Comparison of Dimethoate and Dimethoxon Residues in Citrus Leaves and Grapefruit following Foliar Treatment with Dimethoate Wettable Powder with and without Surfactant

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Residues of dimethoate and its oxygen analog (a dimethoate degradation product) on and in grapefruit peels, pulp, and leaves were investigated by a gas chromatographic-flame photometric detection (gc-FPD) procedure following treatment of citrus trees with dimethoate wettable-powder spray solution with and without surfactant. Addition of a surfactant to the spray mixture resulted in a more rapid penetration of the insecticide into the leaf than when a surfactant was not used. Disappearance was about equal, but residues on leaves through the 24-hr post-treatment period were less subject to removal by rainfall and other weathering factors because of their location in the internal portion of the leaves. No appreciable residues of dimethoate were detected in grapefruit pulp 14 days following treatment, well below the 2 ppm allowable tolerance for grapefruit. Residues in the peels were significantly higher with an average of 3.29 ppm detected 14 days following treatment.

Dimethoate, *O,O*-dimethyl *S*-(*N*-methylcarbamoyl methyl) phosphorodithioate, is an insecticide exhibiting both systemic and contact action against certain insect pests attacking animals and plants. Investigations by Hewitt *et al.* (1958), Dauterman *et al.* (1959), Drummond (1959), and Marquardt and Lovelace (1961) demonstrated the importance of this insecticide in the control of insects attacking animals. Reports have been published concerning the systemic action of dimethoate in the control of insect pests attacking plants, such as those by Dauterman *et al.* (1960), Santi and Giacomelli (1962), Hacskaylo and Bull (1963), Van Middelem and Waites (1964), and Enos and Frear (1964).

Studies of this systemic insecticide by de Pietri-Tonelli and Barontini (1963) and Gunther *et al.* (1965) have demonstrated its importance in the control of citrus insects. However, their studies did not include the use of surfactants to allow faster penetration of the insecticide into the waxy leaf surface.

This report concerns the persistence of surface and internal residues of dimethoate and its oxygen analog in grapefruit pulp, peels, and leaves following foliage treatment with an aqueous 25% wettable-powder suspension containing 0.50 lb of dimethoate and 8 fluid oz of surfactant per 100 gal of spray solution for control of the citrus blackfly in the lower Rio Grande Valley (Brownsville, Tex.). The surfactant was added to reduce the penetration time of the insecticide into the citrus foliage and also to possibly increase the residual life of the insecticide. Fruit samples were also collected to determine if dimethoate and/or the oxygen analog were absorbed and/or translocated into the peels and pulp of the fruit in appreciable quantities.

Samples of leaves and fruit were also collected following a spray treatment as described above without the surfactant.

EXPERIMENTAL SECTION

Type of Citrus, Application Rate, and Procedure. Separate test plots were designed with Texas Ruby Red grapefruit trees with three replicates, two trees per replicate. The trees were treated with two spray solutions, an aqueous spray suspension of 2 lb of dimethoate 25% wettable-power (0.5 lb actual) per 100 gal and the same spray suspension, but with the addition of 8 fluid oz/100 gal of Ortho HDD surfactant (alkylphenoxypolyoxyethylene, 100% active). The citrus trees were treated using John Bean sprayers with No. 785 spray guns, saturating the trees at a rate of *ca.* 2.5 lb/A. Trees receiving no treatment were reserved for controls.

Sampling Procedures. *Leaves.* Citrus leaves were collected at intervals of 2, 4, and 8 hr and 1, 2, 7, and 14 days following treatment. Representative samples of 150 leaves were randomly collected from each tree, compositing the leaves from the two trees in each replication. Leaves at all stages of development were collected from various locations on the tree in order to maintain representative samplings. Control samples from untreated trees were collected as needed.

Fruit. Random samples of grapefruit were collected from treated trees at intervals of 2 and 14 days following treatment with the spray mixture containing the surfactant and 7 and 14 days post-treatment without the surfactant.

Analytical Procedures. Extraction procedures utilized in this work were modifications of existing methods. The water extraction procedures of Chilwell and Beecham (1960) and Beck *et al.* (1966 and 1968) were modified for extraction of surface and total residues of dimethoate and its oxygen analog from the citrus leaves, while the procedure of Stellar and Curry (1964) was modified for the grapefruit extractions. The procedures involved the extraction of the insecticide and oxygen analog from the plant material, utilizing an aqueous solution of glacial acetic acid for extracting the citrus leaves, a mixture of acetone and water for extracting the grapefruit pulp, and an acetonitrile extraction of the peel samples.

