Evaluation of Systemic Fungicides and Mineral Oil Adjuvants for the Control of Mal Secco Disease of Lemon Plants

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ABSTRACT

The performance of systemic fungicides in controlling mal secco caused by *Deuterophoma tracheiphila* was evaluated in bioassays and in 1-year-old seedlings of Rough lemon , an extremely susceptible species.

In bioassay tests, carboxin and cycloheximide were the most toxic compounds to *D. tracheiphila*, preventing germination at 2 μg/ml.

Sprays applied before inoculation were superior to those applied afterward. Moreover, the efficacy of postinoculation sprays declined rapidly as application was delayed. Foliar sprays with aqueous benomyl suspension markedly decreased disease severity, whereas Thiabendazole (TBZ) and carboxin treatments were ineffective. Soil drenches with cycloheximide semicarbazone, benomyl, TBZ, and oxycarboxin prevented disease development in stems inoculated 1 month later. The first two compounds and carboxin inhibited infection in leaves inoculated soon after treatment.

Certain mineral oils added to the aqueous leaf sprays of TBZ and carboxin greatly enhanced their performance, probably by increasing uptake. Carboxin suspended in undiluted mineral oil, applied to a band at the base of the stem, suppressed systemic development of the disease when stems were subsequently inoculated above the application band, and inhibited disease development in inoculated leaves.

These results justify the evaluation of TBZ and carboxin with oils in mal secco-infected lemon groves, in a combined stem and foliar application.

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Mal secco, a vascular disease caused by the fungus *Deuterophoma tracheiphila* Petri, inflicts severe damage to lemon trees in the Mediterranean region (14). The first symptom is leaf chlorosis, followed by leaf drying and drop, drying of twigs and trunk, and finally death of the tree. No satisfactory control measure is available (14). In recent years, control of vascular diseases by means of systemic fungicides has been attempted (4). Mal secco has been controlled by soil application of benomyl in pot experiments (3), but similar treatments under natural conditions gave negative results (M. Elia, *personal communication*). Control of the disease in potted seedlings was observed with cycloheximide semicarbazone drenches to the soil (J. Pinkas & Mathilda Chorin, *unpublished data*). However, this fungicide is undesirable due to its phytoxicity and toxicity to humans.

Other vascular diseases have been controlled by soil application in pot experiments, but no satisfactory control has been achieved under natural conditions (4, 6). The report, by Jepson et al. (7), that leaf sprayings or trunk treatment of a systemic acaricide gave better control of lemon bud mites than soil drenches, prompted us to evaluate the application of systemic fungicides to epigeal organs of the plant.

In the present study, known systemic fungicides were bioassayed for their toxicity toward *D. tracheiphila*, and the most active were evaluated for their control of mal secco in 1-year-old Rough lemon seedlings. Different methods of application and the effects of adding mineral oils and other adjuvants to the fungicides were also tested.

MATERIALS AND METHODS.—*Chemicals.*—The fungicides tested were: benomyl (Benlate, WP 50%, E. I. Du Pont de Nemours, Wilmington, Del.); Thiabendazole (TBZ) (Mertect flowable, 42% Merck & Co. Inc., Rahway, N.J.); a dichloro derivative of Thiabendazole, 2-(4'-thiazolyl) dichlorobenzimidazole (ClTBZ); fuberidazole (Farbenfabriken Bayer AG, Leverkusen, Germany); carboxin (Vitavax, 75% WP, UniRoyal Chemical, Bethany, Conn.); oxycarboxin (Plantvax, 75% WP, UniRoyal Chemical); cycloheximide (technical, Upjohn Co., Kalamazoo, Mich.); cycloheximide semicarbazone, a semicarbazone derivative of cycloheximide (6.7% WP, Upjohn Co.); triphenyl tin acetate (TPTA) (Bedilan, 60% WP, Makhteshim Chemical Works, Beer Sheba, Israel).

