Table III. Rainfall during NDPA Field Soil Column Dissipation Experiment

sampling no.	date sample collected	total days from initiation of experiment	rainfall since last sampling period, cm	cumulative rainfall, cm
1	4/12/77	0		0
2	4/27/77	15	4.4	4.4
3	5/06/77	24	2.7	7.1
4	6/20/77	69	6.1	13.2
5	8/01/77	111	20.6	33.8
6	8/16/77	126	6.6	40.4

dissipation of NDPA also occurred rapidly. Little or no radioactivity was observed in the 20–30-cm column fraction and the soil leachates. The disappearance of NDPA and radioactivity from the soil is shown in Figure 8a,b with half the initially applied NDPA dissipated in approximately 21 and 40 days for the sandy loam and silty clay loam soils, respectively.

DISCUSSION

Leaching of NDPA. The laboratory leaching study showed that the compound will leach under rather heavy simulated rainfall conditions, which is consistent with Dean-Raymond and Alexander (1976) who reported on the leachability of dimethylnitrosamine (DMNA). The field study indicated that NDPA did not leach below 20 cm. This suggests that NDPA dissipated readily before extensive leaching occurred and that the high water solubility (approximately 10000 ppm) of NDPA (Mirvish et al., 1976) caused it to move with the soil moisture. During periods of rain, the compound was leached downward, but during periods of drying it may have returned to the soil surface as the water evaporated (Spencer and Cliath, 1973). Some NDPA was observed in the 10–20-cm soil section but the compound was mixed initially in the 0–10-cm section. The observed differences in leaching between laboratory and field studies demonstrate the value of conducting soil experiments under simulated field conditions whenever possible.

Dissipation of NDPA in Soil. Half-lives of approximately 21 and 40 days for NDPA in sandy loam and silty clay loam soil, respectively, were found in the field dissipation study. Tate and Alexander (1975) reported that NDPA was resistant to microbiological degradation; however, their data for NDPA in Williamson silt loam indicated that half of the initially applied NDPA disappeared in 20–30 days. The aerobic laboratory soil study indicated a half-life of approximately 10–15 days for NDPA in both soils. Oliver et al. (1978) reported a half-life of 2–3 weeks for NDPA in active soils in the laboratory.

These studies demonstrate that the trace quantities of NDPA which have resulted from the application of trifluralin can dissipate from the soil types used in this study.

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Dislodgable Residue of Supracide on Citrus Leaves

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Dislodgable residues of *S*-[(2-methoxy-5-oxo-Δ^2-1,3,4-thiadiazolin-4-yl)methyl] *O,O*-dimethyl phosphorodithioate (Supracide) and a monoxone metabolite were analyzed on citrus leaves at various intervals following application during a dry season (May, 1974) and a wet season (July–August, 1974). Applications were made at one, two, and three times the recommended rate and during the wet season additional applications were made which included oil. Leaves were collected from which leaf disks were punched for analysis. Disks were water/detergent washed for removal of dislodgable residues. Supracide was analyzed by FPD/GC and the monoxone metabolite was quantified by thin-layer chromatography using fly-head cholinesterase. There was a rapid decrease of Supracide and the monoxone metabolite during the first week after application. In those samples in which total residues were checked, total residue paralleled dislodgable residues.

The dislodgable residues of chemicals sprayed in agricultural fields and groves are of high importance with respect to field-worker reentry. This is particularly true of organophosphorus compounds with high mammalian toxicity. Spear et al. (1975) have reported work dealing with worker hazard in California citrus treated with parathion. They found the paraoxon accounted for a significant portion of the residue found and that it decayed more slowly than parathion. Dislodgable residue studies by this research group (Leffingwell et al., 1975) have also concerned Ethion and zolone on grape foliage. Westlake

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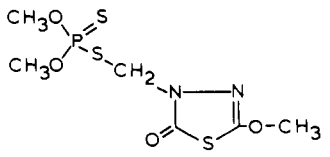

Figure 1. Structure of Supracide.

