indicated by the differences in percentage leaves diseased. In terms of percent leaf diseased, greasy spot control improved overall with increasing DT of the oil applied. Nevertheless, only in Experiment 3 were distinct differences in efficacy exhibited between all oils. The 455 oil was significantly more effective than 435 oil in reducing the percent leaves diseased in Experiments 3 and 4, but not in Experiments 1, 2 and 5.

If a copper fungicide is not applied to grapefruit trees in the summer, there might be some incentive for using an oil of higher DT than 435 oil to enhance greasy spot control on leaves, provided that the heavier oil imposed no adverse physiological effect on the trees or fruit. It should be noted, however, that basic copper sulfate alone or mixed with 0.5% oil was more effective in reducing greasy spot on leaves than 1% 435 or 455 oils in Experiments 1, 3 and 4.

Most grapefruit groves are managed with the intention of producing a high packout of fruit for the fresh market, which means ensuring adequate control of GSRB. Because the heavier oils do not adequately control GSRB, it would still be necessary to include a copper fungicide in the summer oil spray mix when the fruit is intended for the fresh market, even if a heavier oil is used. Where copper is needed anyway, there would be little incentive for using a heavier oil such as the one with 455 DT specifications, unless it is considered essential for enhanced control of various insects or mites.

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EFFECTIVENESS OF FUNGICIDE SUPPLEMENTS TO SPRAY OIL FOR IMPROVING GREASY SPOT CONTROL ON GRAPEFRUIT LEAVES AND FRUIT

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Abstract. **An oil spray in the summer provides the mainstay for control of greasy spot (*Mycosphaerella citri* Whiteside) in Florida citrus groves. For grapefruit trees, a copper fungicide is commonly added to the spray mix to improve control, particularly on the fruit. Copper fungicides are highly effective for greasy spot control but darken scar tissue on the rind, which further detracts from the appearance of fruit intended for the fresh market. Copper fungicides have another disadvantage in that if their application is delayed from July to August to protect more summer growth flush, their effectiveness in controlling greasy spot on the spring growth flush may be reduced. One of the more promising materials studied in recent years was an experimental triazole derivative and ergosterol biosynthesis inhibitor, CGA 169374. It controlled greasy spot well and provided a bright fruit finish. However, despite some curative kickback action, as demonstrated on inoculated plants in the greenhouse, it still controlled greasy spot on the spring growth flush better when it was applied in July than when it was delayed until August.**

The multipurpose oil spray that is commonly applied to Florida grapefruit groves in the summer is useful for reducing the severity of greasy spot, caused by *Mycosphaerella citri* Whiteside, on leaves, but it has little or no value for preventing the development of greasy spot rind blotch (GSRB) (5). When control of GSRB is needed, as on grapefruit grown for the fresh market, it is necessary to add a fungicide to the spray mix to protect the rind from this blemish. Applications of oil alone can also be relatively poor sometimes for controlling greasy spot on leaves, particularly when disease pressure is heavy (6).

Little is known about the mode of action of oil in controlling greasy spot or of the factors that influence its effectiveness. Oil has little direct effect on *M. citri* or on the infection process. Rather, oil prevents the symptoms of greasy spot developing mainly through some alteration of the host physiology (2). Because oil has little or no effect on the greasy spot pathogen, spray timing with oil is less critical than it is with fungicides. Most fungicides have to be applied before major infection occurs, whereas oil can still be effective if applied long before or after infection (5).

Currently, many grapefruit growers include a copper fungicide in the summer oil spray when greasy spot control on leaves needs to be enhanced and when GSRB has to be controlled (1). No other material has proven as reliable as copper for greasy spot control (6). However, when the fruit is intended for the fresh market, copper fungicides can have an undesirable side effect: they emphasize existing injuries, such as those caused by wind scar and melanose, thereby causing further downgrading of fruit.

In the 1970's, when benomyl became available to citrus growers, it seemed this material would be a useful and safe alternative to copper fungicides for greasy spot control. However, control failures soon began to occur because of fungal tolerance to benomyl. In some groves, benomyl failed to provide significant control of greasy spot after it was applied once a year for only 4 consecutive yr (4).