Surface Residues (Leaves). Leaves were thoroughly mixed immediately upon receipt in the laboratory and representative 25-g samples were weighed into half-gallon Mason jars. To this was added 300 ml of a 2.0% (v/v) solution of glacial acetic acid in distilled water. The jars were sealed tightly with screw caps and Teflon liners and then rotated on a concentric rotator for 4 hr. The samples were filtered through glass wool, collecting 180-ml aliquots, which were transferred into 500-ml separatory funnels and extracted three times with fresh 100-ml portions of dichloromethane. The extracts were filtered through glass wool–anhydrous sodium sulfate filters into 500-ml erlenmeyer flasks, two glass beads and 1 ml of a 0.01% (v/v) Nujol in hexane solution were added, and the solvent was evaporated to *ca.* 5 ml through Snyder columns on a warm (40–50°) water bath. The concentrated extracts were then transferred to 15-ml graduated centrifuge tubes and evaporated to 1 ml in a warm (40–50°) water bath

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Table I. Residues of Dimethoate and Its Oxygen Analog in Citrus Leaves Following Treatment of the Trees with a Spray Solution of Dimethoate Wettable Powder with and without Surfactant[a]

Sampling interval	Residue, ppm[b-d]			
	Without surfactant[e]		With surfactant[e]	
	Surface	Internal	Surface	Internal
2 hr	38.38	4.42	14.37	29.64
4 hr	50.92	<0.01	12.84	23.96
8 hr	41.66	10.71	8.61	26.09
1 day	7.14	7.89	1.61	20.88
2 days	1.60	17.14	0.74	22.43
7 days	0.12	16.42	0.11	15.93
14 days	0.54	1.96	0.11	2.07

[a] Refer to text for spray mixture information. [b] Total of dimethoate and dimethoxon residues. [c] Corrected for insecticide recovery from fortified samples. [d] Lower limits of sensitivity = 0.01 ppm for dimethoate, 0.05 ppm for dimethoxon. [e] Average of three replicates.

Table II. Residues of Dimethoate and Its Oxygen Analog on and in Grapefruit Peels Following a Dimethoate Wettable-Powder Treatment (with Surfactant)[a]

Sampling interval, days	Residue, ppm[b-d]		
	Dimethoate	Dimethoxon	Total
2	5.07	0.18	5.25
	5.43	0.26	5.69
	8.92	0.28	9.20
	5.22	0.17	5.39
	6.75	0.25	7.01
	Av 6.28	0.23	6.51
14	4.15	0.15	4.30
	2.28	0.16	2.44
	3.41	0.15	3.56
	2.75	0.23	2.97
	3.08	0.12	3.20
	Av 3.13	0.16	3.29

[a] Refer to text for spray mixture information. [b] Corrected for dimethoate and dimethoxon recovery from fortified samples. [c] Lower limits of sensitivity for dimethoate = 0.01 ppm, dimethoxon = 0.05 ppm. [d] Average of five trees.

with a gentle stream of dry air. The extracts were diluted to 10 ml with ACS grade benzene and evaporated again to 1 ml, and then diluted to the desired volume with benzene. Caution was taken in the final evaporation step, being certain all traces of dichloromethane were removed. This extraction procedure also removed subsurface residues which would be susceptible to removal by rainfall and other weathering processes.

The acetic acid was utilized in this procedure to stabilize the residues during storage of the aqueous extracts, and also to improve extraction efficiency.

Total Residues (Leaves). Representative 25-g leaf samples (from the preceding samples for surface residues) were weighed into 1000-ml blender jars, 300 ml of a 1.0% (v/v) glacial acetic acid in distilled water solution and one spoonful of Celite (Johns-Manville) filter aid were added, and the samples were macerated for 30 sec at low speed and then 2.5 min at high speed. The aqueous extracts were filtered through glass wool and a layer of Celite into graduated cylinders, collecting 180-ml aliquots which were transferred into 500-ml separatory funnels. The aqueous extracts were extracted with dichloromethane and evaporated as described in the previous section for surface residues.

A 1% acid solution was utilized in this procedure instead of the higher concentration used for extraction of surface residues. This was necessary because of small amounts of acid being extracted when the aqueous solution was extracted with dichloromethane. This problem was not encountered when the lower concentration was used, apparently attributable to the maceration procedure utilized.

