Timing of Single-Spray Treatments for Optimal Control of Greasy Spot on Grapefruit Leaves and Fruit

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ABSTRACT

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Spray-timing experiments for control of citrus greasy spot caused by *Mycosphaerella citri* were conducted in a grapefruit grove at Lake Alfred, FL, for 4 consecutive years. In all years, basic copper sulfate gave better control of greasy spot on leaves and fruit when applied in June or July, during or after peak ascospore release, than in late April or early May, before major ascospore discharge began. Benomyl controlled greasy spot as well as the copper fungicide did prior to a shift in pathogen population from benomyl sensitive to benomyl tolerant. Greasy spot control on leaves with oil was affected little by time of spraying, and it often equaled that provided by a well-timed copper fungicide treatment. However, unlike summer application of basic copper sulfate, oil gave variable and often no control of greasy spot rind blotch.

Citrus greasy spot, caused by *Mycosphaerella citri* Whiteside, is particularly severe in Florida on grapefruit (*Citrus paradisi* Macf.), on which it causes an unsightly rind blemish called greasy spot rind blotch (GSRB) (6), as well as serious premature defoliation. Ascospores released by rainfall (and to a minor extent by dew) from perithecia formed on decomposing, fallen citrus leaves are the major inoculum (4).

Economic considerations limit the number of spray treatments that can be applied for the control of greasy spot. Currently, the summer oil spray that is applied routinely to almost all Florida citrus groves is the mainstay of control. This spray is applied in June or July to control several insect and mite pests as well as greasy spot, and it is particularly timely for sooty mold control. Other materials used to control greasy spot include copper fungicides and benomyl, but development of tolerance to benomyl has limited its use (10).

Copper fungicides and benomyl gave better control of greasy spot when applied in June or July than when applied in May or August (1,3,5). The relatively high rate of copper fungicide commonly applied to grapefruit trees in April or May to control melanose caused by *Diaporthe citri* provided some greasy spot control, but not enough when the disease was severe (3,5). Greasy spot is usually well controlled on the spring growth flush in groves receiving a copper fungicide postbloom spray plus an oil spray in the summer, but GSRB is not always controlled by this program. This has raised doubts about the value of the postbloom copper and summer oil treatments for controlling greasy spot on leaves and fruit, respectively.

This paper reports the results of spray-timing experiments on greasy spot control in relation to ascospore release. A major purpose of these experiments was to develop a spray program for the reliable control of GSRB.

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MATERIALS AND METHODS

Experimental design. The Marsh grapefruit trees used for these experiments were about 3 m high and spaced 7.3 × 4.6 m apart. Spray treatments were applied with a handgun using 30 L/tree to single-tree plots replicated six or eight times in a randomized complete block design. The spray materials used were basic copper sulfate, 53% Cu (Cities Service Co., Atlanta, GA 30302); benomyl 50W (E. I. du Pont De Nemours & Co., Wilmington, DE 19898); and spray oil (Sunspray 7E, Sun Oil Co., Philadelphia, PA 19103) containing 99% refined petroleum distillate and meeting FC 435-66 specifications (2). The only other spray material applied to the trees during the 4-yr period was ethion, which was applied separately from the other materials and up to three times each year if rust mites became sufficiently numerous to require control.

Ascospore trapping. A Kramer-Collins 7-Day Drum Spore Sampler (G. R. Manufacturing Co., 1317 Collins Lane, Manhattan, KS 66502) was operated continuously at the center of the experimental area from 1 April to 30 September each year. The trap orifice was 1 m above the ground, and the suction flow was maintained at 10 L/min. Daily segments of the tape were mounted on microscope slides, stained with lactophenol-cotton blue, and examined at ×1000 magnification. The number of ascospores of *M. citri* present over a single 180 μm-width pass was recorded as the daily count.

Rainfall data were obtained from the National Weather Service Lake Alfred Recording Station, located 500 m from the spore trap.

Disease assessments. Greasy spot severity on leaves was based on the amount of leaf drop caused by the disease and number of leaves still attached to the shoots with greasy spot symptoms. A significant difference ($P = 0.05$) in either of these disease severity components was regarded as a positive difference in treatment efficacy. Disease assessments were confined to the spring growth flush because this is the most important one of the year and the one most severely affected by the disease.