Table I. Rainfall (in inches) Recorded during Two Experimental Periods Beginning May 1 (Dry Season) and July 23 (Wet Season), 1974[a]

dry			wet				
day	6	0.60	day	2	0.28	day 11	1.26
	7	1.35		5	0.20	13	0.44
	15	1.20		6	0.68	14	0.19
				7	0.35	19	1.08
				8	0.49	20	0.23

[a] Amounts <0.1 in./day not indicated.

et al. (1973) reported dislodgable residues of dioxathion on California citrus as well as methodology for their analysis. Dislodgable residues of parathion, ethion, azinphos-methyl, carbophenothion, and dioxothion on Florida citrus during wet and dry periods have been reported (Thompson and Brooks, 1976; Thompson et al., 1976). They stated that the respective oxone metabolities formed less than 10% of the dislodgable residues found.

This report concerns dislodgable residues of *S*-[(2-methoxy-5-oxo-Δ^2-1,3,4-thiadiazolin-4-yl)methyl] *O,O*-dimethyl phosphorodithioate (Supracide) and the monoxone metabolite GS 13007 on Florida orange foliage.

MATERIALS AND METHODS

Supracide (Figure 1) was applied at 1200 gal/acre with high-pressure handgun at 600 lb/in.^{2} to Valencia orange trees in Lake Alfred, FL, until trees were wet at rates of 57 g/362 L of water (×), 2×, and, 3× using a commercial type sprayer during two periods of the growing season of 1974, dry season (May) and wet season (July). Rainfall amounts are listed in Table I. In addition, during the wet season formulations containing oil (0.5%) at the ×, 2×, and 3× rates were applied. Samples consisted of 2.5-cm-diameter disks punched from each of 50 leaves from each treatment on days 0, 1, 3, 5, 7, 14, and 21 following treatment. Zero-day samples were taken when the spray had dried. Each treatment was replicated four times and untreated checks were also sampled. Samples were frozen until analysis.

The extraction procedure consisted of a water + wetting agent wash and partition of the insecticide into hexane following the procedure of Gunther et al. (1973). Supracide was analyzed by flame photometric gas chromatography under the same conditions reported by Thompson and Brooks (1976). Supracide monoxone was analyzed by thin-layer chromatography using fly-head cholinesterase following the method of Mattson et al. (1969). Recoveries averaged 70%, and the minimum detectable quantities were 0.5 ng of Supracide and 0.035 ng of Supracide Monoxone.

Certain 3× samples taken during the wet season were stripped with 50 mL of benzene for 30 min following the water wash. The residues in these samples were used in estimations of total residue and percent dislodgable residue. Data presented are averages of four replicates. Standard error for the entire experiment was 20%.

RESULTS AND DISCUSSION

Dislodgable residues of Supracide disappeared with time after application following a pattern similar to other organophosphates previously reported in studies with Florida citrus (Thompson and Brooks, 1976). Figures 2–4 illustrate the levels of dislodgable residues during dry and wet seasons. Other than the sample taken on the day of application, dislodgable residues on early sampling days

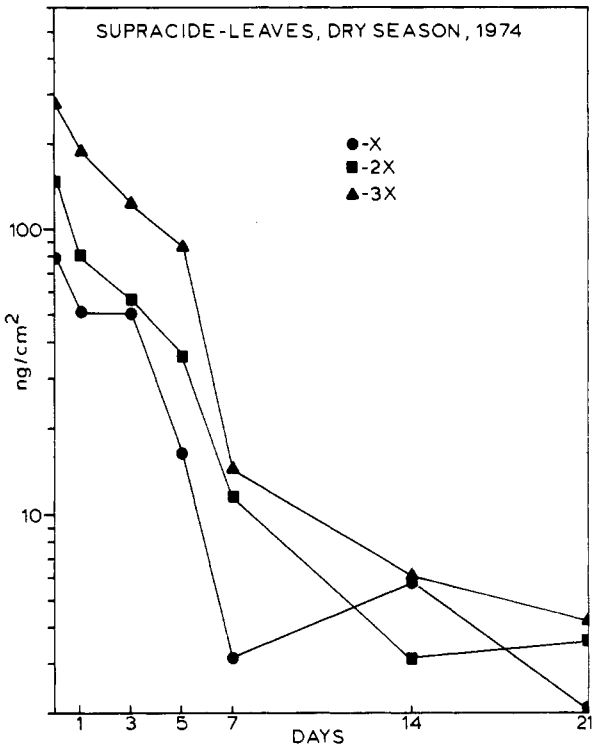

Figure 2. Dissipation of dislodgable residues of Supracide on Valencia orange leaves after application rates of ×, 2×, and 3× during dry season.

