Fungicidal Effects of Some Acaricides on *Mycosphaerella citri*

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ABSTRACT

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Among acaricides applied to citrus trees in Florida, chlorobenzilate, cyhexatin, dicofol, and sulfur were fungicidal to *Mycosphaerella citri*, the greasy spot pathogen. Carbophenothion, dialifor, dioxathion, ethion, fenbutatin oxide, and propargite exhibited relatively little or no such activity, even in vitro at 200 μg a.i./ml. In greenhouse tests on leaves of rough lemon (*Citrus jambhiri*) inoculated with *M. citri*, applications of chlorobenzilate, cyhexatin, dicofol, and sulfur during a 10- to 12-day infection period consistently reduced greasy spot severity. Only sulfur and cyhexatin, and sometimes dicofol, reduced greasy spot severity when applied 4–7 days before inoculation. In the field, two foliar spray treatments of grapefruit (*Citrus paradisi*) trees with sulfur or dicofol, but not chlorobenzilate, reduced greasy spot severity. This effect, however, was too slight to consider sulfur or dicofol as possible substitutes for copper fungicides or spray oil for greasy spot control.

In 1980, Timmer et al (5) reported in Texas that grapefruit (*Citrus paradisi* Macf.) trees sprayed with the acaricides chlorobenzilate and dicofol showed less greasy spot caused by *Mycosphaerella citri* Whiteside than untreated trees. Previously, Van Brussel (6) in Surinam had reported a reduction in greasy spot severity by treating the leaves of citrus trees with chlorobenzilate. He concluded this response was due to the control of citrus rust mites (*Phyllocoptruta oleviora* (Ashmead)), based on a belief that rust mites promoted greasy spot attack. Timmer et al (5), however, noted that the acaricide treatments reduced greasy spot even when the rust mite populations were very low. They suggested, therefore, that the treatments may have a direct effect on *M. citri*.

Except for some acaricides fungicidal to powdery mildews, acaricides other than sulfur are generally regarded as having no action against fungal pathogens; however, a fungicidal action of cyhexatin on *Cercospora arachidicola* has been reported (3).

This paper reports on the fungicidal action of some acaricides on *M. citri*. Claims of the possible impact of rust mites on greasy spot severity are reexamined in the light of some of the results obtained.

MATERIALS AND METHODS

Most of the acaricides currently used in Florida citrus groves to control citrus rust mites and other mites were tested: carbophenothion (Trithion 4EC), chlorobenzilate (Chlorobenzilate 4EC), cyhexatin (Plictran 50WP), dialifor (Torak 4EC), dicofol (Kelthane 1.6EC), dioxathion (Delnav 8EC), ethion (Ethion 4EC), fenbutatin oxide (Vendex 50WP), propargite (Comite 6.75EC), and wettable sulfur. The currently recommended treatments for control of greasy spot are a copper fungicide or citrus 435 spray oil. Therefore, basic copper sulfate (containing 53% Cu) and spray oil were included in all the field experiments and the copper fungicide was also included in some in vitro and greenhouse tests.

Laboratory in vitro studies. Each test material was suspended or dissolved in sterile distilled water and added to sterile cooled Difco Bacto cornmeal agar (CMA) before being poured into four-compartment 90-mm-diameter petri dishes. All materials were tested at 0, 50, 100, and 200 μg a.i./ml. Each compartment was inoculated at two locations with 4-mm^2 blocks of mycelium from 2-wk-old colonies of *M. citri* on CMA. Colony diameters were measured after 10–14 days of incubation in the dark at 25 C. Four replicate dishes were used for each material. Four separate dishes with CMA alone were inoculated in each test and the growth of these colonies was compared with that on the sector in the four-compartment dishes that contained CMA alone. This was done to determine if there was any vapor action from acaricides present in the other compartments of the same dish.