The following mineral oils were used as adjuvants: Blancol, a summer oil spray consisting of 80% white medium-light oil and 20% water and emulsifiers (Pazchem, Tel Aviv, Israel); Citgo Agricultural Spray Base Oil (CASBO); and Sun Superior Spray Oil 7E, and 11EL (SSSO 7E and 11EL) paraffinic oils with viscosity of 71 and 100, unsulfonated residue over 90%, temperature range of 62 and 93 F between 10% and 90% distillation, and 1% and 0.5% emulsifier, respectively (Sun Oil Co., Philadelphia, Pa.). Additional adjuvants tested were: dimethylsulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), and urea, c.p. grade.

Acidified formulations of TBZ and benomyl were prepared according to the procedure described by Buchenauer & Erwin (2).

Bioassay.—Inhibition of germination of *D. tracheiphila* conidia was tested with the cellophane

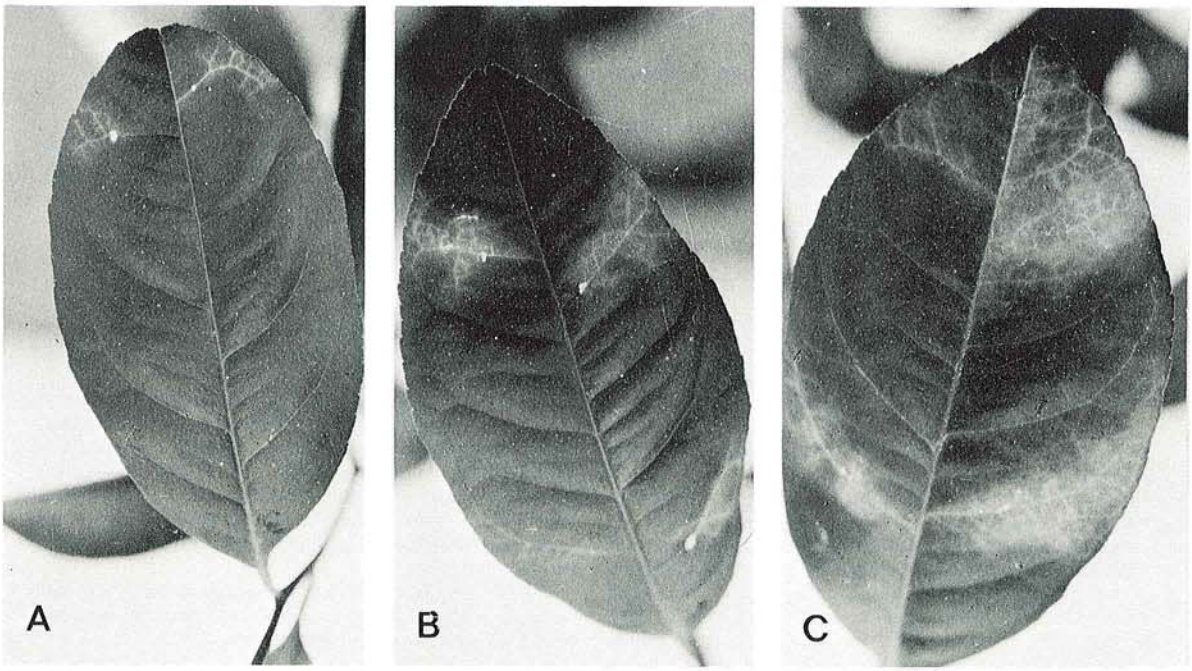

Fig. 1. Infection degrees of mal secco on Rough lemon leaves inoculated by injecting conidia of *Deuterophoma tracheiphila*. **A)** Bottom, degree 0, no infection; top, degree 1, local light chlorosis. **B)** Top, degree 2, spreading light chlorosis. **C)** Degree 3, spreading severe chlorosis.

disc method (9). Germination was rated after 40-hr incubation at 20 C, on a scale of values as follows: 0 = no germination; 1, 2, 3, 4 = germ tube length not exceeding 10, 20, 40, and 80 μ, respectively; 5 = germ tube longer than 80 μ; and 6, in which an elongated germ tube develops secondary branching. Germination value was established from 100 conidia/disc, three discs/test; each fungicide was bioassayed in two repeated tests.