After the leaves had fully expanded, shoots to be sampled were tagged and the leaves were counted. Shoots were labeled in groups of 10 at four locations arranged equidistantly around the canopy, at the same compass points on each tree. This arrangement facilitated retrieval and reduced variability resulting from canopy location. Leaf abscission induced by greasy spot occurs earlier on the shaded north side of the tree than on more brightly illuminated parts of the tree canopy (Whiteside, *unpublished data*).

In the first three experiments, a final assessment of greasy spot on tagged shoots was made during the early emergence of the following year's spring growth flush in late February or March. In the fourth experiment, data were obtained immediately after the major freeze of 12–14 January 1981, before freeze-induced defoliation began.

Each year, 150–200 fruits were picked randomly from each tree, washed, and examined for GSRB. In experiments 3 and 4, the rind symptoms were also rated as a percentage of fruit surface area affected, which was then averaged for the total number of fruits sampled from each tree.

Percentage data were converted by arc sine transformations to degrees of an angle before being subjected to an analysis of variance and Duncan's multiple range test.

RESULTS

Experiment 1. 1977–1978. Rainfall was below normal in April but near normal for the other months when ascospores were trapped (Fig. 1). Little leaf litter, and hence perithecial substrate, was observed on the grove floor after July. Ascospore releases were heavy during June and fell to low levels by August.

Trees sprayed with basic copper sulfate on 28 April had more defoliation than untreated trees. Basic copper sulfate and benomyl reduced defoliation most when applied on 21 July 1977 (Table 1). GSRB was controlled better with single treatments of basic copper sulfate on 21 July and 31 August than by the ones on 28 April and 7 June. The application of benomyl on 7 June controlled GSRB better than the basic copper sulfate treatment applied on that date. With the 31 August application date, only basic copper sulfate controlled GSRB. Time of application did not significantly affect the level of greasy spot control on foliage obtained with the oil spray. Oil reduced GSRB only at the application date of 7 June.

Experiment 2. 1978–1979. Rainfall was again below normal in April; above normal in May, June, and July; and well below normal in August and September (Fig. 1). Very little leaf litter was present on the ground after June. Ascospore

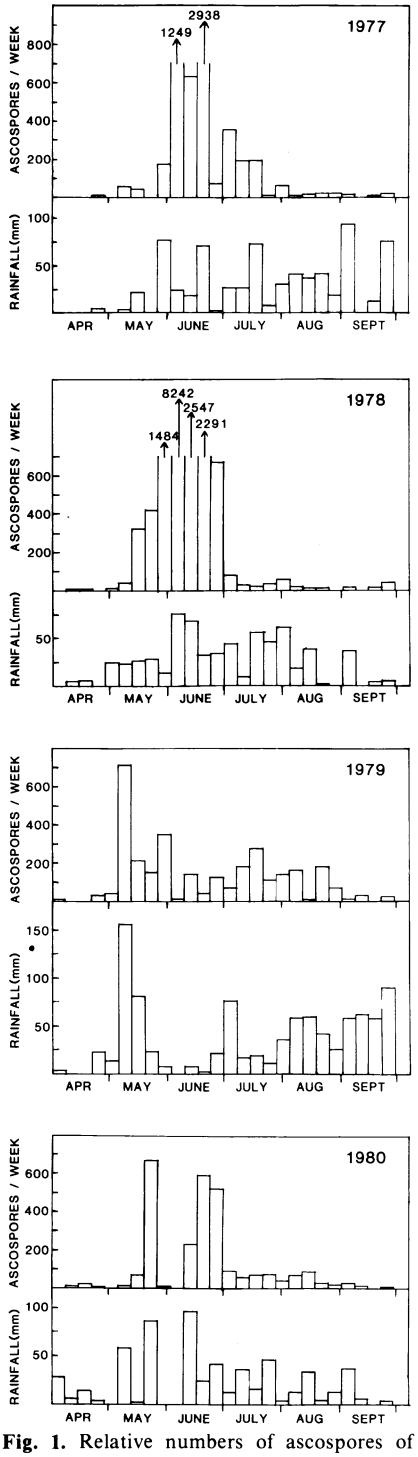

Fig. 1. Relative numbers of ascospores of *Mycosphaerella citri* released into the atmosphere each week at Lake Alfred, FL, from 1 April to 30 September in 1977–1980. Rainfall is indicated for the same weekly period as the spore trapping data. Average monthly rainfalls (based on 1941–1970) were: April, 69 mm; May, 99 mm; June, 208 mm; July, 201 mm; August, 183 mm; and September, 175 mm.