Greenhouse studies on inoculated leaves. Rough lemon (*Citrus jambhiri* Lush.) was used because of the relatively short incubation period for greasy spot symptoms on this host (7). The container-grown plants were cut back to about 8 cm above soil level to induce new shoot growth. Only two of the new shoots were retained. After at least 10 leaves had expanded fully on each shoot, all shoot growth above the uppermost fully expanded leaf was removed from all plants on the same date. Thus, the 10 uppermost leaves on each shoot fell within a similar age range. This minimized variations in disease severity due to differences in the age of leaves at time of inoculation. Even minor variations in leaf age had been observed to affect symptom severity.

Inoculum of *M. citri* consisted of a suspension of mycelial fragments in 1% sucrose solution prepared from colonies grown on a modified Fries solution (sucrose 30 g, ammonium tartrate 5 g, ammonium nitrate 1 g, potassium dibasic phosphate 1 g, magnesium sulfate (anhydrous) 0.25 g, sodium chloride 0.1 g, calcium chloride 0.1 g, and distilled water to 1 L). After 10–14 days of growth, the fungal colonies were strained and washed on cheesecloth and fragmented for 5 sec in a Waring Blendor. The resulting suspension was poured through a 500-μm (32-mesh) sieve to remove excessively large fragments.

The inoculum was sprayed onto the lower surfaces of the leaves and inoculated plants were placed immediately in a moist chamber at near 100% relative humidity for 2 days. For the next 8–10 days, the plants were exposed on the greenhouse bench during the day and placed in the moist chamber only overnight.

The time from application of the inoculum to the time the plants were removed permanently from the moist chamber was defined as the infection

Table 1. Effect of basic copper sulfate and various acaricides at 200 μg a.i./ml on the growth of *Mycosphaerella citri* in vitro

Material	Growth inhibition[a] (%)
Basic copper sulfate[b]	62
Carbophenothion	12
Chlorobenzilate	48
Cyhexatin	46
Dialifor	12
Dicofol	82
Dioxathion	4
Ethion	11
Fenbutatin oxide	20
Propargite	8

[a] Results represent the means for at least two tests on cornmeal agar.

[b] Concentration expressed as metallic copper content.

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period. During this period, hyphal fragments formed a branching extramatrical growth and hyphal tips grew into and through the stomata after forming appressoria in the outer stomatal chambers. The sucrose in the inoculum promoted extramatrical growth, thereby increasing the chances for numerous stomatal penetrations essential for disease development (8).

The materials tested on the inoculated plants included all those causing more than 40% inhibition in fungal growth in vitro (Table 1) and two commonly used acaricides, ethion and fenbutatin oxide, which showed little or no fungicidal activity in vitro. The materials were mixed with water at the recommended field rates of application (Tables 2 and 3) and sprayed onto the lower leaf surfaces with a pressure-retaining hand sprayer. The treatments were applied either 4 or 7 days before inoculation to detect any protectant effect or 4 days after inoculation to determine the on-contact sensitivity of extramatrical mycelial growth to the materials. Disease severity was based on the amount of greasy spot-induced defoliation of the top 10 leaves on each shoot and was recorded at a time when about 50% of the leaves on untreated plants had abscised, which varied from 13 to 22 wk after inoculation.

Field studies. Treatments were applied as dilute (high-volume) sprays to runoff by handgun at 30 L/tree to single-tree plots of Marsh grapefruit and replicated six to eight times in a randomized block design. Each treatment (Table 4) was applied only once in tests 1 and 3 on 9 July 1980 and 16 July 1981, respectively. In test 2, the copper fungicide and oil treatments were applied only on 30 June, but the other treatments were applied on 12 August as well as on 30 June. Rust mites were controlled in each experiment by applying ethion routinely in early May and again during the summer and fall. Thirty or 40 shoots of the current year's spring growth flush were tagged on each tree to assess greasy spot-induced defoliation and greasy spot symptoms on surviving leaves. These evaluations were made on 13 January 1981, 13 January 1982, and 16 February 1982 in tests 1, 2, and 3, respectively. In evaluating the data, a significant difference at $P = 0.05$ in either defoliation or the percentage of remaining leaves with symptoms was regarded as a positive difference between treatments.