Inhibition of mycelium growth was tested on potato-dextrose agar (PDA) in petri dishes. A 10-mm disc of *D. tracheiphila* mycelium growing on PDA was placed in the center of a dish, and two cellulose discs soaked with 0.08 ml of the fungicide were placed on the opposite margins of the dish, so that the center of the disc was on a straight line between them. After a 15-day-incubation at 20 C, a colony had developed, and its area was determined by drawing its contour on a sheet of plastic with a known ratio between area and weight, and the surface so marked was cut and weighed.

Inoculation.—One-year-old seedlings of Rough lemon, *Citrus jambhiri* Lush., a species extremely susceptible to mal secco, were used. In all experiments, four plants were used for each treatment. The experiments were carried out in a greenhouse maintained at 20 C. Leaves were inoculated at four points by injecting aqueous conidial suspensions of *D. tracheiphila* (10^6 conidia/ml) through the lower epidermis by means of a hypodermic syringe. A wound of approximately 1 mm in diam was formed, often over a vein, and the water was infiltrated over a surface surrounded by the bigger veins; most conidia were retained close to the injection point. Infection was rated weekly for 8 weeks as follows: 0 = no infection; 1 = local light chlorosis (Fig. 1-A); 2 = spreading light chlorosis (Fig. 1-B); and 3 = spreading severe chlorosis (Fig. 1-C). Preliminary trials demonstrated that susceptibility of leaves decreases with aging. Therefore, only the five uppermost, fully expanded leaves of actively growing plants were inoculated. A relative disease index was calculated expressing the average infection degree of the treated plants as per cent of the check.

For stem inoculations, three tiny holes were drilled in leaf axils above the fungicide treatment zone, a few drops of the conidial suspension were injected into them, and the holes were sealed with adhesive tape. The first symptoms, leaf veinal chlorosis, appeared several weeks later; subsequently, the leaves dried up and dropped.

RESULTS.—*Bioassay screening of fungicides.*—Eight fungicides were bioassayed for spore inhibition at three concentrations (Table 1). Carboxin and cycloheximide, the most active, were highly inhibitory at a concentration of 2 μg/ml and prevented any spore germination at 10 μg/ml. TPTA was somewhat less fungitoxic, and oxycarboxin was much less active. The benzimidazole fungicides were moderate in toxicity, and even at 50 μg/ml did not prevent germination. However, they caused malformation of the germ tubes. The semicarbazone derivative of cycloheximide was weakly fungitoxic, but after incubation for 6 hr with an aqueous lemon leaf homogenate, toxicity increased to a degree similar to that of free cycloheximide.

In the colony growth inhibition test, 16 μg of the fungicides were absorbed by each of two cellulose discs. Growth inhibition by cycloheximide, CITBZ, and carboxin was 93, 41, and 8%, respectively. The other fungicides did not show any inhibition of mycelium growth.

Foliage treatments.—Aqueous suspensions of the fungicide, with or without supplemented adjuvants, were sprayed until runoff onto both sides of the leaves with a DeVilbiss No. 15 atomizer. A preliminary trial indicated that the wound formed by the hypodermic needle in the inoculation procedure did not affect fungicidal performance. Treatments with fungicides applied 1, 2, 3, or 4 days before inoculation were equally effective in disease control. The results are summarized in Table 2. The most effective fungicide without adjuvants was benomyl; the other benzimidazole derivatives were either partially (TBZ) or not at all (fuberidazole) active. Carboxin and cycloheximide semicarbazone gave moderate control of the disease; and TPTA, only slight control. Carboxin treatment at 0.1% resulted in negligible control. One supplementary application of 0.1% carboxin 15 or 23 days after inoculation resulted in a relative disease index of ca. 82, whereas three applications, 4, 15, and 23 days after inoculation, lowered the relative disease index to 64.1. The addition of mineral oils to spray suspensions greatly enhanced the fungicidal performance of TBZ and carboxin, SSSO 7E being superior to Blancol. Increasing the oil concentration in the spray from 1 to 5% slightly improved fungicidal performance. Applying 2% SSSO 7E in water separately 2 days before carboxin or TBZ also enhanced fungicidal performance, in both pre- and postinoculation treatments. Adding DMSO, EDTA,