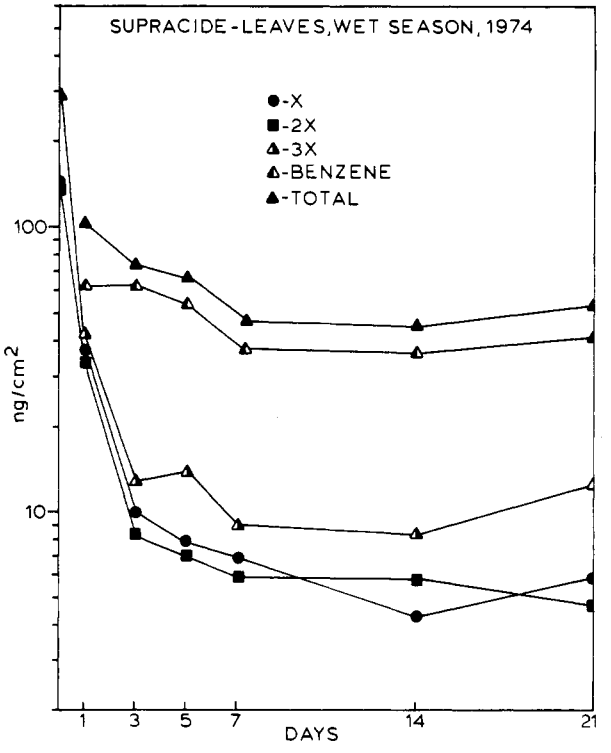

Figure 3. Dissipation of dislodgable residues of Supracide on Valencia orange leaves after application rates of ×, 2×, and 3× during wet season including benzene extractable Supracide following water wash and total Supracide residues: without addition of oil to spray mixture.

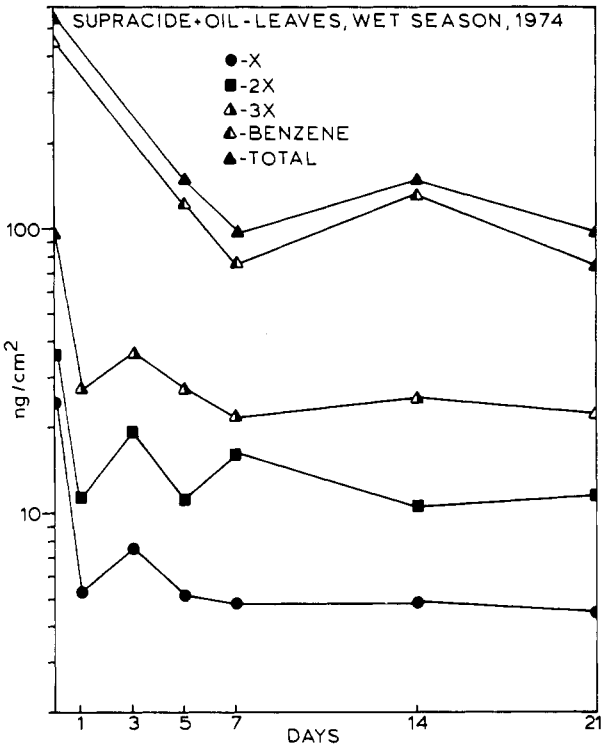

Figure 4. Same conditions as Figure 3 except including oil in spray mixture.