Comparative studies were made for recoveries of the insecticide from fortified citrus leaves treated with the surfactant *vs.* recoveries from fortified citrus leaves without the surfactant. No significant difference (*ca.* 5%) was noted between recoveries from the two tests. This variation was within experimental error of the analytical procedure.

Grapefruits were first peeled, then quartered, and opposite quarters collected, chopped, and mixed. Representative 100-g samples were weighed into 1000-ml blender jars; 150 ml of a 1:2 acetone–distilled water solution was added and macerated for 30 sec at low speed and then for 1.5 min at high speed. The macerate was filtered through glass wool into 1000-ml separatory funnels, the filter cake washed with 250 ml of acetone into the funnel, and the blender jar then rinsed with 100 ml of dichloromethane and drained through the filter cakes into the separatory funnels. Funnels were gently swirled to avoid emulsions and then gently shaken for *ca.* 30 sec. The dichloromethane–acetone layer was filtered through sodium sulfate into 500-ml erlenmeyer flasks. The extraction procedure was repeated two additional times with fresh 100-ml portions of dichloromethane, draining the extracts through sodium sulfate into the erlenmeyer flasks. The sodium sulfate was then rinsed with *ca.* 25 ml of dichloromethane into the flasks and aqueous layers were discarded. Glass beads and 1 ml of a 0.01% Nujol in hexane solution were added to each sample and the solvent was evaporated to *ca.* 5 ml on a warm (40–50°) water bath through Snyder columns. The concentrated extracts were transferred to glass-stoppered 50-ml graduated centrifuge tubes with ACS grade benzene which was evaporated to *ca.* 1 ml in a warm (40–50°) water bath with a gentle stream of dry air. Ten milliliters of benzene was added and the solvent was again evaporated to 1 ml as before and then diluted to the appropriate volume with benzene. Caution was taken to be certain all traces of dichloromethane and acetone were removed.

Grapefruit peels corresponding to the pulp samples were chopped thoroughly; then representative 50-g samples were weighed into 1000-ml blender jars. Three hundred milliliters of Nanograde acetonitrile (Mallinckrodt Chemical Works, St. Louis, Mo.) and one spoonful of Celite filter aid were added and macerated for 30 sec at low speed and then for 2.5 min at high speed. The acetonitrile extracts were filtered through glass wool and a layer of Celite, collecting 180-ml aliquots, and then concentrated on hot plates to exactly 25 ml and stored in a refrigerator pending gas chromatographic analysis.

Gas Chromatographic Analysis. Detection of the dimethoate and its oxygen analog was performed on a gas–liquid chromatograph (glc) equipped with a dual channel Melpar flame photometric detector (FPD) with both the sulfur (394 mμ) and the phosphorus (526 mμ) interference filters. Since dimethoate and the oxygen analog contain thio and phosphorus groups, identification of peaks was possible as the samples were chromatographed. No special columns were necessary for these materials. Both the parent compound and oxygen analog were chromatographed without special derivitization.

Instrumental operating parameters were: columns: dimethoate, 6 × 0.25 in. o.d. glass column packed with 3% DC-200 on 100–200 mesh Gas Chrom-Q (Applied Science Laboratories, State College, Pa.); dimethoxon, 6 × 0.25 in. o.d. glass column packed with 10% DC-200 on 100–200 mesh Gas Chrom-Q; carrier gas, nitrogen (55 cm^3/min for the 3% DC-200 column, 110 cm^3/min for the 10% DC-200 column); detector gases, hydrogen (50 cm^3/min), oxygen

(15 cm^3/min), and air (40 cm^3/min); isothermal temperatures, column, 200°; detector, 200°; and injector, 250°; recorder speed, 30 in./hr.

Sensitivity was adjusted to obtain half-full scale deflection of the recorder pen with an injection of 4.5 ng of dimethoate and 15 ng of the oxygen analog.

A series of control samples consisting of solvent check, untreated sample, and untreated sample fortified with dimethoate and dimethoxon was carried through the complete procedure with each group of treated samples. No interfering peaks were detected in any of the solvents, reagents, or untreated material. Average recovery values of 62.6% dimethoate and 76.0% dimethoxon were obtained on the surface extraction of the citrus leaves and values of 62.2% dimethoate and 70.9% dimethoxon were obtained for the internal extraction. For the grapefruit pulp recovery, values were 54.0% for the dimethoate and 62.0% for the dimethoxon. Average recovery values for the grapefruit peels were 69.1% for the dimethoate and 66.2% for the dimethoxon. All residues were corrected for these values. The lower limits of sensitivity were determined to be 0.01 ppm for the dimethoate and 0.05 ppm for the dimethoxon.