Table 1. Influence of time of application of basic copper sulfate, benomyl, and oil on the severity of greasy spot on grapefruit leaves and fruit, 1977–1978

Date of spraying Treatment and rate (g a.i./L)	Disease severity (%)[x] Leaves: Fallen	Leaves: Remaining, with symptoms	Fruit with greasy spot rind blotch
28 April 1977			
Basic copper sulfate 0.48 g[y]	71.6 e	59.7 d	32.0 bcde
Benomyl 0.11 g	37.8 c	32.3 ab	26.2 bcd
Oil 10 ml	27.7 bc	29.2 ab	31.2 cde
7 June 1977			
Basic copper sulfate 0.48 g	31.2 cd	34.3 b	17.0 b
Benomyl 0.11 g	32.0 cd	32.6 b	6.4 a
Oil 10 ml	29.2 bc	32.0 ab	20.5 bc
21 July 1977			
Basic copper sulfate 0.48 g	14.5 a	30.2 ab	2.0 a
Benomyl 0.11 g	16.9 ab	19.2 a	7.3 a
Oil 10 ml	40.2 cd	31.3 ab	36.0 de
31 August 1977			
Basic copper sulfate 0.48 g	29.2 bc	41.4 bc	2.8 a
Benomyl 0.11 g	32.6 c	34.0 b	27.8 bcd
Oil 10 ml	34.3 cd	34.9 b	45.0 e
Control (untreated)	50.2 d	50.7 cd	40.1 de

[x] Recorded for foliage on 14 March 1978 and for fruit on 16 January 1978. Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[y] Active ingredient for basic copper sulfate is expressed as metallic Cu.

Table 2. Influence of time of application of basic copper sulfate, benomyl, and oil on the severity of greasy spot on grapefruit leaves and fruit, 1978–1979

Date of spraying Treatment and rate (g a.i./L)	Disease severity (%)[x] Leaves: Fallen	Leaves: Remaining, with symptoms	Fruit with greasy spot rind blotch
5 May 1978			
Basic copper sulfate 0.48 g[y]	51.6 d	77.7 f	37.7 h
Benomyl 0.11 g	52.0 d	78.0 f	24.4 efg
Oil 10 ml	8.7 ab	22.5 ab	13.9 cde
15 June 1978			
Basic copper sulfate 0.48 g	4.7 a	11.4 a	1.0 a
Benomyl 0.11 g	28.1 c	52.9 de	11.3 c
Oil 10 ml	8.0 a	21.5 ab	22.9 defg
14 July 1978			
Basic copper sulfate 0.48 g	12.4 abc	35.0 bc	2.8 ab
Benomyl 0.11 g	21.0 bc	56.3 de	11.5 cd
Oil 10 ml	14.8 abc	43.9 cd	28.0 fgh
21 August 1978			
Basic copper sulfate 0.48 g	21.8 bc	63.3 ef	8.4 bc
Benomyl 0.11 g	29.2 c	64.6 ef	17.3 cdef
Oil 10 ml	21.4 bc	53.8 de	61.4 i
Control (untreated)	43.4 d	76.7 f	33.4 gh

[x] Recorded for foliage on 14 March 1979 and for fruit on 11 December 1978. Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[y] Active ingredient for basic copper sulfate is expressed as metallic Cu.

numbers were exceptionally high in May and June, but dropped to low levels by July.

Basic copper sulfate provided better control of greasy spot on leaves when applied on 15 June than on 14 July, poor control when applied on 21 August, and no control on 5 May (Table 2). GSRB was controlled better by basic copper sulfate applied on 15 June than on 21 August. That year, June or July applications of basic copper sulfate were more effective than similarly timed applications of benomyl in controlling greasy spot on leaves and fruit. Oil sprays applied on 5 May and 15 June controlled greasy spot on leaves better than those applied in July and August. Oil reduced GSRB only when applied on 5 May. The incidence of GSRB was higher on trees sprayed with oil on 21 August than on the untreated trees.