(days 1, 3, and 5) were lower during wet season application but after 2 weeks were similar to dry season samples. During a dry season in Florida it is estimated the leaves have some moisture on them about 70% of the time. The difference in actual leaf moisture during dry and wet seasons is not known. It is clear that there is rapid disappearance of dislodgable residues during the 5–7-day period after spraying, 92–97% for dry and wet season lacking oil and 56–80% when oil was included during wet season.

The amount of dislodgable Supracide present on the day of spray was less when oil was included in the spray mixture during the wet season which is probably accounted for by increased run-off. Oil was not included in the dry season spray routine. Samples taken from day 1 and following show little change in the dislodgable Supracide residues in the plus oil formulation. When the effect of oil is evaluated, there appears to be little difference in the × rate treatments. However, at the 2× and 3× rates dislodgable residues were higher at the later sampling dates (Figures 3 and 4).

Benzene extracts of 3× wet season samples after water washing contained additional residues of Supracide which followed a dissipation pattern similar to that of dislodgable residues (Figures 3 and 4). When oil was included in the spray, the extractable residue was about twice that of the no-oil formulation.

The total Supracide residue present in certain of the 3X treated samples was determined by adding the dislodgable residue to the remaining extractable residue. The percent contribution of dislodgable residue is shown in Tables II and III. It appears that the dislodgable residues comprise about 20% of the total residue, 80% being either absorbed or bound to the leaf surface so as not to be removed by a water detergent wash.

Dislodgable residues of Supracide monoxone generally decrease with time after the day of application (Table IV). It is estimated that quantitation based on comparison of a sample spot size and density to that of standards could

Table II. Relative Contribution of Dislodgable Residue to Total Residue of Supracide (ng/cm²) after 170 g/362 L of Water Was Applied to Orange Leaves during the Wet Season

days	dislodgable (a)	benzene extractable (b)	total, $a + b$	$\frac{a}{a+b} \times 100$	b/a
0	294				
1	43	62	105	40.9	1.4
3	13	62	75	17.3	4.8
5	14	54	68	20.5	3.9
7	9	38	47	19.1	4.2
14	8	37	45	17.8	4.6
21	13	41	54	24.1	3.2

Table III. Relative Contribution of Dislodgable Residue to Total Residue of Supracide (ng/cm²) after 170 g + oil/362 L of Water Was Applied to Orange Leaves during the Wet Season

days	dislodgable (a)	benzene extractable (b)	total, $a + b$	$\frac{a}{a+b} \times 100$	a/b
0	96	448	544	17.6	4.7
1	27				
3	37				
5	28	124	152	18.4	4.4
7	22	76	98	22.4	3.5
14	25	125	150	16.7	5.0
21	22	74	96	22.9	3.4

Table IV. Relative Contribution of Dislodgable Residues to Total Residue of Supracide Monoxone (ng/cm²) after Supracide at 170 g/362 L of Water Was Applied to Orange Leaves during the Wet Season

days	dislodgable (a)	benzene extractable (b)	total, $a + b$	$\frac{a}{a+b} \times 100$	a/b
0	3.3				
1	2.9	4.1	7.0	41	1.4
3	0.8	1.4	2.2	57	1.8
5	1.2	4.6	5.8	36	3.8
7	0.2	3.1	3.3	6	15.5
14	1.1	2.3	3.4	32	2.1
21	2.3	2.5	4.8	48	1.1

contain error up to 30%. Considering the residue level and the high sensitivity of the thin-layer chromatography method (0.035 ng), this amount of error is not considered severe. Occasionally, spots were visible from 0.020-ng applications and this accounts for levels reported less than 0.035 ng. The dislodgable monoxone constitutes about twice the fraction of benzene extractable residue as does the thion. This could occur as a result of less penetration of the monoxone into leaf tissues and a surface conversion of Supracide to the monoxone is also supported by the data. Supracide Monoxone residues are of a sufficiently low order of magnitude that they appear not to contribute to the total residue in a significant way.

The disappearance of dislodgable residue of Supracide follows the pattern of other organophosphates tested on citrus in Florida (Thompson and Brooks, 1976; Thompson et al., 1976) and most closely approximates carbophenothion and ethion. The recommended application rate of

Supracide is, however, one-third that of ethion and carbophenothion.