The search has continued over the years for safer alternatives to copper fungicides, but without much success.

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The dithiocarbamates and chlorothalonil were much less effective than copper for greasy spot control, and folpet, captan, dodine, iprodione and dithianon provided little or no control (6). In recent years, several of the sterol-inhibiting fungicides have been tested against greasy spot, including triforine, propiconazole, prochloraz, sisthane, fenarimol, nuarimol and pyrifenox (6, 7, 8), but none of them showed sufficient promise or reliability to justify further development for use against this disease.

This paper reports the results of experiments with two experimental sterol-inhibiting fungicides, diniconazole (Spotless) and CGA 169374, for greasy spot control. Applications of these materials were made to plants in the greenhouse at various times after inoculating them with *M. citri*, to detect any postinfection curative activity. Other experiments were conducted in grapefruit groves to determine the field performance of these two materials.

Materials and Methods

Materials used. The spray materials used were diniconazole (Spotless 25W) supplied by Valent U.S.A. Corp., Tallahassee, FL., CGA 169374 (3-chloro-4-[4methyl-2-(1H-1,2,4-triazole-1-ylmethyl)-1,3-dioxolan-2 yl] phenyl 4-chlorophenyl ether) 3.5 EC, supplied by Ciba-Geigy Corp., Greensboro, NC, basic copper sulfate 53% Cu (Tribasic copper sulfate) supplied by Tennessee Chemical Company, Atlanta, GA, and Sunspray 7N 435 oil, supplied by Sun Oil Company, Philadelphia, PA. The spray oil was emulsified by adding Thompson-Haywood T-MULZ-FLO emulsifier to it at the rate of 30 ml/gal.

Greenhouse experiments. 'Marsh' grapefruit plants on rough lemon rootstock in 1 gal pots were cut back close to the bud union to induce new growth. Only 2 of the new shoots were retained. After at least 10 leaves had expanded on each shoot, the shoots were cut back above the last fully expanded leaf, thus retaining at least 10 leaves on each shoot, with the difference in age between the oldest and youngest leaves being only about 2 weeks. To minimize experimental variability, it was important to conduct the tests on leaves of similar age because greasy spot develops earlier on leaves infected while young than on those that are only a few weeks older at the time of infection.

Inoculum of *M. citri* was cultured and prepared as previously described (3), thereby providing a suspension of mycelial fragments. This suspension was deposited on the underside of the leaves with a pressurized hand sprayer. Immediately after inoculation, the plants were placed in a damp chamber and kept there for 2 days. During this period, hyphae growing from the mycelial fragments formed a branching fungal growth over the leaf surface. However, during this 2-day period, there was little leaf penetration. To encourage infection, the plants were allowed to dry during the daytime and returned to the damp chamber only overnight. This drying and wetting procedure continued for 12 days and it promoted more leaf penetration, which was through stomata, than would have occurred if the leaves had been kept continuously wet (3). After the 14-day inoculation and infection period, the leaves were kept dry to prevent further infection.

The spray materials were applied to both the upper and lower sides of the leaves with a pressurized hand sprayer at specified times after the end of the infection period.

Assessments for disease severity were made before greasy spot-induced defoliation began on the untreated plants. The area of each leaf with symptoms, which included the associated chlorotic tissue, was estimated, and disease severity was expressed as the mean percentage area diseased on the 10 uppermost leaves on each shoot.

Field experiments. These experiments were conducted on 'Marsh' grapefruit trees at Lake Alfred and Davenport, Florida. The trees at Lake Alfred were 6 yr old and 6 ft high, and spaced 17 × 12 ft, whereas the trees at Davenport were 30 yr old and 18 ft high and spaced 25 × 25 ft.