Experiment 3. 1979–1980. Rainfall was below normal in April but well above normal in May—353 mm compared with 99 mm (Fig. 1). Breakdown of leaf litter proceeded rapidly in May because of the unusually wet conditions, leaving much less substrate than normal for continued fungal development. Frequent wetting of the leaves favored biodegradation, and physical battering by some exceptionally heavy downpours hastened their disintegration. Dry weather in June, when rainfall was 46 mm compared with the average of 208 mm, probably delayed the seasonal exhaustion of the perithecia; this caused ascospore numbers, though low, to be higher in August than in the other 3 yr (Fig. 1).

Relatively little defoliation induced by greasy spot occurred by late winter (Table 3). Basic copper sulfate applied on 11 May at the higher rate of 0.96 g of Cu/L reduced disease severity on leaves but not nearly as well as the copper treatment applied at even half this rate on 16 July. The July application of basic copper sulfate gave better control of greasy spot on leaves than the July oil spray, but it was no better than the oil treatment applied on 11 May. GSRB was reduced significantly only by those July spray treatments that contained copper.

Experiment 4. 1980–1981. Rainfall was near normal in April but above normal in May (185 mm compared with an average of 99 mm). This hastened maturation of ascospores, and the highest weekly spore count for the year was recorded in May (Fig. 1). However, the highest monthly total still occurred in June. Ascospore releases were relatively low after June because most of the fallen leaves had completely decomposed by this time.

The application of basic copper sulfate on 7 May gave no control of GSRB and less greasy spot control on leaves than any of the other treatments at the other application dates (Table 4). Oil controlled greasy spot to a similar extent at all times of application, and it also reduced GSRB except when applied on 7 May. However, basic copper sulfate applied on 8 August controlled GSRB better than the oil spray applied on that date.

DISCUSSION

Greasy spot control with benomyl in experiments 2 and 3 was inferior to that obtained in experiment 1. This resulted from the buildup of resistance of *M. citri* to benomyl (10), which was why this material was dropped from the last experiment.

In 1977, 1978, and 1980, counts of discharged ascospores increased rapidly through May, reached a peak in June, and then declined rapidly through July because of rapid decomposition of the fallen leaves and an observed lack of leaf drop to provide further perithecial substrate. This seasonal discharge pattern was similar to that previously reported (4,5). A different discharge pattern occurred in 1979 because of the unseasonally wet weather during May, which reduced the overall ascospore-producing potential of the leaf litter. In 1979, the highest weekly ascospore count was in early May. Thus a copper fungicide application in May might have been expected to control greasy spot better than one applied in July. This was not the case. Basic copper sulfate at 0.48 g of Cu/L applied 16 July still controlled greasy spot on the leaves better than the

Table 3. Influence of different spray treatments applied in July and different times of application of basic copper sulfate and oil on the severity of greasy spot on grapefruit leaves and fruit, 1979–1980

	Disease severity (%)[x]			
	Leaves		Rind blotch	
Date of spraying Treatment and rate (g a.i./L)	Fallen	Remaining, with symptoms	Fruit affected	Surface area with symptoms
11 May 1979				
Basic copper sulfate 0.96 g[y]	2.4 bc	16.7 d	4.6 b	0.2 b
Oil 10 ml	0.8 ab	4.6 bc	3.7 b	0.2 b
13 June 1979				
Oil 10 ml	1.8 ab	9.7 cd	3.6 b	0.2 b
16 July 1979				
Basic copper sulfate 0.48 g	0.5 ab	2.2 ab	0.0 a	0.0 a
Benomyl 0.11 g	2.0 ab	15.5 d	4.0 b	0.2 b
Oil 5 ml	1.3 ab	17.4 d	6.9 bc	0.6 bc
Oil 10 ml	1.0 ab	12.6 cd	8.4 c	0.7 c
Basic copper sulfate 0.24 g + oil 5 ml	1.2 ab	0.5 a	0.9 a	0.0 a
Basic copper sulfate 0.48 g + oil 5 ml	0.4 a	1.1 a	0.2 a	0.0 a
Benomyl 0.11 g + oil 5 ml	0.8 ab	6.3 c	4.4 b	0.4 b
20 Aug 1979				
Oil 10 ml	0.9 ab	10.1 cd	7.4 bc	0.5 bc
Control (untreated)	4.3 c	42.4 e	6.3 bc	0.5 bc

[x] Recorded for foliage on 28 February 1980 and for fruit on 4 March 1980. Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[y] Active ingredient for basic copper sulfate is expressed as metallic Cu.