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Orlandin: A Nontoxic Fungal Metabolite with Plant Growth Inhibiting Properties

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A new metabolite, bis[8,8′-(7-hydroxy-4-methoxy-5-methylcoumarin)], trivial name orlandin, was isolated from *Aspergillus niger* found growing on orange leaves. It was nontoxic to day-old cockerels but significantly inhibited wheat coleoptile growth at 10^{-3}, 10^{-4}, and 10^{-5} M. Orlandin may be a precursor of kotanin, bis[8,8′-(4,7-dimethoxy-5-methylcoumarin)], isolated from *Aspergillus clavatus*. The latter was toxic to day-old cockerels, but did not inhibit the growth of wheat coleoptiles.

In March 1978, while visiting a citrus orchard in Orlando, Florida, we collected leaves from orange trees on which colonies of *Aspergillus niger* were visible. While many trees were diseased, no gross anatomical or physiological differences were observed on leaves infected with *A. niger*. Nevertheless, the organism was examined for plant growth regulator metabolites. Subsequent isolation and extraction of the fungus, from shredded wheat cultures, yielded a crystalline metabolite that inhibited the growth of etiolated wheat coleoptile sections. The metabolite, when compared with the plant growth inhibitor (±)-abscisic acid was as active at 10^{-3} and 10^{-4} M in the coleoptile bioassay, but was less active at 10^{-5} M. (+)-Abscisic acid has been found in many higher plants (Addicott and Lyon, 1969; Milborrow, 1974) and was initially isolated from immature cotton fruits, *Gossypium hirsutum* L. (Ohkuma et al., 1963) and sycamore leaves, *Acer pseudoplatanus* L. (Cornforth et al., 1965). Chemical and physical analyses showed that the new metabolite was bis[8,8′-(7-hydroxy-4-methoxy-5-methylcoumarin)] which we have given the trivial name orlandin (I) (Figure 1).

The simple coumarins have shown a wide range of growth regulating responses in plants from growth promotion to growth inhibition depending on the plant species or plant part treated (Mayer and Poljakoff-Mayber, 1961). In animals, the simple coumarins induce a hypnotic and narcotic response (Dean, 1952; Wawzonek, 1951) and the furanocoumarins are highly toxic to fish (Späth, 1936).

Orlandin is closely related to kotanin (III), bis[8,8′-(4,7-dimethoxy-5-methylcoumarin)], a fungal metabolite isolated from *Aspergillus glaucus* (Büchi et al., 1971) which was later identified as *A. clavatus* (Büchi et al., 1977) found on mold-damaged rice collected from a village in Baan Kota, Thailand. The rice, which was also contaminated with *A. flavus*, *A. niger*, and unidentified *Penicillium*, was implicated in the death of a young boy. However, neither kotanin, nor desmethylkotanin was toxic in rat bioassays. Kotanin did not inhibit the growth of wheat coleoptiles but it was toxic to day-old cockerels in our assay.

MATERIALS AND METHODS

Production and Isolation of Orlandin. *Aspergillus niger* (ATCC accession no. 36626) was isolated from the surface of orange leaves and cultured on potato dextrose agar slants at 26 °C for 7 days. Cultures were maintained at 5 °C until transferred to Fernbach flasks (2.8 L), each containing 100 g of shredded wheat, 200 mL of Difco mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose (Kirksey and Cole, 1974) for production of the metabolite. After 19 days growth at 27 °C, 300 mL of acetone was added to each flask (ethyl acetate in later extractions). Mycelia and substrate were macerated with a Super Dispax Homogenizer. The suspension was strained through cheesecloth to remove the pulp. The filtrate was filtered through Whatman No. 1 filter paper on a Buchner funnel to yield a crude liquid extract. Solvent was removed from the crude extract under vacuum at 50 °C and the resulting aqueous phase was extracted twice with two volumes of ethyl acetate, each equal to the volume of the aqueous phase. Combined ethyl acetate extracts were dried over anhydrous sodium sulfate, reduced

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