Spray treatments were applied by handgun to drip off using 4 gal/tree (850 gal/acre) at Lake Alfred and 11 gal/tree (750 gal/acre) at Davenport. The only other spray materials used during the year of test were acaricides, which were applied to all trees, separately from the test treaments, to control rust mites. The experimental designs consisted of single-tree plots, replicated 7 or 8 times, arranged in a randomized complete block.

Greasy spot was assessed only on the spring growth flush. In May or June, prior to the application of the spray treatments, groups of shoots were labeled with white plastic tags. At Lake Alfred, 15 shoots were labeled on the east and west side of each tree. At Davenport, 8 shoots were labeled at the same 4 compass points on each tree. The original number of leaves on each shoot was recorded. In February and early March, the remaining leaves were counted and examined for greasy spot. A leaf was regarded as diseased even if it carried only one lesion. All leaves that had already abscised were assumed to have done so because of greasy spot and they were included in the total count of diseased leaves.

Only in one out of the 4 field experiments (Experiment 4, 1988-89) did enough GSRB develop to provide data on the control of this rind blemish. In this experiment, 100 fruit were picked randomly from each tree and the percentage area of fruit covered with GSRB was estimated.

Results

Greenhouse experiments. In Test 1 (Table 1), where the treatments were applied only 5 days after the end of the 14-day infection period, all treatments except diniconazole alone reduced greasy spot. When mixed with oil, CGA 169374 was more effective than oil alone, but diniconazole + oil was not significantly more effective than oil alone.

Where treatments were delayed until 14 days after the infection period (Table 1, Test 2), neither diniconazole nor basic copper sulfate reduced greasy spot severity, but CGA 169374 and oil did. When applied after 14 days, diniconazole + oil and CGA 169374 + oil did not reduce greasy spot below that obtained with oil alone.

In Test 3 (Table 1), where treatments were delayed until 25 days after the infection period, only the 1% oil spray significantly reduced disease severity.

Field experiments. In Experiment 1 (Table 2), basic copper sulfate controlled greasy spot better than oil and its performance was not enhanced by adding oil to the spray mix. In contrast, diniconazole provided relatively little greasy spot control unless oil was added to the spray mix. In Experiment 2 (Table 2) disease pressure was exceptionally heavy. Diniconazole alone provided no control, but a mixture of diniconazole + oil was more effective than copper or oil alone.

Table 1. Greenhouse experiments on grapefruit plants inoculated with *Mycosphaerella citri* to detect postinfection activity of spray materials.

Material and rate/100 gal	% leaf area diseased[z]		
	Test No. and period in days (in parenthesis) from end of 14-day infection period to treatment.		
	1(5)	2(14)	3(25)
Basic copper sulfate 0.75 lb.	—[x]	40.2 c[y]	—
Basic copper sulfate 0.75 lb. + oil 0.5 gal	—	—	37.4 ab
Diniconazole 25W 0.9 lb.	65.2 c	44.9 c	—
Diniconazole 25W 0.9 lb. + oil 0.5 gal	10.7 ab	15.5 a	42.0 ab
CGA 169374 3.5EC 0.5 pt	12.4 ab	27.8 b	—
CGA 169374 3.5EC 0.5 pt + oil 0.5 gal	3.8 a	18.2 ab	35.6 ab
Oil 0.5 gal	22.0 b	22.4 ab	37.2 ab
Oil 1.0 gal	—	—	23.3 a
Check (untreated)	60.5 c	47.0 c	58.2 b

[z]Disease severity rated at 133, 61 and 70 days after the end of the 14-day infection period in Tests No. 1, 2 and 3, respectively.
[y]Mean separation within columns by Duncan's multiple range test at P = 0.05.
[x]Treatment not included in test.

Table 2. Field evaluation of spray materials for controlling greasy spot on grapefruit leaves in 1987-88.