TABLE 1. Germination inhibition of conidia of *Deuterophoma tracheiphila* by fungicides as shown by cellophane disc bioassay (9)

Fungicide	Germination value[a] at indicated fungicide concentration (μg/ml)		
	2	10	50
Benomyl	5.5	2.5	2.0
Thiabendazole	5.5	3.5	2.0
Thiabendazole-dichloro	4.0	3.5	1.5
Carboxin	0.5	0.0	0.0
Oxycarboxin	5.5	2.5	2.0
Cycloheximide	1.0	0.0	0.0
Cycloheximide semicarbazone	4.5	3.0	2.5
Triphenyl tin acetate	3.0	0.3	0.0

[a] Germination value was rated on a scale of values as follows: 0 = no germination; 1, 2, 3, 4 = germ tube length not exceeding 10, 20, 40, and 80 μ, respectively; 5 = germ tube longer than 80 μ; and 6 = elongated and branched germ tubes. Each figure is an average of two tests, each consisting of three replicates of 100 spores.

TABLE 2. Effect of fungicidal sprays, with and without adjuvants, on disease development in lemon leaves inoculated with *Deuterophoma tracheiphila* after treatment

Fungicide	Concentration in spray (%)	Adjuvant	Concentration in spray (%)	Relative disease index[a]
Benomyl	0.5			20.7
Carboxin	0.1			93.3
Carboxin	0.5			50.1
Carboxin	0.5	Blancol	1.0	38.5
Carboxin	0.5	DMSO[b]	2.0	68.6
Carboxin	0.5	EDTA[c]	2.0	73.2
Carboxin	0.5	SSSO 7E[d]	1.0	31.6
Carboxin	0.5	SSSO 7E	2.0	27.8
Carboxin	0.5	SSSO 7E	5.0	22.5
Carboxin	0.5	Urea	2.0	74.3
Cycloheximide semicarbazone	0.05			42.3
Fuberidazole	0.5			109.0
Thiabendazole	0.5			71.4
Thiabendazole	0.5	SSSO 7E	1.0	10.1
Thiabendazole	0.5	SSSO 7E	5.0	7.6
		Blancol	1.0	93.1
		SSSO 7E	2.0	82.6

[a] Infection degree was rated on the following scale: 0 = no infection; 1 = local light chlorosis; 2 = spreading light chlorosis; and 3 = spreading severe chlorosis; and related to check (100%). Each figure is an average of two tests, each consisting of 80 replicates (4 plants × 5 leaves × 4 inoculation points).

[b] Dimethylsulfoxide.

[c] Ethylenediaminetetraacetic acid.

[d] Sun Superior Spray Oil 7E.

and urea to carboxin reduced its ability to control the disease.

For elucidating the effect of the mineral oils, leaves treated with 0.05 or 0.5% aqueous carboxin suspension either with or without supplemented Blancol at 1 ml/100 ml were sampled 0.5 and 96 hr after treatment. The leaves were extracted by water (13), and the extracts were bioassayed for carboxin content by the cellophane disc method. Inhibition rate was calculated by the formula:

$$\text{Inhibition factor} = 1 - \frac{\text{sample germination value}}{\text{check germination value}};$$