When the dimethoate and dimethoxon recovery values were subjected to the statistical analysis of Bauer (1971), standard deviations of 0.23 and 0.19 were obtained for the surface and internal residues, respectively, for dimethoate recoveries and 0.08 and 0.16 for surface and internal residue recoveries of dimethoxon. These values were based on fortification levels of 1.11 ppm for dimethoate and 1.85 ppm for dimethoxon.

RESULTS

Table I presents residue data on and in grapefruit leaves following a foliar treatment of trees with a dimethoate wttable-powder suspension, with and without surfactant. Essentially all residues were surface and subsurface when the surfactant was not utilized, 38.38 ppm for surface and 4.42 ppm for internal, 2 hr following treatment. The ratio was essentially the same for samples collected 4 and 8 hr post-treatment, with an indication of penetration showing in the 1-day samples at which time surface and internal residues were approximately equal. The 2-, 7-, and 14-day samples indicated a decrease in surface and subsurface residues with a corresponding increase in internal residues.

In contrast, residues on and in leaves from trees treated with the insecticide containing a surfactant indicated a rapid penetration of dimethoate, within 2 hr following treatment of the trees. Average residues were 14.37 and 29.64 ppm of dimethoate and its oxygen analog for the surface (including subsurface) and internal residues, respectively. This was true for all samples through the 2-day post-treatment which showed 0.74 ppm for the surface and subsurface residues and 22.43 ppm for internal residues. The 7- and 14-day samples were essentially the same as for the dimethoate treatment without surfactant.

Table II presents residue data in grapefruit peels following a dimethoate wettable-powder treatment with surfactant. Two days following treatment, residues of 6.28 ppm of dimethoate and 0.23 ppm of dimethoxon were detected on and in the peels. After 14 days, these residues had decreased to 3.13 and 0.16 ppm for the dimethoate and dimethoxon, respectively. These residues were apparently due to either translocation of the insecticide from the treated leaves, direct deposition on the surface of the fruit, or a combination of the two.

Table III presents residue data in grapefruit pulp following the dimethoate wettable-powder treatment with and without surfactant. No appreciable translocation of the pesticide or oxygen analog was noted. After 2 days, only 0.09 ppm of dimethoate and no detectable dimethoxon was found in the grapefruit following a dimethoate spray treatment containing surfactant and 0.12 ppm of dimethoate, and no detectable residues of dimethoxon were found 7 days following dimethoate treatment without surfactant. After 14 days, residues were reduced to 0.03 ppm of dimethoate, no detectable residues of dimethoxon with surfactant and 0.09 ppm of dimethoate, and no detectable dimethoxon without the surfactant. These residues were well below the allowable tolerance level of 2 ppm, even 7 days following the dimethoate treatment.

Table III. Residues of Dimethoate and Its Oxygen Analog in Grapefruit Pulp Following a Dimethoate Wettable-Powder Treatment, with and without Surfactant[a]

Sampling interval, days		Residue, ppm[b,c]		
		Dimethoate	Dimethoxon	Total
		Without Surfactant		
7		0.06	<0.05	0.06
		0.19	<0.05	0.19
		0.10	<0.05	0.10
	Av	0.12	<0.05	0.12
14		0.07	<0.05	0.07
		0.12	<0.05	0.12
	Av	0.09	<0.05	0.09
		With Surfactant		
2		0.09	<0.05	0.09
		0.08	<0.05	0.08
		0.12	<0.05	0.12
		0.05	<0.05	0.05
		0.09	<0.06	0.15
	Av	0.09	<0.05	0.10
14		0.04	<0.05	0.04
		0.01	<0.05	0.01
		0.04	<0.05	0.04
		0.03	<0.05	0.03
		0.01	<0.05	0.01
	Av	0.03	<0.05	0.03

[a] Refer to text for spray mixture information. [b] Corrected for dimethoate and dimethoxon recovery from fortified samples. [c] Lower limits of sensitivity for dimethoate = 0.01 ppm, dimethoxon = 0.05 ppm.

DISCUSSION

The use of a surfactant with a dimethoate wettable-powder treatment resulted in more rapid initial penetration of the insecticide into citrus leaves. Within 2 hr after application, a higher percentage of residues was located in the internal portion of the leaf, with smaller amounts detected as surface and subsurface residues. Residues followed a similar pattern through the 2-day post-treatment sampling.

