95
13	40	103	34.3	-23.3	97
14	37	92	35.4	-15.0	96
15	35	33	25.7	-26.1	82
16	41	0	23.2	-45.5	80
17	43	3	22.5	-40.0	80
18	44	106	32.3	-15.0	91
19	47	102	32.1	-17.8	92
20	53	235	21.3	-12.2	0
21	53	215	21.1	- 3.9	0
22	49	255	20.0	-15.0	0
23	49	249	20.4	-20.4	0
24	54	215	22.6	1.6	0
25	51	235	21.5	- 9.4	0
26	52	220	21.2	- 9.4	0

Appendix I (continued)

Oil no.	Percent Aromatics	Flash Point C	Fire Point C	Mol. wt.	Refract. Index
1	20	174	202	290	1.483
2	22	185	213	350	1.486
3	11	182	--	300	1.467
4	11	182	--	300	1.467
5	10	202	--	390	--
6	--	47	--	145	--
7	--	54	--	175	--
8	--	96	--	200	--
9	96	85	--	165	--
10	14	185	204	328	1.4704
11	13	199	229	358	1.4720
12	6	168	191	310	1.4659
13	8	202	227	359	1.4706
14	8	174	213	324	1.4675
15	26	149	171	315	1.4870
16	38	168	191	325	1.5015
17	39	174	196	330	1.5039
18	16	207	229	399	1.4757
19	16	227	249	419	1.4771
20	0	291	346	828	1.4750
21	0	307	357	799	1.4723
22	0	299	354	868	1.4814
23	0	329	360	757	1.4818
24	0	296	299	799	1.4703
25	0	291	352	777	1.4756
26	0	296	349	916	1.4755

Appendix I (continued)

Oil no.	Distillation temperatures C @ 10 mm Hg					
	Initial	5%	10%	50%	90%	Final
1	--	--	--	--	--	--
2	--	--	--	--	--	--
3	--	--	--	--	--	--
4	--	--	--	--	--	--
5	--	--	--	--	--	--
6	--	--	--	--	--	--
7	--	--	--	--	--	--
8	--	--	--	--	--	--
9	--	--	--	--	--	--
10	186	201	207	222	244	259
11	203	220	226	243	264	277
12	176	187	194	212	233	246
13	204	221	226	242	261	269
14	179	196	203	222	245	266
15	140	152	160	187	220	242
16	167	178	187	217	271	303
17	163	176	186	222	286	306
18	191	209	221	272	304	318
19	219	234	240	278	329	338
20	--	--	--	--	--	--
21	--	--	--	--	--	--
22	--	--	--	--	--	--
23	--	--	--	--	--	--
24	--	--	--	--	--	--
25	--	--	--	--	--	--
26	--	--	--	--	--	--

Appendix II

Aromatics: Percent by weight of aromatic type compounds in the oil.

Distillation temperatures: Temperature at which a certain portion of the oil distills off.

Fire point: Temperature at which a combustible mixture with air will ignite in the absence of a free flame.

Flash point: Temperature to which a compound must be heated before the vapors will ignite in the presence of a free flame.

Gravity (API) @ 15.6 C: Gravity in the reverse order to specific gravity; high values are associated with volatile petroleum fractions and low values with heavy fractions.

Molecular weight: The sum of the atomic weights of the atoms making up oil molecules; figures in Appendix I are average values of numerous compounds within the complex mineral oil mixtures.

Pour point: Temperature at which oil will not pour from a jar; pour point evaluates flowability and solidification in cold weather.

Refractive index: Indicates the purity of hydrocarbons; saturated hydrocarbons have relatively low refractive indices; aromatic hydrocarbons have relatively high refractive indices; naphthenic hydrocarbons are intermediate.

Unsulfonated residue: The percent of oil that does not react with 37 N sulfuric acid; this gives an approximation of the saturated structures present in the oil.

Viscosity index: Empirical number that indicates the effect of change of temperature on the viscosity of an oil; a high viscosity index signifies a smaller change in viscosity with change in temperature than a low index; viscosities at 37.8 C and 98.9 C are used in its calculation.

Viscosity (SUS) @ 37.8 C or 98.9 C: Measures resistance to flow and is reported as Saybolt Universal Seconds (SUS); viscosity is used to evaluate the lubricating properties of oils.