Material and rate/100 gal	Experiment 1 (Lake Alfred)[z]		Experiment 2 (Davenport)[y]	
	% defoliation	% leaves diseased	% defoliation	% leaves diseased
Basic copper sulfate 0.75 lb.[w]	2.2 a[x]	3.8 a	24.1 b	71.7 b
Basic copper sulfate 0.75 lb. + oil 0.5 gal	2.3 a	2.4 a	—	—
Diniconazole 25W 0.4 lb.	8.0 ab	27.4 b	60.2 c	92.1 c
Diniconazole 25W 0.4 lb. + oil 0.5 gal	2.0 a	4.4 a	14.1 a	43.4 a
Oil 0.5 gal	14.6 b	37.3 b	29.1 b	66.3 b
Oil 1.0 gal	11.2 b	27.8 b	26.5 b	64.1 b
Check (untreated)	32.7 c	66.6 c	58.5 c	93.4 c

[z]Spray materials were applied on 28 July 1987 and disease severity was assessed on 22 Feb. 1988.
[y]Spray materials were applied on 14 July 1987 and disease severity was assessed on 28 Feb. 1988.
[x]Mean separation within columns by Duncan's multiple range test at P = 0.05.
[w]Basic copper sulfate contained 53% Cu.

In the 1988-89 experiment at Lake Alfred (Table 3), greasy spot pressure was relatively light and basic copper sulfate and CGA 169374 performed better than diniconazole + oil. CGA 169374 + oil controlled greasy spot better when applied on 21 July than when it was delayed until 22 Aug., whereas time of application did not significantly affect the efficacy of the 1% oil and basic copper sulfate + oil treatments.

In the 1988-89 experiment at Davenport (Table 4), greasy spot severity was relatively mild on leaves but moderate on fruit. There was little greasy spot-induced defoliation and no differences in efficacy between treatments were discernible in this respect. Based on the number of leaves with symptoms, the July applications of basic copper sulfate, CGA 169374 and 1.0% oil performed about equally well. Diniconazole alone was less effective. Mixtures of basic copper sulfate, diniconazole or CGA 169374 with 0.5% oil were no more effective than 1.0% oil alone at either time of application. All treatments were less effective when delayed until 15 Aug. than when applied on 19 July.

GSRB was controlled well by basic copper sulfate and CGA 169374, and time of application did not significantly affect the efficacy of these materials. Oil alone provided no control of GSRB. Diniconazole was less effective than CGA 169374 at both times of application, even when mixed with oil.

Darkening of scar tissue on rind occurred after spraying with basic copper sulfate, but not with the other materials.

Discussion

Whereas oil alone failed to control GSRB, it provided as good control of greasy spot on leaves as basic copper sulfate in 3 out of the 4 field experiments. The reason for the poor results with oil in Experiment 1 of 1987 (Table 2) is unknown.

From the results reported in this paper, it appears that CGA 169374, applied either alone or mixed with oil, could be a potential alternative to copper fungicide for controlling greasy spot on both leaves and fruit. Therefore, continuing development work with CGA 169374 for use in the control of greasy spot in citrus seems justified. Diniconazole showed relatively little promise for greasy spot control, even at the high rates used in these experiments.

The results of the greenhouse tests demonstrated that whereas CGA 169374, applied alone or mixed with oil, and diniconazole + oil had some postinfection action when applied at 14 days after the end of the infection period, basic copper sulfate showed no such action when applied at this time. Nevertheless, there seemed to be little possibility of a mixture of diniconazole + oil or CGA 169374 + oil providing better greasy spot control on leaves of the spring growth flush than oil alone if the application was

Table 3. Field evaluation of spray materials at different times of application for controlling greasy spot on grapefruit leaves at Lake Alfred in 1988-89.