When the insecticide was applied without a surfactant, significant penetration was not noted until 1 day following treatment. No appreciable translocation or absorption of dimethoate or its oxygen analog into the fruit was noted in the analysis of the grapefruit. All residues were well below the 2-ppm tolerance level for the fruit, even 7 days following treatment.

The dimethoate wettable-powder spray treatment with surfactant is currently being utilized in the lower Rio Grande Valley in Brownsville, Tex., as a means of controlling the citrus blackfly. Since essentially all of the residues were detected within the leaves, a greater resistance to rainfall and weathering would be produced.

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Translocation of Pesticides as Affected by Plant Nutrition

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The nutritional effects upon absorption and translocation of two organophosphate insecticides and two systemic fungicides into hydroponically grown bean plants are described. The insecticides included Guthion (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl] phosphorodithioate) and parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate). The fungicides included the major degradation product of benomyl (MBC) methyl 2-benzimidazole carbamate and thiophanate-methyl [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)]. Translocation of the four compounds was related to the total root-absorbed activity in a complete nutrient solution.

The degree of penetration of a synthetic compound into plant roots and the extent of its subsequent translocation into other plant parts are both functions of the particular plant, soil type, and physicochemical properties of the compound, *e.g.*, water solubility, polarity, and/or its stability within the living cells (Lichtenstein *et al.*, 1970; Reynolds and Metcalf, 1962). Several investigators have reported to have observed nutritional influences relative to the penetration and translocation of pesticidal compounds within plants. Casida *et al.* (1952) reported a decreased schradan absorption by pea plants with a concomitant increase of available phosphorus, while Hacskaylo *et al.* (1961) observed a reduced dimethoate absorption by cotton plants grown in a phosphorus-deficient nutrient solution. More recently, Yu and Morrison (1969) discovered the alteration in uptake of mevinophos and phosphamidon by bean plants when the supply levels of phosphorus, potassium, magnesium, nitrogen, and calcium were varied. Finally, Talekar and Lichtenstein (1971) witnessed an increased penetration of lindane into the root system of pea plants grown in nitrogen-, sulfur-, or boron-deficient media. Actual translocation of lindane into the aerial parts of the pea plant, however, was reduced.

The present paper describes such nutritional effects upon absorption and translocation of four different systemic compounds. The test compounds used in this investigation included two organophosphate insecticides, Guthion (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate) and parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), and two broad-spectrum fungicides, MBC (methyl 2-benzimidazole carbamate) and thiophanate-methyl [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)].

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REAGENTS AND APPARATUS

Chemicals. Guthion (benzenoid-ring-U-^{14}C) (sp act. 1.0 μCi/mmol) was synthesized by White *et al.* (1972). The radiolabeled MBC-2-^{14}C (sp act. 2.83 μCi/mmol) was synthesized according to White and Kilgore (1972). Parathion (1,2-^{14}C ring labeled) (sp act. 1.52 μCi/mmol) was purchased from International Chemicals and Nuclear Corp., Irvine, Calif., and thiophanate-methyl (ring-U-^{14}C) (sp act. 2.9 μCi/mmol) was generously provided by the Biological Research Institute, Nippon Soda Co., Ltd., Japan. Analytical reagent grade chemicals and double-distilled solvents were used throughout this investigation.

Instruments. The Polytron, a high specific intensity ultrasonic generator (Type PT 3500, Brinkmann Instruments, Inc., Westbury, N. Y.) equipped with a saw tooth cutting head, was used to extract the labeled compounds from the plant tissues. Infrared spectra were obtained from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer. The radioactivity (^{14}C) was measured in a Model 2425 Packard Tri-Carb liquid scintillation spectrometer. The scintillator fluid was composed of 15 g of 2,5-diphenyloxazole, 2 l. of toluene, and 1 l. of ethylene glycol monomethyl ether.

Thin-Layer Chromatograms. Precoated glass plates (silica gel UV-254, with fluorescent indicator) and precoated plastic sheets (polyamide II/UV-254, with fluorescent indicator) were purchased from Brinkmann Instruments, Inc., Westbury, N. Y.

PROCEDURE

Propagation of Plants. Bean seeds (*Phaseolus vulgaris* L. Tenderbest) were grown as described by Al-Adil *et al.* (1972).