RESULTS

In vitro tests. Results of the in vitro agar tests are given only for the highest concentration of each material tested, 200 μg a.i./L (Table 1). None of the materials tested had enough vapor action to retard growth of the fungus in untreated sections of dishes in which other compartments contained a test material. Basic copper sulfate, chlorobenzilate, cyhexatin, and dicofol substantially reduced mycelial growth of *M. citri*. Dicofol showed more activity than chlorobenzilate or cyhexatin. Carbophenothion, dialifor, dioxathion, ethion, fenbutatin oxide, and propargite showed little or no fungicidal activity.

Greenhouse tests. No fungicidal action was exhibited by ethion or fenbutatin oxide at either time of application. Preinoculation spray treatments with sulfur, basic copper sulfate, and cyhexatin reduced greasy spot severity in all tests where they were included (Tables 2 and 3). When applied before inoculation, chlorobenzilate never reduced greasy spot severity and dicofol reduced disease severity significantly in only one of three tests (Table 3). When applied 4 days after inoculation during the infection period, basic copper sulfate, sulfur, dicofol, and cyhexatin, and to a lesser extent, chlorobenzilate consistently reduced greasy spot severity (Tables 2 and 3).

Field tests. In the three field tests (Table 4), only the standard treatments with basic copper sulfate or oil consistently reduced greasy spot severity. Dicofol and sulfur only reduced greasy spot severity in test 2, which was the only test in which they were applied twice.

DISCUSSION

Despite timely treatments with ethion to control rust mites, some of these pests

Table 2. Greenhouse tests to determine the effect of spray materials on greasy spot severity on rough lemon leaves inoculated with *Mycosphaerella citri*

		Greasy spot-induced defoliation (%)			
		Applied 7 days before inoculation		Applied 4 days after inoculation	
Material	Rate (mg a.i./L)	Test 1	Test 2	Test 3	Test 4
Basic copper sulfate	485[y]	0 c[z]	1 c	10 c	4 bc
Sulfur	6,000	1 c	14 b	25 bc	0 c
Chlorobenzilate	300	41 ab	56 a	38 b	13 b
Cyhexatin	225	12 bc	...	26 bc	...
Dicofol	384	64 a	49 a	29 bc	1 bc
Ethion	450	34 ab	...	40 ab	...
Fenbutatin oxide	300	50 a	...	...	...
Control (untreated)		50 a	54 a	55 a	48 a

[y] Concentration (mg a.i./L) expressed as metallic copper content.
[z] Numbers in each column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Effect of spray materials on greasy spot severity on rough lemon leaves inoculated with *Mycosphaerella citri* in the greenhouse

		Greasy spot-induced defoliation (%)	
Material	Rate (mg a.i./L)	Applied 4 days before inoculation	Applied 4 days after inoculation
Chlorobenzilate	300	67 a[z]	41 b
Cyhexatin	225	19 c	1 c
Dicofol	384	32 bc	9 c
Ethion	450	52 ab	54 ab
Fenbutatin oxide	300	65 a	60 ab
Control (untreated)		64 a	

[z] Numbers followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Effect of various foliar spray treatments on greasy spot severity on Marsh grapefruit trees

		Disease severity (%)					
		Test 1		Test 2		Test 3	
Material[v]	Rate (mg a.i./L)	DEF[w]	RLD[x]	DEF	RLD	DEF	RLD
Basic copper sulfate	485[y]	4 b[z]	28 b	0 d	9 d	11 d	14 cd
Oil	10,000	13 b	33 b	1 cd	20 cd	12 cd	28 c
Chlorobenzilate	300	58 a	71 a	5 ab	56 a	45 a	67 ab
Dicofol	384	50 a	70 a	3 bc	30 bc	28 abc	66 ab
Sulfur	6,000	50 a	70 a	2 bc	45 b	39 ab	58 ab
Control (untreated)	...	49 a	68 a	6 a	61 a	40 ab	76 a