Material and rate/100 gal	Date of application	Disease severity[z] % defoliation	Disease severity[z] % leaves diseased
Basic copper sulfate 0.75 lb.[y]	21 July	0.0 a[x]	1.0 abc
Basic copper sulfate 0.75 lb. + oil 0.5 gal	21 July	0.0 a	0.2 ab
Basic copper sulfate 0.75 lb. + oil 0.5 gal	22 Aug.	0.0 a	2.3 abcd
Diniconazole 25W 0.88 lb.	21 July	0.4 a	17.3 ef
Diniconazole 25W 0.44 lb. + oil 0.5 gal	21 July	0.1 a	9.7 de
Diniconazole 25W 0.44 lb. + oil 0.5 gal	22 Aug.	3.2 b	27.2 f
CGA 169374 3.5EC 0.75 pt	21 July	0.0 a	0.5 ab
CGA 169374 3.5EC 0.38 pt + oil 0.5 gal	21 July	0.0 a	0.2 a
CGA 169374 3.5EC 0.38 pt + oil 0.5 gal	22 Aug.	0.0 a	5.8 bcd
Oil 1.0 gal	21 July	0.0 a	2.5 abcd
Oil 1.0 gal	22 Aug.	0.5 a	6.1 cd
Check (untreated)	—	8.5 c	57.5 g

[z]Disease severity was assessed on 2 Feb. 1989.
[y]Basic copper sulfate contained 53% Cu.
[x]Mean separation within columns by Duncan's multiple range test at P = 0.05.

Table 4. Field evaluation of spray materials with different times of application for controlling greasy spot and greasy spot rind blotch (GSRB) at Davenport in 1988-89.

Material and rate/100 gal	Date of application	Disease severity[z] % defoliation	Disease severity[z] % leaves diseased	Disease severity[z] % fruit with GSRB	Disease severity[z] % area of GSRB on diseased fruit
Basic copper sulfate 0.75 lb.[y]	19 July	0.0 a[x]	0.2 a	0.3 ab	1.3 ab
Basic copper sulfate 0.75 lb. + oil 0.5 gal	19 July	0.0 a	0.2 a	0.6 ab	2.3 ab
Basic copper sulfate 0.75 lb. + oil 0.5 gal	15 Aug.	0.3 a	4.7 bc	2.0 bc	4.4 bc
Diniconazole 25W 0.88 lb.	19 July	0.3 a	16.0 e	4.1 cd	11.2 cd
Diniconazole 25W 0.44 lb. + oil 0.5 gal	19 July	0.0 a	1.2 ab	7.1 de	15.5 d
Diniconazole 25W 0.44 lb. + oil 0.5 gal	15 Aug.	0.3 a	13.6 de	6.1 cde	12.2 cd
CGA 169374 3.5EC 0.75 pt.	19 July	0.0 a	0.3 a	0.0 a	0.0 a
CGA 169374 3.5EC 0.37 pt + oil 0.5 gal	19 July	0.0 a	0.1 a	0.1 a	0.4 a
CGA 169374 3.5EC 0.37 pt + oil 0.5 gal	15 Aug.	0.7 a	10.9 de	0.6 ab	2.4 ab
Oil 1.0 gal	19 July	0.1 a	1.1 ab	11.0 ef	7.3 cd
Oil 1.0 gal	15 Aug.	0.3 a	8.7 cd	12.1 f	12.7 d
Check (untreated)	—	4.9 b	27.6 f	9.7 def	15.4 d

[z]Disease severity on foliage was assessed on 27 Feb. 1989 and on fruit on 17 Jan. 1989.
[y]Basic copper sulfate contained 53% Cu.
[x]Mean separation within columns by Duncan's multiple range test at P = 0.05.

long delayed. For example, when treatment was delayed until August (Tables 3 & 4) there was no significant difference in effectiveness between CGA 169374 + 0.5% oil and 1% oil. In the 1987-88 experiment at Lake Alfred (Table 3) the mixture of diniconazole + 0.5% oil applied on 22 Aug., gave even poorer control of greasy spot than the 1% oil spray applied at this time.

Overall, there appeared to be little possibility of the postinfection activity of CGA 169374 having much practical impact on the control of greasy spot on leaves. In practice, more efficient use of CGA 169374 to control greasy spot on the spring growth flush would probably be achieved by applying it in July, instead of by delaying it until August to await the emergence of more summer growth flush. However, the good control of GSRB provided by the 15 Aug. application of CGA 169374 (Table 4) suggested that some flexibility in spray timing with this material might be permissible where protection of the fruit is the primary concern.

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