thus, a factor of 0 would designate no fungitoxic activity, while 1 would indicate strong activity. The presence of carboxin was suggested by R_F value in thin-layer chromatography (13). In the first sampling, only carboxin at 0.5% with Blancol inhibited spore germination (inhibition factor 0.16). In the second sampling, inhibition factors of treatments with carboxin 0.05% unsupplemented and supplemented were 0.20 and 0.30, respectively. The corresponding inhibition factors for carboxin 0.5% were 0.50 and 0.54, respectively. The inhibition factor of leaves treated with 1% Blancol itself was 0. These values imply that the addition of Blancol to spray suspension both expedited and increased carboxin uptake via leaves. In another experiment, inhibition factors of leaves sprayed with 0.05% carboxin alone or with added 5% DMSO or 2% urea 8 days after treatment were 0.60, 0.63, and 0.48, respectively, and with 0.5% carboxin, the corresponding inhibition factors were 0.85, 0.84, and 0.69. Although both adjuvants decreased carboxin performance, only urea diminished its uptake. Results of both experiments show that higher concentrations of carboxin in the spray suspension enhanced its uptake, and that carboxin content in leaves increased with time.

The results of treatments 2 days after inoculation are summarized in Table 3. None of the unsupplemented fungicides reached a high level of control, but benomyl and cycloheximide semicarbazone were the most effective. Supplemented oils considerably enhanced the fungicidal performance of carboxin and TBZ, whereas that of benomyl was only moderately improved. SSSO 11EL was inferior to other oils. Acidified TBZ solution gave better control than the aqueous suspension, without any visible phytotoxic effect. Acidified benomyl was, however, inferior to the aqueous suspension. Foliage treatments that reduced severity of the disease also delayed its development.

Control declined as the time interval between inoculation and treatment increased. The relative disease index was 48.6, 38.0, and 51.5, respectively, when 0.5% benomyl, carboxin, or TBZ, all plus 2% SSSO 7E, were applied 4 days after inoculation. When applied after 6 days, relative disease indices increased.

Application to stem.—The lower leaves were removed, and a band of 5 cm at the base of the stem was wrapped with 1-cm-thick absorbent cotton, oversaturated with the fungicide suspension, and tied with plastic tape. The diameter of treated stems was 6 mm, and bark width was 460 μ. The effect of stem treatment was studied with both leaf and stem

TABLE 3. Effect of fungicidal sprays, with and without adjuvants on disease development in lemon leaves inoculated with *Deuterophoma tracheiphila* 2 days before treatment

Fungicide[a]	Adjuvant[b]	Relative disease index[c]
Benomyl		50.2
Benomyl	CASBO[d]	35.9
Benomyl	SSSO 7E[e]	26.1
Benomyl	SSSO 11EL[f]	49.2
Benomyl	Hydrochloric acid	69.2
Carboxin		85.9
Carboxin	Blancol	35.2
Carboxin	CASBO	31.9
Carboxin	SSSO 7E	18.9
Carboxin	SSSO 11EL	43.8
Cycloheximide semicarbazone		50.0
Thiabendazole		76.0
Thiabendazole	CASBO	32.2
Thiabendazole	SSSO 7E	10.8
Thiabendazole	SSSO 11EL	46.5
Thiabendazole	Hydrochloric acid	36.1
Triphenil tin acetate		78.8

[a] Concentration in spray suspension was 0.5% except for that of cycloheximide semicarbazone, which was at 0.05%.

[b] Concentration of mineral oils in spray suspension was 2%. That of hydrochloric acid was 2% of a normal solution.

[c] Infection degree was rated on a scale from 0 = no symptom to 3 = spreading severe chlorisis, and related to check (100%). Each figure is an average of two tests, each consisting of 80 replicates (4 plants × 5 leaves × 4 inoculation points).

[d] Citgo Agricultural Spray Base Oil.

[e] Sun Superior Spray Oil 7E.

[f] Sun Superior Spray Oil 11EL.

inoculation. In leaves inoculated 8 days after treatment with 0.5% carboxin in undiluted SSSO 7E 20-25 cm above the application band, disease development was inhibited during the 1st month (relative disease index = 72). Treatments with either 0.5% aqueous carboxin suspension or 0.5% benomyl suspension in SSSO 7E were completely ineffective.

When stems were inoculated 4 weeks after treatment with aqueous carboxin or with benomyl suspended in oil, severe infections were observed after 5 weeks, whereas those treated with 0.5% carboxin in undiluted SSSO 7E did not show any symptoms up to 8 weeks after inoculation; at that time, slight chlorosis was observed in leaves.