Table 4. Influence of time of application of basic copper sulfate and oil on the severity of greasy spot on grapefruit leaves and fruit, 1980–1981

	Disease severity (%)[x]			
	Leaves		Rind blotch	
Date of spraying Treatment and rate (g a.i./L)	Fallen	Remaining, with symptoms	Fruit affected	Surface area with symptoms
7 May 1980				
Basic copper sulfate 0.96 g[y]	5.0 b	28.7 c	23.5 d	6.8 d
Oil 10 ml	1.5 a	13.5 ab	15.0 cd	4.3 cd
16 June 1980				
Basic copper sulfate 0.48 g	1.2 a	6.9 a	5.4 ab	0.6 ab
Oil 10 ml	1.1 a	13.5 ab	9.6 bc	1.9 bc
8 July 1980				
Basic copper sulfate 0.48 g	1.8 a	7.0 a	3.2 ab	0.5 ab
Oil 10 ml	1.1 a	12.2 ab	5.4 ab	0.7 ab
8 August 1980				
Basic copper sulfate 0.48 g	0.8 a	14.2 ab	0.5 a	0.0 a
Oil 10 ml	1.8 a	17.8 b	9.6 bc	1.7 bc
Control (untreated)	6.1 b	54.9 d	22.0 d	5.5 d

[x] Recorded for foliage on 16 January 1981 and for fruit on 26 January 1981. Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[y] Active ingredient for basic copper sulfate is expressed as metallic Cu.

treatment with twice as much copper applied on 11 May.

In all four experiments, the copper fungicide controlled greasy spot much better when delayed until June or July, during or after peak ascospore release, than when applied in May before major ascospore release normally begins. This appears to contradict the principle that a protectant material such as copper should be deposited on the plant ahead of inoculum deposition. The apparent anomaly with greasy spot control can be explained on the basis that host penetration by *M. citri* in Florida does not necessarily coincide with the time of major ascospore release. A key feature in greasy spot epidemiology is the extramatrical mycelial growth that *M. citri* produces on the surface of the host following germination of ascospores, greatly enhancing the potential for host penetration later (8). This growth is dependent on high humidity or dew wetting with accompanying high temperature.

In Florida, the conditions for extramatrical growth of *M. citri* occur mostly at night and more frequently from July to September than in May or even June (8,9). A copper fungicide treatment applied in June or July can kill existing extramatrical mycelial growth and still provide protection against later releases of ascospores. In contrast, the residual value of a copper fungicide applied in April or May could be lost by erosion before major ascospore discharge ceases and before the humidity-temperature regimes become favorable for extramatrical mycelial growth and infection.

The reason for the significant increase in defoliation on trees sprayed with the low rate of basic copper sulfate on 28 April 1977 is unknown.

The relative effectiveness of June versus July applications of copper fungicide for greasy spot control varied from year to year. In experiment 1, treatment on 21 July gave better control of greasy spot on leaves and fruit than one applied on 7 June. In experiment 2, greasy spot on leaves was controlled better by a treatment applied on 15 June than by one on 14 July; however, they both controlled GSRB well. In 1980, there was no substantial difference in effectiveness between the copper fungicide treatments applied in June and July. GSRB was controlled satisfactorily by copper treatments applied in August, even in years when such late application did not control the disease well on the leaves. The reason for this difference in treatment response is unknown.

Time of spraying had no significant effect on the efficacy of oil for greasy spot control on leaves except in experiment 2, where better control resulted from treatment in May or June than in July or August. The results corroborated previous contentions that oil controls greasy spot more by decreasing host predisposition than by disrupting infection (7). Oil sprays often performed as well as a suitably timed copper fungicide in reducing foliar greasy spot. However, oil often gave little or no control of GSRB.

The reason for the substantial increase in severity of GSRB in experiment 2 following the oil treatment of 21 August is unknown, but it could have been caused by some phytotoxic effect of oil on cells previously weakened by *M. citri*.

In summary, these results highlighted the unreliability of oil for the control of GSRB. They indicated the need for including a relatively low rate of copper fungicide in the oil spray to fortify the control of GSRB and to assure a high packout of blemish-free grapefruit for the fresh market. Overall, the best month to apply a copper fungicide-oil spray routinely for optimal control of greasy spot on both leaves and fruit appeared to be June or July, which is the time of year when an oil spray is best applied for another important purpose, sooty mold control.

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