[v] In test 2, chlorobenzilate, dicofol, and sulfur were applied twice. Otherwise, the materials were applied only once.
[w] DEF = Percent defoliation.
[x] RLD = Percent remaining leaves diseased.
[y] Concentration (mg a.i./L) expressed as metallic copper content.
[z] Numbers in each column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

were always present in the field tests, making it impossible to determine whether they had any complicity in greasy spot attack or development. In contrast, no rust mite infestations occurred on plants grown in the greenhouse. The results from the greenhouse tests supported the idea that reduced greasy spot severity in the field after applying dicofol or sulfur was probably due to fungicidal action by these materials. The effect of these materials, however, was too slight and variable for them to be considered as possible alternatives to fungicides or spray oil for greasy spot control, particularly if applied only once.

Chlorobenzilate and dicofol probably acted mainly on the epidemiologically important extramatrical hyphal growth of *M. citri* on the leaf surface (8), either through on-contact lethal action or as an inhibitor of hyphal growth. Chlorobenzilate reduced greasy spot only when applied during the infection period, whereas dicofol also showed some protectant action. Timmer et al (5) were probably correct in concluding that reduced greasy spot severity on trees sprayed with chlorobenzilate and dicofol may have been due to a direct action of these materials on *M. citri* rather than to an indirect effect through rust mite control. The reduced greasy spot severity reported from the Texas experiments, however, may have been due more to the dicofol treatments than the treatments with chlorobenzilate.

Suggestions that greasy spot might be promoted by citrus rust mite first appeared in the literature (4) before greasy spot was determined to be a fungus disease (2,7). Originally, greasy spot was even considered to be a direct result of the feeding of rust mites (4). This conclusion was based on an observed reduction in greasy spot severity when trees were sprayed with sulfur, which controls rust mites. Now it seems that the previously observed reduction in greasy spot severity was probably due to a direct fungicidal action of sulfur on *M. citri*.

Ideas that punctures in the epidermis by rust mites might assist access of *M. citri* to the host were dismissed as unlikely after studies on the mode of entry of the pathogen into leaves (8) and more detailed histological studies of rust mite injuries (1). In 1975, however, Van Brussel (6) revived the idea of rust mite involvement in greasy spot epidemiology. He concluded that in Surinam, there was such a close association between rust mite populations and greasy spot severity that greasy spot might be controllable merely by preventing a buildup of rust mites. These conclusions were based on some experiments in which the canopies of young grapefruit trees were dipped every 10 days for several months (number unspecified) in aqueous mixes of chlorobenzilate or trichlorphon to prevent rust mite infestations. Other batches of plants were left untreated. Relatively little greasy spot developed on the treated plants compared with the heavily rust mite-infested untreated plants. Van Brussel (6) concluded from these results that rust mites promoted greasy spot attack. The results of my tests indicated that the response was more likely due to a direct fungicidal effect of chlorobenzilate on *M. citri*, an effect probably accentuated by the high frequency of treatment. The insecticide trichlorphon, which Van Brussel (6) also used to control rust mite in his experiments, is also fungicidal to *M. citri* (J. O. Whiteside, *unpublished*).

In Florida at least, severe greasy spot can occur even when rust mite populations are low. In some citrus groves, however, heavy rust mite infestations and severe greasy spot attacks tend to occur simultaneously year after year, hence the popular belief of a possible interrelationship between rust mites and greasy spot. An explanation for such occurrences is that some microclimatic effect or cultural practice might favor, independently, a buildup of rust mites and promote more infection by *M. citri*. Previous conclusions (4,6) of an interrelationship between rust mites and greasy spot disease were based on results of tests with acaricides, which are now known to have some fungicidal activity against *M. citri*. Therefore, no convincing evidence exists that rust mites can increase greasy spot severity.

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