Application to soil.—Fungicides were suspended in water and applied as a soil drench, 2 g/4 kg dry wt sandy loam (cycloheximide semicarbazone 0.6 g only) in 20-cm plastic pots with a single lemon seedling. The soil was watered every other day throughout the experiment. Cycloheximide semicarbazone, benomyl, and carboxin in descending order were effective when leaves were inoculated 7 days after soil treatment (Fig. 2). After 2 months, both disease rate and speed of development were affected. When stems were inoculated 4 weeks after soil treatment and observed 10 weeks after inoculation, cycloheximide semicarbazone, benomyl, TBZ, and oxycarboxin drenches prevented disease development. Carboxin controlled the disease in half the plants, whereas all TPTA treated and untreated plants were infected. No phytotoxic symptoms were visible in foliage, except with oxycarboxin where interveinal chlorosis was observed. However, growth was completely suppressed by cycloheximide semicarbazone, and markedly inhibited by oxathiin compounds.

DISCUSSION.—The performance of the tested fungicides reflects their fungitoxic as well as systemic properties. The latter depend on the plant organ through which uptake occurs, and on the physicochemical properties of both the fungicide and the carrier fluid. Thus, aqueous suepensions of cycloheximide semicarbazone and carboxin were most fungitoxic towards *D. tracheiphila*, and highly effective in controlling the disease when applied as a preinoculation soil drench, but less so when applied as a foliage spray, presumably due to low leaf uptake. Three months after soil drench, carboxin had lost half

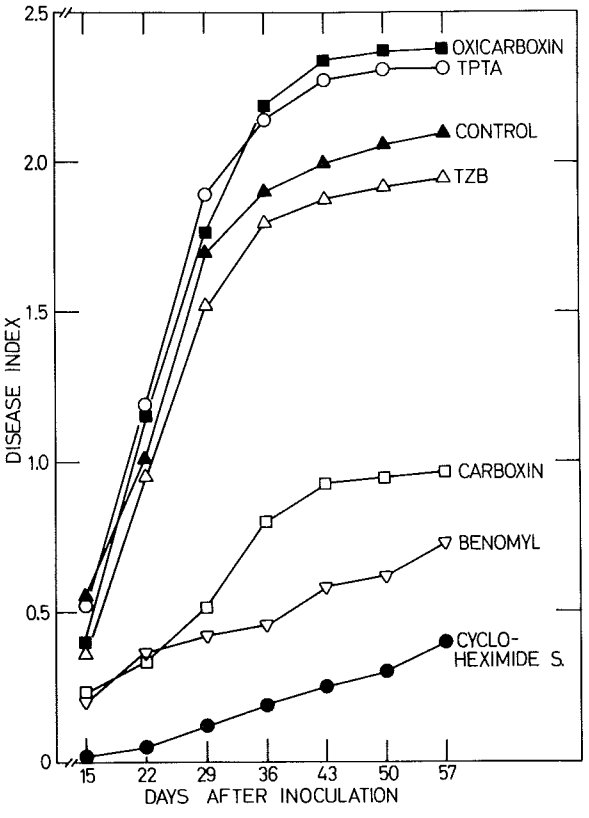

Fig. 2. Mal secco development in Rough lemon leaves inoculated with *Deuterophoma tracheiphila*. Fungicides were drenched onto soil, 2 g/4 kg dry wt (cycloheximide semicarbazone 0.6 g only) 7 days before inoculation. Disease index is the average infection degree of 80 replicates (4 plants × 5 leaves × 4 inoculation points) rated on a scale from 0 = no symptom to 3 = spreading and severe chlorosis. Cycloheximide S. = cycloheximide semicarbazone.

its activity. Conversely, the sulfone analogue, oxycarboxin, was ineffective in controlling the disease 1 week after soil treatment, but later on, an effective and lasting activity was reached. Since both oxathiin compounds are relatively water-soluble, one would expect them to be available to the plant's root system. The difference in their performance may be interpreted on the basis of studies by Snel & Edgington (11), who found in bean plants that both uptake and metabolism of oxycarboxin were slower than that of carboxin. Due to its superior fungitoxicity and presumably rapid uptake, carboxin was effective soon after the time of treatment, whereas oxycarboxin gave better long-lasting protection. Although aqueous benomyl was weakly fungitoxic, it nevertheless was markedly fungicidal when applied as a soil drench or as a foliar spray, suggesting a superiority in both uptake and translocation. Although similar in toxicity to benomyl, TBZ was ineffective in both soil and foliage treatments. The inefficacy of TBZ as a soil drench may be interpreted on the basis of results obtained with cotton plants (4), where it was suggested that TBZ translocates to the terminal portions of the plant less freely than benomyl or carboxin. Retention of this chemical by soil particles is not likely (12). The in vitro effect of benzimidazole fungicides, causing malformation of germ tubes, is similar to that reported with other fungi (5, 12). The distinct toxicity of CITBZ in the colony growth inhibition test may be attributed to its exceptional water solubility, which increased its diffusion rate in the agar plate, an important factor in this type of assay. TPTA was moderate in fungitoxicity and evidently limited in systemicity; thus, it was ineffective when applied to soil, but gave some control when sprayed on leaves. The semicarbazone derivative of cycloheximide was used in the control experiments rather than the free cycloheximide, since a derivative was reported to be less phytotoxic, although less fungitoxic in vitro. In vivo it undergoes chemical cleavage to the parent molecule (8).

TBZ and carboxin applied to leaves attained marked fortification from oil adjuvants, whereas benomyl was affected to only a small extent. Highest increase in performance of those three fungicides was obtained with SSSO 7E. This oil also enhanced the performance of carboxin, but not of benomyl, when the two were suspended in it and applied to stem bark. Peterson & Edgington (10) found that oils enhanced benomyl performance in controlling Dutch elm disease when applied to the bark of twigs. They excluded a direct effect of the emulgator present in the oil formulation (C. A. Peterson & L. V. Edgington, *personal communication*).

The effect of oils on pesticide performance has been studied extensively with herbicides. Oil-in-water emulsions or oils as carriers enhance the performance of herbicides by increasing the latter's penetration (1). Likewise, our results imply that the oil expedited and increased fungicide penetration. We studied only a limited number of mineral oils, and the specifications conferring fortification ability are not yet clear. However, it is clear that oils differ in that activity. SSSO llEL, e.g., is an ineffective oil, has relatively high viscosity, a wide distillation range, and an especially low rate of emulsifier.

Benzimidazoles in acidified solutions varied in performance; the activity of TBZ was increased, but that of benomyl was decreased. Whereas results with TBZ are in agreement with those reported by Buchenauer & Erwin in cotton (2), those with benomyl do not agree. Thin-layer chromatography confirmed their findings that in the HCl solution, TBZ remained chemically unchanged, while benomyl hydrolyzed to methyl 2-benzimidazole-carbamate (MBC). Although TBZ penetration into leaves is increased in the soluble state, it appears that penetration of MBC is inferior to that of the low-soluble authentic benomyl. Low transcuticular movement of MBC was observed with isolated apple cuticles (Z. Solel & L. V. Edgington, *unpublished data*).

Only suggestions can be made at the present time as to the practical implications of our findings. It seems that preventive treatments are more effective than therapeutic ones. In nature, primary infection may occur via leaves as well as through twigs. Soil and stem treatments, which effectively inhibited the systemic development of the disease, revealed only partial control of the disease in leaves, probably due to a low rate of fungicide accumulation in leaves. Bioassay studies seem to confirm this assumption (13). As mentioned in the introduction, soil treatments were ineffective under natural grove conditions. It therefore seems worthwhile to evaluate, in affected lemon groves, carboxin and TBZ with oil in a combination of band "painting" on the trunk and major branches, together with foliage spraying.

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